THE IMPACT OF INTENSIVE GENETIC SELECTION FOR IMPROVED PERFORMANCE IN THE BROILER CHICKEN ON METABOLIC RATE, MORPHOLOGY AND BODY COMPOSITION

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ABSTRACT

The intensive genetic selection for fast growth rate, improved feed conversion ratio and increased breast meat yield that broilers have undergone has resulted in an increased incidence of metabolic disorders. It has been hypothesised that this is due to an imbalance in form and function between supply and demand tissues, known as symmorphosis. Such a breakdown in symmorphosis may also have consequences in limiting future performance. The effects of genetic selection on the gross morphology (organ mass), metabolic rates and carcass composition of three genotypes of chicken, which have undergone selection on different criteria, were examined. In addition, the use of near-infrared spectroscopy (NIRS) was investigated as an alternative technique for assessing the chemical composition of chicken carcasses.

The experimental studies described within this thesis investigated the effects of the intensive genetic selection in the modern broiler by comparing it with two other genotypes of chicken that have undergone different levels of selection. These included: a modern commercial broiler (Ross 308, FB), a broiler that has not been selected for fast growth since 1972 (Ross 1972, SB) and a layer chicken which has never been selected for fast growth rate (Euribrid HISEX, L).

A thermal load was placed on the birds to evaluate the effects of genetic selection on metabolic rate. Metabolic rate was determined using open-circuit indirect calorimetry to measure oxygen consumption (VO_2). Resting (RMR) and peak metabolic rate (PMR) were measured in each individual bird on the same day during the first 2 weeks of life. Resting metabolic rate was determined by measuring VO_2 of the birds at thermoneutrality (28-32 °C), whilst PMR was determined at the point that VO_2 began to decline with decreasing temperature (0 to –7 °C). There was no significant effect of genotype on relative RMR ($ml.O_2/hr$). However, the FB genotype had larger PMR and consequently a larger metabolic scope compared to the L genotype. This could be attributed to the greater relative muscle mass of the broiler, which is used primarily for heat production during shivering thermogenesis.

The FB genotype also had a larger relative pectoral and leg muscle mass, but also a larger water content within these muscles, indicating a lack of maturity in the muscle growth. The FB genotype had larger relative intestine and caeca mass, whereas the relative gizzard and brain masses and ash content were greater in the L genotype. Relative liver mass did not vary with genotype and the variation in relative heart mass between genotypes depended on the covariate used in the analysis. This suggests that during the genetic selection process that the broiler chicken has undergone, the mass of these organs has been sacrificed to increase meat yield. The residuals of RMR correlated significantly and positively with the residuals of heart mass in those FB birds above 80 g. The broiler industry is continually trying to keep energy costs down, therefore they are likely to select birds with lower energy costs and therefore lower RMR. In turn this may potentially be selecting for modern broilers with a lower heart mass.

The morphological differences observed in the first 14 days of life were further investigated. Each of the three genotypes was grown until their respective maximum growth rate was achieved, and then their organ development compared. As expected, the FB genotype had a larger body mass and grew at a faster rate than the L genotype, with the SB genotype being intermediate. The relative pectoral and leg muscle masses were greater in the FB genotype and there was a larger water content within the muscles of the FB, which has been previously shown to indicate a lack of maturity in the muscle. The relative intestine, liver, heart and lung masses were all larger in the FB genotype compared to the other genotypes. The intestine and liver are required to absorb and metabolise nutrients and the heart and lungs are required to pump oxygenated and nutrient rich blood around the body. It is not surprising that these supply organs are larger in the FB genotype in response to the increased growth rate and breast meat yield. In contrast, the relative carcass, gizzard and brain masses were larger in the L genotype and the relative caeca mass was greater in the SB genotype compared to the FB. This suggests that the mass of these organs has been sacrificed during the selection process that the FB has undergone, with the possible consequence of metabolic disorders.

A large detailed database of body composition in these three genotypes was generated. NIRS was used as an alternative technique for predicting the chemical composition of poultry carcasses. The use of NIRS provided a rapid, reliable and nonhazardous method of determining genotype, age, body mass, growth rate, and robustly predicted both crude protein and fat content of whole milled chicken carcasses. In addition, NIRS was also able to successfully discriminate between the different genotypes used within this study and also between birds from the same genotype (L) that had been fed diets with different energy and protein levels.

It was concluded that the intensive genetic selection that has occurred within the broiler breeder industry to produce the modern broiler has led to a bird with an increased muscle mass but has also led to a reduction in relative mass of the brain, gizzard and carcass. There is a possibility that genetic selection could continue at the present rate but that a physiological limit could soon be reached due to the evidence from the present study which suggests that symmorphosis has broken down. If the level of intensive selection used currently to produce broilers does continue at the present rate without a concomitant increase in supply organs, there will ultimately be a further increase in metabolic disorders or the emergence of more metabolic disorders. Since the heart, as well as other supply organs, is being placed under a much greater metabolic workload. The inclusion of increased mass of the supply organs such as the heart, liver and lung as a selection criterion may be necessary to produce future improvements in muscle yield.

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DECLARATION

This thesis has been composed by myself and has not been presented in any previous application for a degree. The work, of which this is a record, has been done by myself (unless otherwise stated) and all sources of information have been specifically acknowledged by means of references.

Amanda Jane Gavin

GLOSSARY OF SYMBOLS AND ABBREVIATIONS

AFP	Abdominal fat pad
AHI	Ascites heart index
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BAT	Brown adipose tissue
BM	Body mass
BM-OM	Body mass minus organ mass
BMR	Basal metabolic rate
CP	Crude protein
DEA	Daily energy assimilation
DEI	Daily energy intake
DIT	Dietary induced thermogenesis
DLW	Doubly labelled water
DM	Dry body mass
DM-OM	Dry body mass minus organ mass
DXA	Dual-energy x-ray absorptiometry
ECG	Electrocardiograms
EE	Energy expenditure
FA	Fatty acids
FB	Fast-growing broiler genotype
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
FMR	Fasting metabolic rate
GIT	Gastro-intestinal tract
GR	Growth rate
HP	Heat production
L	Layer genotype
LBM	Lean body mass
LBM-OM	Lean body mass minus organ mass

M:O	Muscle mass to organ mass ratio
MPLS	Modified partial least squares
MR	Metabolic rate
MS	Metabolic scope
n	Number of samples
NIRS	Near-infrared reflectance spectroscopy
NST	Non shivering thermogenesis
1-VR	Coefficient of determination in cross validation in NIRS
OLLN	Number of outliers removed to perform the calibration models in NIRS
PCA	Principal component analysis
PHS	Pulmonary hypertension syndrome
PLS	Partial least squares
PMR	Peak metabolic rate
P_aO_2	Partial pressure of oxygen
PSE	Pale, soft and exudative meat
r^2	Coefficient of multidetermination in calibration in NIRS
RV:TV	Right ventricle to total ventricle ratio
RMR	Resting metabolic rate
SB	Slow-growing broiler genotype
SD	Standard deviation from the mean
SDS	Sudden death syndrome
SEC	Standard error of calibration in NIRS
SECV	Standard error of cross validation in NIRS
SEL	Standard error of laboratory
SEM	Standard error of the mean
SNV	Standard normal variate
ST	Shivering thermogenesis
STP	Standard temperature and pressure
SusMR	Sustainable metabolic rate
SusMS	Sustainable metabolic scope
T	Number of PLS factors used to perform the calibration models in

	NIRS
T_a	Ambient temperature
TAG	Triacylglycerides
TC	Thermal conductivity
TNZ	Thermoneutral zone
VO_2	Oxygen consumption
VO_2max	Maximal oxygen consumption

PUBLICATIONS OF EXPERIMENTAL DATA

The following publications were derived from this research:

A. Gavin, I. Murray and R. M. McDevitt (2000). The use of near-infrared reflectance spectroscopy (NIRS) for poultry carcass analysis. *Abstracts and Proceedings of the XXI World Poultry Congress, Montreal, Canada.*

M. Konarzewski, A. Gavin, R. McDevitt and I. Wallis (2000). Metabolic and organ responses to selection for high growth rates in the domestic chicken (*Gallus domesticus*). *Physiological and Biochemical Zoology* 73:237-248.

A. Gavin and R. McDevitt (1999). Intraspecific variation in muscle and organ growth in three strains of chicken with differential genetic selection for fast growth rate. *British Poultry Science* 40:S19-S20.

I. Wallis, M. Konarzewski, R. McDevitt and A. Gavin (1999). Metabolic and organ mass responses to selection in chickens (*Gallus domesticus*). *Australian Poultry Science Symposium* 11:53-56.

A. Gavin, M. Konarzewski, I. Wallis and R. McDevitt (1998). The relationship between metabolic rate and organ size in two strains of chicken. *British Poultry Science* 39:S51-S52.

CHAPTER 1

1. INTRODUCTION

1.1 Genetic Selection in Chickens, (*Gallus gallus domesticus*)

Chickens are thought to have been domesticated over 4000 years ago, after centuries of being hunted as wild jungle fowl for food. The early domesticated fowls were also used in religious ceremonies (Parkhurst & Mountney, 1988). The domestic fowl as we know it today originated from the Red Jungle Fowl (*Gallus gallus*), in Southeast Asia. They reached Persia about 1000 BC and Greece around 500 BC, where the Greeks raised chickens for the sport of cockfighting (Parkhurst & Mountney, 1988). The Romans were probably the first poultrymen. They kept and bred chickens specifically for food, which is the first record of breeding and genetic selection in the domestic fowl (Parkhurst & Mountney, 1988).

Most breeds and varieties of fowl were developed in the 19th century. Initially little attention was given to eggs or meat as food items, as breeding objectives were primarily aesthetic, for the establishment of feather patterns and body conformation. It was the more affluent of society which had an interest in poultry breeding and which produced the majority of the standard breeds currently dominating the world's poultry industry (Marks, 1995).

During the early part of the 20th century two important events, one practical and the other scientific knowledge-based, aided the overall development of today's poultry industry. These were the rediscovery of Mendelian principles of hereditary and the invention of the trapnest which lead to individual egg production records (Marks, 1995). The domestic fowl's high reproductive capacity i.e. an egg per day, made it an ideal model for demonstrating the application of Mendelian genetics. The introduction of the trapnest allowed for selection to be based on the performance of

individual birds (Marks, 1995).

The commercial broiler industry started around 1935. Two of the major companies that have evolved into today's leading commercial poultry breeders are Ross Breeders and Cobb Breeding Company. From 1935 to the present day there has been genetic selection within and between breeds (industrial breeds e.g. White Cornish, Rhode Island Red) and genotypes (the lines that different commercial companies produce e.g. Ross 308, Cobb 500). The main aim of this selection for growth, is to improve the profitability of broiler production by a variety of related means such as increasing the lean muscle yield and increased efficiency of food utilisation. According to Havenstein (1991a,b), genetic selection accounts for approximately 80% of the increased performance of modern broilers and nutrition only accounts for 20%.

1.1.1 Genetic Selection in Meat-Type Chickens

1.1.1.1 Selection for Growth Rate

Initially, investigators attempted to determine the importance of heredity in growth and the nature of the genetic factors involved by studying the performance of chickens of various breed and genotype crosses (Chambers, 1990). Early studies provided evidence of the heritability of growth rate, but results indicated that the factors involved are complex (Lerner & Asmundson, 1932).

Evidence of the success of this selection for growth rate and its effect on broiler performance is shown by the drastic reduction in the age of the chicken to reach slaughter weight over the past 50 years. In the year 1950 broilers reached 1.82 kg live body weight at 12 wk of age and consumed 3.25 kg feed for every kg of liveweight gain. In 1988, broilers weighed 1.82 kg at 6 wk 2 days of age after consuming only 1.95 kg feed per kg gain (Gyles, 1989). In 1999, Ross broilers averaged 1.82 kg in 5 wk on 1.56 kg feed per kg gain (Ross Breeders, 1999). This

reduction in time for the broiler to reach a slaughter weight is linked to profit. Apart from the obvious increase in profit through reduction of feed conversion ratio (FCR), the industry benefits by rearing more flocks each year in each poultry house (Mallard & Douaire, 1988). The production of poultry within the United Kingdom has consequently increased dramatically over the past four decades (Figure 1.1).

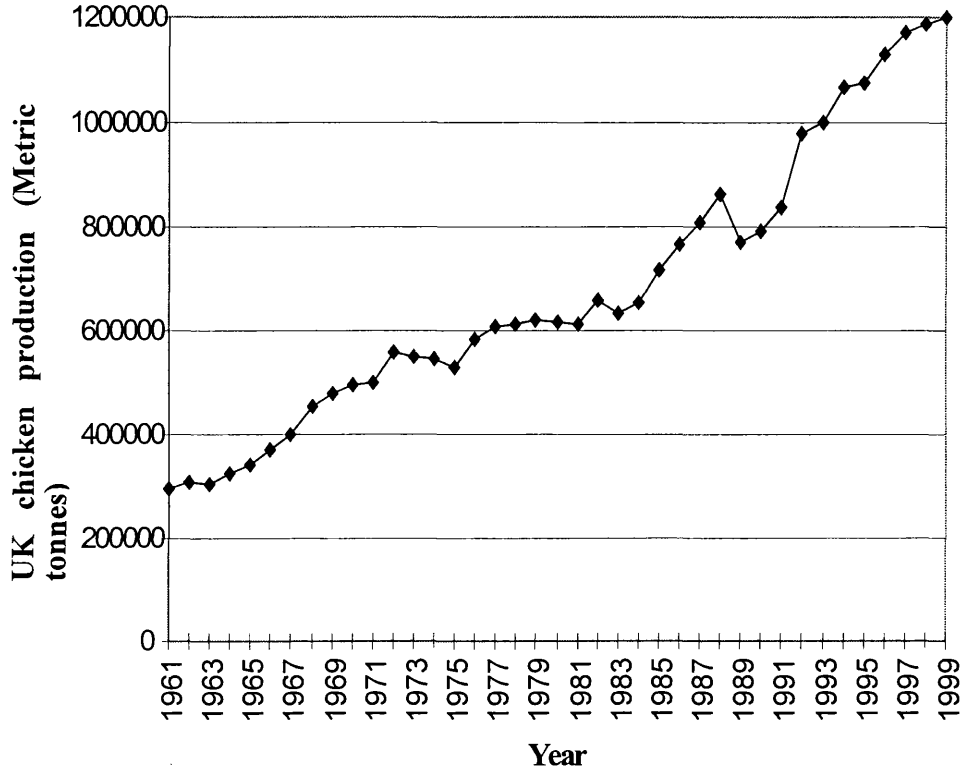


Figure 1.1 Poultry production within the UK. Data taken from the Food and Agriculture Organization (FAO) (www.http://apps.fao.org/).

Chickens from a common origin can be classed together. There are four major classes; the American, the Mediterranean, the English and the Asiatic. The American class includes chickens that were bred to forage and survive on farms with a minimum of care e.g. Rhode Island and New Hampshire Reds, White and Barred Plymouth Rocks, Wyandottes and Jersey Giants. The Mediterranean class originated in Italy and Spain. They are generally active, nervous birds e.g. Leghorns, Minorcas, Anaconas, Blue Andalusians and White-Faced Black Spanish. Chickens in the

English class generally had very white skin e.g. Orpingtons, Sussex, Australorps, Dorkings and Cornish. The Asiatic class have chickens which are slow and lethargic in temperament e.g. Cochins, Brahmas and Langshans (Parkhurst & Mountney, 1988). Various breed and genotype crosses have been used during the selection process to produce a modern broiler genotype, but today most of the world's commercial broilers are a four-way cross. The paternal grandparents are synthetic lines derived in part from White Cornish (Crawford, 1990). These lines are called synthetic because the have resulted from mixing of several breeds over many generations (Gibson, 1996). The maternal grandparents are also synthetic lines, based heavily on White Plymouth Rock (Crawford, 1990).

Measurements of Growth

Growth is a complex physiological process of cell division that begins at fertilization and continues until mature body weight is reached. The individual's genetic code defines the beginning and end of the developmental phases, although changes in environmental factors could influence the development. Neurological development occurs initially and is then followed by the formation of bone, muscle and adipose tissue (Marks, 1995). Early transition of the digestive system from embryonic absorption of yolk to digestion of exogenous feed seems essential to maximize early growth (Nitsan *et al.*, 1991). Muscle fibre hyperplasia (increase in cell number) is the dominant growth process before hatching, which gradually becomes fibre hypertrophy (increase in radius and length of fibres) during the post-embryonic period (Swatland, 1995). Hyperplasia of the muscle cells at hatch could be used as an indicator for the potential lean growth in the mature bird.

If a chicken is to reach its genetic growth potential then it must be reared in a favourable environment (ventilation, temperature and humidity control within the poultry house) and under adequate nutrition. The accurate measurement of the entire growth phase cannot be easily performed because this would require continual measurements of a chickens body weight. This is impractical, as it would impede on the chickens movement and activity. Therefore, simplified and practical measures are used to evaluate the growth of chickens and other animals (Chambers, 1990) of

which there are three main examples. The easiest, the most commonly used and the crudest measure of growth rate is body mass at different ages. Unfortunately this measurement gives no indication of the growth rate for the intervening ages, but simply shows the accumulated growth. The second method, body weight gain during a given interval is also a good measure of average growth rate during the interval measured, but again gives little indication of any changes in growth rate during the measurement period (Chambers, 1990). Also, this method gives no indication as to whether the increase in body mass measured was due to muscle or fat production. The final and most exact technique commonly used is to model growth with mathematical functions. The growth curve of the chicken, like other animal, plant and microbial populations is sigmoidal (Chambers, 1990) and can describe the growth over the whole life cycle or part thereof.

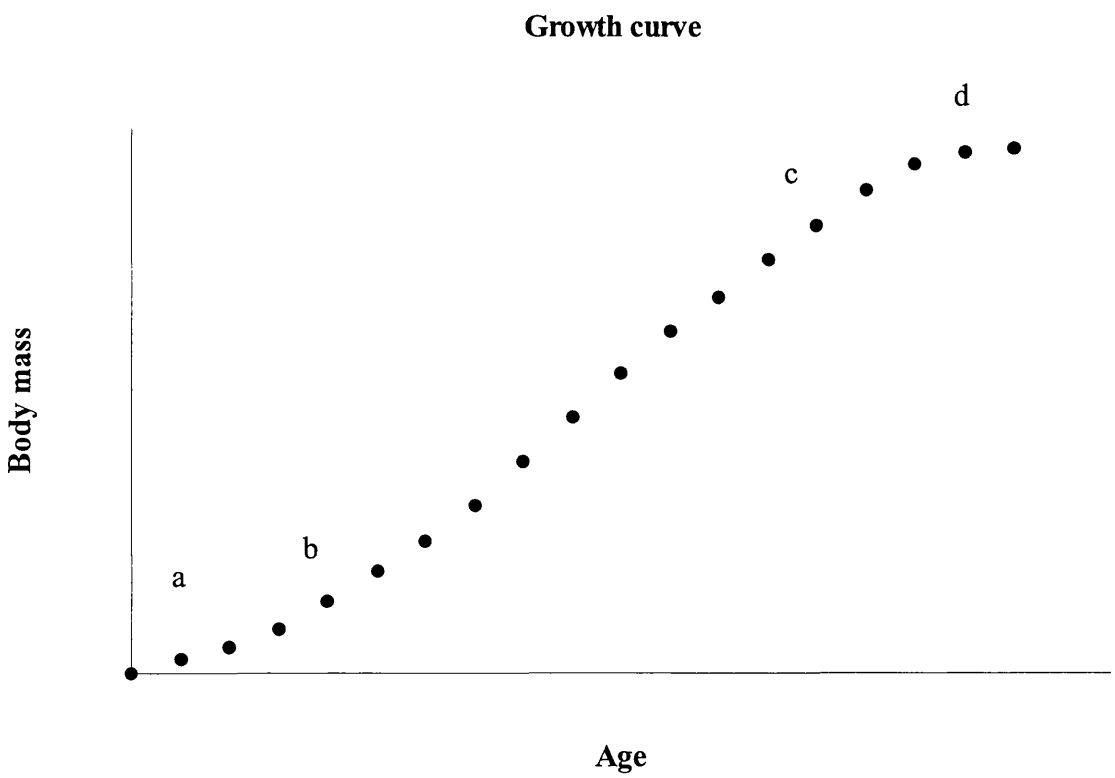


Figure 1.2 Example of an avian growth curve.

Avian growth curves have four characteristics; an initial growth phase following

hatching (a), a point of inflection coinciding with the maximum growth rate (b), a descending growth phase (c), and a mature weight which is referred to as the asymptote (d) (Figure 1) (Baker, 1944, cited in Chambers, 1990).

There are different mathematical models that can be used to calculate the different phases of growth, the Gompertz, Logistic, von Bertalanffy, Morgan-Mercer-Flodin and Weibull. The growth equations for these models are shown in Table 1. In these sigmoidal functions *W(t)* is the weight in grams at the time *t* in days. The parameter A_{max} is the asymptote or final body weight, t^* is the inflection point, *B* and *C* are constants and *e* is base of natural log.

Table 1.1 Equations for growth curve analysis.

Function	Growth equation
Gompertz	$W(t) = A_{max}.e^{-e-B\,(t-t^*)}$
Logistic	$W(t) = \frac{A_{max}}{1+e^{-B(t-t^*)}}$
Von Bertalanffy	$W(t) = A_{max}\left(1-\frac{1}{3}e^{-B(t-t^*)}\right)^3$
Morgan-Mercer-Flodin	$W(t) = A_{max} - \frac{A_{max}-B}{1+\left(\frac{C-1}{C+1}\right)\left(\frac{t}{t^*}\right)^c}$
Weibull	$W(t) = A_{max} - (A_{max}-B)e^{\left(\frac{C-1}{C}\right)\left(\frac{t}{t^*}\right)^c}$

Source Maruyama *et al.*, 1999

Constants in growth curves are equal to the product of elapsed time and the compound interest rate of growth for that phase of growth (Lerner and Asmundson, 1938). Each phase will therefore have a different constant. Constants have been used not only to quantify but also qualify differences in growth curves between sexes, breeds, and strains (Lerner and Asmundson, 1938). To fit and determine the

parameters of the growth curve for a given strain of bird, a combination of actual and predicted body weights and equations are used. In applied poultry breeding it is necessary to evaluate growth during juvenile stages to permit choice of actions long before the chicken achieves maturity (Chambers, 1990). Unfortunately due to the practicalities of application there is limited interest in growth curves for day-to-day use by the poultry breeding industry.

1.1.1.2 Selection for Feed Efficiency

In the domestic animal industries the efficiency of growth is usually defined as either feed conversion efficiency (FCE, which is defined as weight gained per unit of feed consumed) or its inverse, feed conversion ratio (FCR, which is defined as feed consumed per unit of weight gained) (Guenter & Campbell, 1995). From the industry perspective, an ideal chicken growth pattern would include both a high FCE and a low FCR. The feed consumed by the animal to maintain itself accounts for the largest share of the total feed consumed. The proportion of feed that maintenance represents increases with age (Shalev, 1995).

Growing broiler chickens are invariably fed *ad libitum* on a high quality diet formulated to be adequate in energy, macronutrients and micronutrients. They are reared under environmental conditions which minimize heat loss and maximize food intake (Forbes, 1988). The genetic improvement in feed conversion in broilers was achieved initially as a concomitant response to the selection for increased growth rate. Faster growing birds are more efficient at converting food into body mass (BM) than their slower growing counterparts (Marks, 1995). The energy expenditure used for physical activity is regarded as being part of the maintenance requirement. Therefore, these faster growing birds have a shorter lifespan and also have a tendency to be less active, thereby reducing their maintenance costs. It was not until the 1980's that most large breeding companies began to actively select for feed efficiency as an independent characteristic (Lilburn, 1988).

Jorgensen *et al.* (1990) reported that the selection for high feed efficiency only

resulted in birds with much leaner carcasses than birds selected for high growth rate. In a study comparing a high efficiency line (A) and a line selected for greater BM (B) at two age intervals (13 and 46 days of age), analysis of the autoclaved carcass, including blood and feather masses, for dry matter, nitrogen, ash, fat and energy content was conducted. The concentration (g/kg dry matter) of ash and protein, reflecting the amount of skeleton and lean muscle in the carcass respectively, was much higher in line A. In addition, the levels of fat and energy were lowest in line A and these results were found at both ages.

Pym & Nicholls (1979) compared three genotypes of chickens, the first selected for increased body weight gain (W), the second for increased feed consumption (F) and the last for decreased feed conversion ratio (FCR) (E). Selection for reduced FCR in the E line resulted in a moderate increase in weight gain, no effect upon feed intake, and consequently a considerable decrease in FCR. Selection for increased weight gain in line W resulted in a substantial increase in gain, a moderate increase in feed consumption and, as a result, only a moderate improvement in FCR. Lastly, selection for increased feed consumption in line F caused a large increase in food intake, a moderate correlated increase in weight gain and, as a consequence, a substantial increase in FCR.

Therefore, using FCR as the sole selection trait would not appear to be recommended because of the lower than optimal correlated response in growth rate (Scheele, 1997). The most economic and appropriate way to improve bird performance is to combine the selection for both growth and feed conversion (Scheele, 1997). Although selection studies resulting in broilers with different body weights and carcass compositions are interesting biologically, they are not necessarily beneficial to industry because commercial chicken production must consider the responses to selection of many traits. This is because the benefits that may be derived from one trait may also be disadvantageous with regard to other carcass characteristics (Lilburn, 1988).

1.1.1.3 Selection for Reduction of Fat Deposition

An unforeseen indirect physiological response to rapid growth selection in broilers has been excessive fat deposition, which is stored as adipose tissue mainly in abdominal, subcutaneous and intermuscular deposits (Leenstra, 1986). Fat is considered to be a by-product of fast growth and has very low commercial value. It is believed that the consumer has two problems with fat. First, they find it objectionable as the fat must be trimmed and discarded and this is waste that the consumer has to pay for. Secondly, because fat is perceived as unhealthy in an increasingly health conscious society this can affect the "low-in-fat-content" image of poultry meat. Excessively fatty carcasses also represent a loss for the poultry processing industry that package, cut-up and further process poultry products. This loss is due to the added expense in cleaning equipment that increases with fatty carcasses and the financial loss incurred because fat is only a by-product and can not be sold.

Avians break down dietary fat into glycerol and fatty acids (FA) in the liver and these components are then reassembled into triacylglycerides (TAG) for transport into the bloodstream, and are stored in fat cells, as the fat cells cannot themselves synthesize TAG, but simply store it. In broiler chickens, older birds have a higher fat content than younger birds and at a given BM, the carcasses of female broilers contain more fat than those of the male broiler (Leenstra, 1986; Havenstein *et al.*, 1994b). Fat is a costly body component from an energy point of view as its deposition in large amounts can depress feed efficiency (Leclercq, 1988). Feed efficiency is not depressed directly in a biochemical or physiological way, but results from the carcass having a higher fat content and a lower muscle content, and this fat is more costly in energy terms to deposit. This is the opposite of what either the meat-producer or the consumer wants. However, the amount of fat in the bird carcass can be reduced by dietary manipulation (lowering the ratio of energy to protein in the diet) or by adjusting husbandry management (by restrictive feeding techniques), which has been reviewed by Leclercq (1986) and Scheele (1997). The specifically formulated diets and the particular rearing environments used in broiler

production are calculated to ensure the maximum growth at minimum cost. Selection for feed efficiency may provide a practical solution to the abdominal fat problem, since there appears to be a favourable inverse relationship between abdominal fat and feed efficiency (Marks, 1995).

It has been previously reported that the dietary fat source fed to an animal could have consequential effects on the animal's deposition of body fat (Awad, 1981). A diet rich in polyunsaturated fatty acids result in preferential stimulation of thermogenic activity and produce lower body energy accumulation than a diet rich in saturated fatty acids (Sanz *et al.*, 1999). Sanz *et al.* (1999) reported that broilers fed on diets containing animal fat blend or tallow had greater abdominal fat pad weights and intramuscular lipid contents than those birds given diets containing sunflower oil-enriched diets. The energy source of a diet may also affect the fat deposition in a chicken. Rama Rao *et al.* (2001) compared broiler breeders fed different energy sources. Deposition of abdominal fat was significantly greater in bird's fed broken rice compared to those fed on yellow maize, whilst liver fat content was significantly greater in birds fed the pearl millet or broken rice diets than those fed yellow maize.

Chambers *et al.* (1983) reported that feed efficiency improved with rapid weight gain but declined with greater carcass fatness. Their results showed that carcass fatness and feed efficiency had a correlation coefficient of 0.48, which rose to 0.62 after adjusting for weight gain. Similarly the correlation coefficient between carcass fatness and feed consumption rose from 0.40 to 0.63 after adjusting for weight gain. Hence, results from feed efficiency tests at a given age should be corrected for differences in initial weight of the bird to reflect true differences in efficiency.

It is, therefore, generally accepted that a genetic and breeding approach, rather than nutrition alone, is a more feasible long-term solution to the reduction of carcass fat (Leenstra, 1986).

Measurements of Body Fat

Research towards a technique for genetic manipulation to reduce fat has been

hampered by the lack of a suitable and accurate non-invasive technique for determining whole body fat in living birds. Although fat occurs throughout the chicken, the abdominal fat pad is a concentrated mass of fat and therefore easier to access and measure. Pym & Thompson (1980) reported on a caliper technique as a physical method for measuring the amount of abdominal fat, but Whitehead (1988) regarded this method as unreliable. Body fat can be composed of: dietary FA which are deposited in the fat vacuoles of specialised adipose cells without being further metabolised, FA are synthesised from carbohydrates or protein in the liver, or fat in the form of FA released from body stores, or a mixture of all of these. A TAG is formed by the combination of one glycerol molecule and three FA molecules. The evidence for using plasma TAG levels as an accurate means of predicting body fat in broilers is equivocal (Bartov & Bornstein, 1974). It was suggested that plasma TAG measurements were an unsuitable predictor of carcass fat in broilers because Bartov & Bornstein (1974) found no significant difference between carcass fat and plasma TAG levels when they compared two dietary energy levels (with and without dietary fat supplement) and diets with different energy to protein ratios. In contrast, Griffin *et al.* (1982) reported phenotypic correlation (i.e. a correlation between two measurable characteristics which are dependent on the animals genetic makeup) between body fat and total plasma TAG level. The total fat content of the broiler carcasses in this study was determined by ether extraction using a Soxhlet apparatus. The results showed that birds with elevated plasma TAG concentrations were significantly fatter than those with low plasma TAG concentrations. Griffin *et al.* (1982) concluded that plasma TAG levels could be used as an indirect method of selecting for reduced fat content in broilers.

Total body electrical conductivity or bio-impedence is a non-invasive procedure used to predict chemical composition, specifically fat mass, in birds (Skagen *et al.*, 1993; Scott *et al.*, 1991). Staudinger *et al.* (1995) used bioimpedence to predict fat-free mass in broiler chicks during the first two weeks of life. This study found that two calibration equations were necessary to predict growth, the first from hatch to 4 day-old and the second from 6 to 14 day-old. These calibration equations could explain 95% of the variation in fat-free mass. Therefore, it was determined that bioimpedence could be used as a predictive tool for measuring fat-free mass in live

broiler chicks up to two weeks posthatch. However, there is still some speculation as to the confidence of the predictions that bioimpedence can produce. Hinton *et al.* (1998) used the whole body potassium-40 content for estimating the fat mass in pigeons. Body potassium is present in lean tissue but not in fat. They used naturally occurring radioactive potassium-40, which occurs as a fixed ratio to total body potassium, to measure the lean mass and then derived fat mass by subtracting lean mass from total BM. However, this study found that the whole body assay of potassium-40 was not a useful predictor of lean mass.

Measuring the absolute amounts of abdominal fat pad (AFP) in the chicken (by removing and weighing it after slaughter), is an indirect method of predicting body fat, since fat depots in the chicken are positively correlated with AFP (Nir *et al.*, 1988). Measurements made on killed individuals are then related to candidates for selection, generally siblings or progeny. However, the accuracy of this system depends on the number of relatives killed (Mallard & Douaire, 1988).

1.1.2 Genetic Selection in Laying-Type Chickens

Modern layer-stock breeders can have birds that produce either white or brown eggs on a commercial basis. The white-egg bird is generally derived from White Leghorns, whilst the brown-egg commercial stocks are produced from selected genotypes derived from the combination of several breeds such as Rhode Island Reds, Rhode Island Whites, Barred Plymouth Rocks and Light Sussex (Gowe & Fairfull, 1995). Consumer choice determines which egg type is produced. White eggs are the predominant type sold in the USA as they are considered by the consumer to be clean, whereas European consumers favour brown eggs, which they consider more natural.

Since the 1950's, most layer breeding companies have had some form of active selection program. The commercial laying hen industry selects for a number of different traits; increased egg production, reduced BM, early sexual maturity (age at first egg), improved feed efficiency, shortened ovulation cycles, behavioural traits

(reduced broodiness and aggression and adaptability to cages), increased viability (disease and stress resistance) and improved egg quality. This final criterion consists of many different traits, the main ones being; egg shell strength or thickness, shell colour, shape, texture, albumen quality and the absence of blood or meat spots (Gowe & Fairfull, 1995). Most breeding companies are still selecting for a combination of these traits to improve bird livability and optimum egg production at the minimum cost of feed.

The production of both the broiler and the layer chicken has been improved through intensive genetic selection, but for different characteristics. The most important selection criteria in the broiler are fast growth rate, lower FCR and reduced carcass fat. However, in the layer, the main criteria are to increase both egg production and egg weight. A reduction in BM is another selection trait used in layers (Gowe & Fairfull, 1995), but this selection has not been as drastic as that seen in the broiler, where the time for the bird to reach slaughter weight has been halved. Although there has been some selection for reduced BM in the layer chicken, the primary aim was to reduce BM in order to decrease food intake and thus improve FCR. Therefore, the layer makes an ideal control group in a comparative growth study with broiler genotypes, selected for fast growth rate, lower FCR and reduced carcass fat.

1.2 Metabolic Disorders - A Response to Genetic Selection

An unforeseen but important consequence of genetic selection for improved growth rate, feed efficiency and greater meat yield in broilers has been an increase in mortality, which has largely been attributable to metabolic disorders (Groves, 1997). Many of these metabolic disorders may be linked to changes in organ morphology in the modern broiler that have occurred indirectly as a result of the genetic selection process. The growth of individual organs influences the growth of the whole body in the chicken. Tissues can be generally classified as either 'supply' or 'demand'. The definitions given by Mitchell & Smith (1991) are that 'supply' organs or tissues are responsible for meeting the nutritional and oxygen demands of the growing animal.

The organs fulfilling these roles are those that form the gastrointestinal tract (GIT), the cardiovascular, the respiratory systems and other viscera e.g. the liver. In contrast 'demand' tissues are primarily users of energy and nutrients in the growing animal, particularly protein, so these would include muscle, bone, skin and feathers. It has been shown that most of the supply organs are developed by 8 to 10 days posthatch, and thus they can begin to support the growth of the demand tissues (Katanbaf *et al.*, 1988a cited in Dunnington & Siegel, 1995). Fast development of the organs of the digestive system is required to assimilate feed efficiently so that the growth of demand organs can be supported (Katanbaf *et al.*, 1989). As the growth rate of broilers continues to increase with genetic selection, this developmental sequence must continue but also remain in balance to be effective and efficient (Dunnington & Siegel, 1995). Although supply organs are worth less commercially than demand organs, efficient development of supply organs is essential if the chick is to produce the valuable demand tissues, i.e. muscle (Dunnington & Siegel, 1995).

The genetic progress within the broiler industry has been linked with the increased proportion of birds within the total flock developing metabolic disorders (Groves, 1997; Rauw *et al.*, 1998). It has been postulated that the mismatch between the metabolic demands of rapid growth and the bird's cardiopulmonary performance has resulted in the appearance of pulmonary hypertension syndrome (PHS), also known as ascites, in the broiler chicken (Julian, 1997). Another metabolic disorder that is a major cause of death within the broiler industry is sudden death syndrome (SDS) or flip-over disease.

1.2.1 Pulmonary Hypertension Syndrome (PHS) or Ascites Syndrome

Pulmonary hypertension syndrome or ascites first became apparent in areas growing chickens at high altitudes (>1500 m) such as Mexico, South America, South Africa and Kenya, and remained for many years problematic only in these areas (Groves, 1997). However, more recently (over the last 10-15 years) ascites has frequently occurred in birds reared at lower altitudes. A survey conducted for Poultry International in 1996 revealed that in the United States and Britain, the incidence of

ascites was 1.4% (Maxwell & Robertson, 1997). The incidence of ascites in winter in Italy, Malaysia, Pakistan and China ranged from 10-15% (Maxwell & Robertson, 1997). A seasonal decrease in environmental temperature has consequences for the birds thermoregulatory system (this is discussed more fully later in the literature review). Denmark reported the lowest overall incidence at 0.01% (Maxwell & Robertson, 1997). For most countries, the peak of mortalities from ascites occurs between 34 and 38 days of age (Maxwell & Robertson, 1997). Thus, in many countries, ascites is the major cause of mortality contributing to economic loss to the broiler industry. This is not only due to direct mortalities, but also to reduced body weight and increased condemnations of carcasses at slaughter (Shlosberg *et al.*, 1996; Julian & Mirsalimi, 1992). It is estimated that the production losses, attributable to ascites, costs almost one billion American dollars annually, worldwide (Maxwell & Robertson, 1997).

The causes and symptoms of ascites are well documented (Julian *et al.*, 1989; Scheele *et al.*, 1991; Julian & Mirsalimi, 1992; Odum, 1993; Walker, 1994; Leeson *et al.*, 1995; Julian, 1995; 1997). In the healthy bird, the lungs are firm and fixed in the thoracic cavity. The small blood capillaries of the lung can expand only a little to accommodate circulation. Rapid growth in broiler chickens causes an increased oxygen requirement that, in turn, causes increased blood flow. This subsequently causes an increase in blood pressure required to drive blood through the capillaries in the lung (pulmonary hypertension). In birds, the right ventricle of the heart is thin walled and when pulmonary hypertension (high blood pressure in the lung) occurs, the right ventricle enlarges rapidly in response to the increased workload. To further aid in improving oxygen delivery to the tissues, in response to the increased demand for growth, the kidney produces a hormone, erythropoietin (Julian *et al.*, 1989) that stimulates red blood cell and haemoglobin production (Odum, 1993). A disadvantage of this process is that the blood becomes more viscous and resistant to flow as a result of greater friction between the blood vessel wall and the blood cells. If hypertension continues, the right ventricle has to pump against the pressure and the heart muscle wall will thicken and enlarge.

In chickens, the atrio-ventricular valve is a muscle flap that is part of the right

ventricular wall. As the right ventricle thickens and dilates, the valve also thickens and becomes rigid until it no longer seals the opening back to the veins. Hypertrophy and dilation of the right ventricle result in valvular insufficiency and right ventricular failure (Julian, 1995; 1997). When the out-flow of blood from the liver to the inferior vena cava is restricted, the blood pressure in the liver increases. This causes an extremely high pressure in the liver sinusoids and in turn causes seepage of fluid from the surface of the liver. This fluid is almost pure plasma so it is high in protein. In turn, the protein causes a high colloid osmotic pressure in the abdominal fluid, and consequently additional fluid from the surfaces of the gut and mesentery are pulled into the peritoneal cavity through the process of osmosis (Walker, 1994). This condition can lead to high levels of mortality and morbidity.

1.2.1.1 Causative Factors in Ascites Syndrome

The factors that predispose a flock of broilers to ascites are generally associated with the circulatory and respiratory systems. These include increased oxygen requirement, decreased rate of transfer of oxygen in the lungs, increased blood volume, reduced oxygen carrying capacity of the blood, increased blood viscosity due to a higher haematocrit, lung pathology where capillaries are narrowed or occluded, increased red cell rigidity and interference with blood flow through the lungs (Odum, 1993; Julian, 1997). Outbreaks of ascites in broiler flocks may be the result of any one of these factors, with one factor leading to the next.

Altitude

The incidence of ascites is influenced by altitude and is more frequent in areas located more than 1300 m above sea level (Hernandez, 1987). At high altitudes barometric pressure is lower, so there is less oxygen available in the air. At sea level the barometric pressure averages 1013 millibars, but at 3658 m above sea level it is only 643 millibars (Curtis, 1999). All animals are affected by a lack of oxygen (hypoxia), except those fully adapted to high elevation. Hypoxia causes an increase in the number of red blood cells that, in turn, make the blood more viscous. Broilers

are very susceptible to right ventricle failure and ascites at high altitudes. This is due to the chicken's inherent ability to increase blood cell numbers very rapidly in response to lack of oxygen. The chicks cannot respond to increased oxygen demand by increasing lung volume since the heart and lungs are rigidly fixed to the thoracic cavity (Julian, 1997). The hypoxia of high altitude that leads to increased right ventricle failure and ascites is more common in broiler chickens than layers. Males are more susceptible to ascites than females, which is probably due to their relatively larger muscle mass and body mass, with a resultant higher metabolic requirement for oxygen for maintenance (Julian *et al.*, 1987).

Witzel *et al.* (1990) produced a research model for inducing ascites by growing broiler chickens at different simulated altitudes using a hypobaric chamber. The results showed that the major occurrence of ascites was between 3 and 5 weeks and at a simulated altitude of 2,896 m, when 11 out of 15 broilers developed ascites and died. The incidence of ascites was 13, 27 and 80% at simulated altitudes of 1,980, 2,438 and 2,896 m, respectively. This compared to no cases of ascites developing among control broilers that were grown at an altitude of 100 m. This clearly demonstrates that mortality due to ascites increases with increasing altitude. In a review of the causes and prevention of ascites, Julian (1997) stated that over 10% of male chickens raised above 800m might die from ascites, if growth rate is not restricted in order to reduce the requirement for oxygen. However, the incidence of ascites has been steadily increasing in broiler chickens grown at low altitudes since the 1970's, which suggests a close link consistent with continued selection for rapid growth rate (Julian, 1997).

Environmental Temperature

Extreme temperatures (hot or cold) increase oxygen requirement as the bird actively thermoregulates to keep cool or to produce heat. Consequently, both cardiac output and blood flow increase and this may result in pressure overload on the right ventricle (Julian *et al.*, 1989). Chicks have a narrow thermoneutral zone (TNZ) of between 28-32 °C (Jackson & Diamond, 1995). This is the range of temperatures where the chicken does not have to actively thermoregulate in order to maintain a

normal body temperature. Any change in temperature from the optimum TNZ increases the oxygen requirement of the bird. For example, a drop in environmental temperature from 20 °C to 2 °C, almost doubles the amount of oxygen required for heat production (HP) in adult White Leghorn hens (Gleeson, 1986). Julian *et al.* (1989) produced significant increases in the incidence of ascites in broiler chickens by lowering the ambient temperature (T_a) to 13 °C after 22 days of age. Mortality due to ascites during the total 56-day period was higher in the cold (142 chickens, 36.3%) than in the warm (48 chickens, 12.3%). Lubritz *et al.* (1995) also found a high incidence of ascites (33.5%) after subjecting a broiler line, selected for maximum white meat yield and rapid growth, to cold stress. The birds were placed at a temperature of 16.5 ± 2.75 °C from 2-8 weeks of age. Increased cold or longer exposure to constant cold would probably have resulted in higher mortality from right ventricle failure and ascites (Lubritz *et al.*, 1995).

Management of Ventilation Systems

Deficiencies in the management of ventilation systems during rearing are also thought to contribute to ascites, mainly due to poor air quality and the build-up of potentially toxic gases, which can depress oxygen levels in the poultry house. Ascitic chickens have a higher number of nodules (small round lumps or tumours) in their lungs as do chickens grown in poorly ventilated houses (Groves, 1997). However, when Shlosberg *et al.* (1992) compared a well ventilated house (20.7% O_2 concentration) against a poorly (20.4%) ventilated house, they found no differences in the incidence of ascites. Therefore, improvements in ventilation may only assist where low barometric pressure is the major inducing factor for ascites, since at high altitudes the partial pressure of oxygen (P_aO_2) is already reduced, so further respiratory stress caused by poor ventilation increases the chance of hypoxia (Groves, 1997).

Nutrition

Hypoxia can be aggravated by a diet with a high energy content, possibly due to an increase in intestinal nutrient uptake which requires more energy, which stimulates

an increase in oxygen consumption (Hernandez, 1987). A diet with a high energy content also causes the bird to increase in size or growth rate more rapidly, which demands more oxygen for energy, which in turn could cause hypoxia (Acar *et al.*, 1995; Leeson *et al.*, 1995). There is a notable increase in the number of birds affected with ascites after the second week of a standard broiler grower diet (Hernandez, 1987). This is the point in the feeding programe at which the energy content of the feed is usually increased (broiler starter ration 12.6 MJ/kg to a broiler grower ration 13.3 MJ/kg (Ross Breeders, 1996)). Van der Klis (1997) stated that selection for high feed efficiency does not automatically result in an increased susceptibility to ascites, but enhanced growth rate and increased feed efficiency is a combination that can be detrimental. It has been widely demonstrated that slowing the growth rate during certain critical stages of development, generally the two weeks posthatch, through feed and/or energy restriction has been an aid to controlling mortality due to ascites (Walker, 1994). The principle of this restriction is that the growth rates of the muscle mass and the cardiac capacity of the bird increase in proportion to each other by slowing muscle deposition in line with the growth of the cardiac system, i.e., symmorphosis is maintained (Taylor & Weibel, 1981; Weibel, *et al.*, 1991). However, feed restriction has both welfare and adverse economic consequences.

A study conducted by Acar *et al.* (1995) investigated the impact of early feed restriction on whole body mass, skeletal muscle growth and mortality due to ascites in broilers. Three feed regimens were tested: *ad libitum*, limiting daily feed intake from days 4-11 or limiting feed for days 7-14. When the birds were restricted-fed it was by 75% of their metabolizable energy normally required for optimum growth. On day 21, ascites was induced in half of the birds using exposure to cold temperature (17.8 °C), and serial dissections performed. The results showed that the *ad libitum* fed birds had significantly greater percentage of breast muscle than the feed-restricted birds. However, subsequent cold exposure caused a significantly higher percentage of these birds to develop ascites. In addition, the right ventricle weight increased relative to total heart weight, which suggested that the surviving birds were more likely to develop ascites (this is known as the ascites heart index (AHI), which is discussed in more detail in section 1.2.1.2). Thus, although feed

restriction reduced mortality due to ascites, this was at the expense of final BM and more specifically at the expense of the percentage of breast muscle. The birds that were restricted-fed between days 4 and 11 had a greater percentage mortality due to ascites (3.97%) compared to those restricted-fed between days 7 and 14 (0.83%).

Recent research has shown that a reduction in intestinal ammonia, which is the primary product of urea hydrolysis by the bacterial enzyme urease, may have a significant influence in reducing ascites (Walker, 1994). Suppression of urease enzyme activity in the intestine of the chicks decreases intestinal wall thickness, increases weight gain and improves feed efficiency. The inclusion of a urease inhibitor in the daily diet of broilers significantly reduced both mortality and condemnations due to ascites (Walker, 1994). A positive relationship between intestinal ammonia and ascites mortality was therefore observed. This is an unexpected result, since anything which is able to increase growth rate would be expected to promote ascites as opposed to reducing it. A possible answer could be found in the changes in blood chemistry, since a lower blood pH is found in ascitic birds. Changes in intestinal ammonia levels may increase blood ammonia levels, thereby increasing blood pH and the oxygen carrying capacity of the blood. Another possibility may lie in the intestinal wall thickness, which is reduced when urease enzyme is suppressed. An extensive capillary structure in the mucosal wall of the intestine is required for nutrient transport. Mucosal wall thickening may place increased pressure on this capillary structure, which in turn could restrict normal blood flow, increasing intestinal hypertension and vascular congestion. The mode of action by which the urease inhibitor reduces ascites mortality requires further investigation (Walker, 1994)

Another dietary supplement which appears to reduce the incidence of pulmonary hypertension (a physiological factor in the development of ascites), is the essential amino acid L-arginine. Supplemental L-arginine, fed at levels above those required for maximal growth, is required as a substrate for the production of nitric oxide, which is a powerful endogenous pulmonary vasodilator (Wideman *et al.*, 1995b). Fifty-five day-old birds fed diets supplemented with 1% L-arginine HCl had significantly lower right ventricle weight and total ventricle weight ratios than

control birds fed a standard broiler diet. This indicated that L-arginine HCl supplementation reduced right ventricular hypertrophy, probably by reducing pulmonary arterial pressure (Wideman *et al.*, 1995b).

Furosemide is a drug that can be used as a dietary additive that may reduce the incidence of ascites. It acts in two ways, both as a diuretic and also as a pulmonary vasodilator (Wideman *et al.*, 1995a). It is already known that excessive Na^+ supplied in the drinking water or diet increases the incidence of ascites. Julian (1987) proposed that Na^+ from all sources are additive in producing Na^+ toxicity. In particular, young chickens may be at risk because they have low plasma osmolarity and a relatively undeveloped kidney. The mechanism of Na^+ toxicity is thought to be caused by changes in pressure in plasma, which then lead to oedema and ascites. Hypertension in chickens is caused by high Na^+ and it has been suggested that hypertension may result in ascites (Julian, 1987). Therefore, diuretic therapy to reduce sodium and fluid retention may reduce ascites mortalities by reducing the secondary consequences of congestive heart failure (Wideman *et al.*, 1995a). In mammals, furosemide has been shown to act as a pulmonary vasodilator, therefore it may be used in other animals such as poultry to reduce pulmonary vascular resistance. Pulmonary hypertension in broilers exposed to cool environmental conditions (10-15 °C) was significantly reduced when furosemide was added to the grower or to both the grower and finisher rations, when compared to a negative control diet. This may be due to a combination of mechanisms including; reduced growth, reduced fluid and electrolyte retention, and reduced pulmonary vascular resistance.

There are different methods of altering the quantity or quality of the diets fed to chickens to reduce the occurrence of ascites. Restricting the feed appeared to reduce the incidence of ascites but this was at the expense of the body weight gain. Therefore this method is of little value, due to the demands of the consumer and the pressure of the commercial poultry industry to produce chickens of a certain weight by a certain age. The addition of the drug furosemide to the diet also appeared to have a positive effect on reducing ascites. This is not necessarily a beneficial method to utilise, because of the current regulations banning a range of both

antibiotic and probiotic drugs from animal feedstuffs. The addition of another drug, of any sort, to the diet of chickens is not advisable without knowing the exact mode of action of the drug and its effect on the chicken and humans. Other research has indicated that the use of dietary supplements such as the bacterial enzyme urease inhibitor and the amino acid L-arginine, are beneficial in reducing the occurrence of ascites. These methods require further investigation, but because dietary supplements are readily used within the commercial poultry industry, they appear to be the most likely methods to be utilised and research should be continued.

1.2.1.2 Predictive Factors for Ascites

In a genetic selection programme, the ability to predict those individuals most resistant or susceptible to ascites would be a useful selection tool, and this could give rise to the possibility of developing broiler genotypes resistant to ascites.

The AHI is a measure of the hypertrophy of the right ventricle, also known as the ratio of right to total ventricle weight (RV: TV) which, along with erythrocyte packed cell volume are parameters often used to monitor ascites. The AHI is calculated as:

AHI = [Mass of right ventricle/(mass of septum + both ventricles)] *100,

and is expressed as a percentage (Shlosberg *et al.*, 1992). The index assumes an isometric relationship is occurring between the right ventricle and the total heart mass, that is, if one plotted one against the other it would produce a straight line that passes through the origin. This appears to be an assumption made about many different physiological traits and in most cases it is an erroneous assumption. It is more common to find allometric relationships in which the regression line does not pass through the origin or is a curvilinear line.

In normal broilers the AHI is <25%, whereas in birds suffering from ascites it is usually 30 to 40% and may even exceed 50% (Shlosberg *et al.*, 1992; Hernandez,

1987). The changes in AHI could be used as a predictor of ascites and as a selection criterion, but because the birds used to collect the AHI data are euthanased in the process it is then difficult to relate the AHI information to live birds.

Electrocardiograms (ECG) have been routinely used in human and veterinary medicine to assess changes in cardiac size and function. They have been used as a non-invasive tool to identify birds susceptible to pulmonary hypertension (Odom *et al.*, 1991; 1992, Roush *et al.*, 1996, Wideman & Kirby, 1996). For instance, Wideman & Kirby (1996) selected 36 birds aged 14 days, on the basis of hatch weight, body weight gain and with normal ECGs. On day 16, 26 birds were anaesthetized and the left pulmonary artery was exposed. The pulmonary artery clamp model developed by Wideman & Kirby (1995a) was used on 16 of these birds. The pulmonary artery in the other 10 birds was exposed but not clamped. This model causes a progression of symptoms typical of those observed in broilers developing ascites spontaneously during a commercial situation (Wideman & Kirby, 1996). The control group were neither anesthetized nor operated upon. Daily ECGs were recorded until half of the 12 surviving clamped birds exhibited clinical ascites at day 27. During the onset of pulmonary hypertension in the clamped group the ECGs showed many changes. For the purposes of the practical application of identifying susceptible individuals in a large number of birds, multiple ECG leads and complicated calculations would be required, but this technique can be used as a non-invasive criteria for detecting cardiac changes.

Roush *et al.* (1997) proposed that physiological measurements that do not result in the death of the bird might be used as inputs in a neural network to predict the propensity of individual broilers risk of ascites. A neural network is an artificial computerised system which is a set of processing units that simulate biological neurons and are interconnected by a set of weights (analogous to synaptic connections in the nervous system) that allows both serial and parallel processing of information through the network. The neural network analysis contains physiological variables including percentage saturation of haemoglobin with oxygen, ECG, blood samples for microhaematocrit determinations and calculation of packed cell volume. Roush *et al.* (1996) used a set of birds initially for calibration of a

neural network. The predictive ability of the neural network was then verified using two other data sets. The first set of broilers was subjected to cool temperatures (18 °C) and the second set had their pulmonary arteries clamped to stimulate the development of ascites. When the neural network results were compared to the laboratory diagnostic results, the artificial neural networks were found to effectively identify broilers both with and without ascites (Roush *et al.*, 1996).

1.2.2 Sudden Death Syndrome (or Flip-Over Disease)

Sudden death syndrome occurs in apparently healthy, fast growing broiler chickens that die suddenly with a short, terminal, wing beating convulsive attack. Approximately 70% of affected birds 'flip-over' during the convulsion and are found dead on their backs, and over 60% of affected broilers are male (Julian, 1995). Sudden death syndrome has been recognized for over 35 years, although the prevalence has increased recently, and is probably also due to increased feed intake and more rapid growth in broilers. Mortality may start as early as 72 hours after hatching and may continue up to 12 weeks of age in roaster flocks. The time of peak mortality varies but is usually between 21 and 27 days of age (Gardiner *et al.*, 1988). Mortality averages between 0.5 and 2%, but can be as high as 4% in all-male flocks (Julian, 1995). It is not known whether some broilers have a genetic predisposition to the condition or that all broilers are inherently susceptible to SDS due to environmental conditions (e.g. temperature, ventilation, and nutrition).

The cause of SDS is not known. However, the higher incidence of SDS in heavier birds (Gardiner *et al.*, 1988) and its link with a high plane of nutrition (Julian, 1995) suggests that it is a disease of the modern, highly selected broiler. The cause of death is most likely to result from an imbalance of metabolites such as the reactants, intermediates and products of enzymatic reactions in metabolic pathways (Voet & Voet, 1990) or electrolytes (which dissociate into ions when dissolved in water e.g. salts, acids and bases; Eckert *et al.*, 1988). Regardless of its cause the outcome is ventricular fibrillation and heart failure (Julian, 1995). Broilers that die from SDS are generally well fleshed and usually have ingesta in the crop, gizzard and intestine,

indicating that the birds were eating. However, the liver of these birds, as a percentage of body weight, is significantly heavier than the liver of normal broilers of the same age (Julian, 1995). How this contributes to the occurrence of SDS is not clear and warrants further investigation. Julian (1995) reports that the bursa and thymus appear normal showing a lack of an immune response, and the ventricles of the heart are contracted with the atria filled with blood and dilated. The confirmation of SDS at necropsy is difficult, as there are no specific heart lesions associated with the condition, which makes investigation of this syndrome difficult.

The symptoms of the metabolic disorders discussed in detail here (ascites and SDS) indicate that it is the 'supply' rather than the 'demand' organs that are affected. Many of the 'supply' organs are affected but specifically the heart, liver and lungs appear to have a role in the development of these metabolic disorders. Therefore a study of the changes in organ allometry from the 1950's chicken to present-day broiler stocks provides a theoretical platform from which we can study metabolic disorders.

1.3 Organ Allometry in Response to Genetic Selection

Allometry is the principle of relating systematic changes in body proportions to increasing BM (Eckert *et al.*, 1988). Observing changes in both 'supply' and 'demand' organs within the animal as it ages is an investigation into organ allometry. The change of the organ mass can be calculated in absolute (expressed as the actual weight) and/or relative terms (where organ mass is expressed as a proportion of BM), the latter often being inappropriately shown as a percentage or a ratio. Unfortunately, such ratios seldom eliminate the influence of BM on a physiological response and they also introduce major problems with respect to statistical analysis (Poehlman & Toth, 1995; Packard & Boardman, 1999). Since most physiological and life-history traits scale with BM, it is appropriate for this mass dependence to be taken into account when comparing group means or when analysing correlations between traits (Christians, 1999).

Problems may arise when the physiological trait in question is the mass of an organ or tissue and the covariate is BM, because the mass of each individual organ contributes to the overall BM, so there is, therefore, a very high degree of auto-correlation between organ mass and BM. Body mass should therefore be corrected for the contribution of individual organ masses by subtracting the mass of each organ from BM in turn. The most appropriate way of comparing any differences in organ mass among groups of different BM is to use BM minus organ mass (BM-OM) as the covariate, rather than BM alone.

It is important to correctly remove the effect of body mass statistically from the data set so that the correct conclusion of the influence of physiological variable being studied can be made (Poehlman & Toth, 1995). However, few studies do this and instead simply express an organ mass as a percentage of BM. In expressing an organ as a percentage of BM one may find relationships or correlations within the data where in reality there may be none. The relative mass of an organ may increase but this may be due to either the organ mass increasing or because the BM is decreasing. The inappropriate use of ratios and percentages in the statistical analysis of many morphological studies causes a lack of consistency and problems when attempting to compare these studies, this can be seen in the following section 1.3.1.

1.3.1 Organ Morphology in Chickens (*Gallus domesticus*)

1.3.1.1 Genotype Comparisons of Organ Allometry

The organ allometry of chickens has been widely investigated and many of these studies have concentrated on the development of the supply organs, most specifically the GIT (Plavnik & Hurwitz, 1982, Smith *et al.*, 1990, Dunnington & Siegel, 1995). The GIT has been implicated as a limiting factor with regard to food intake and subsequent growth in the broiler chicken (Mahagna & Nir, 1996). Dunnington & Siegel (1995) compared chickens selected for 33 generations for high or low 8-wk

body weight. All organ weights were expressed relative to the carcass weight (BM-total GIT contents) as a percentage. The heart, lungs, liver and pancreas generally remained a constant proportion of BM in both lines throughout the 42-day period. The relative weight of the GIT (without GIT contents) was greater in the high weight line from hatch to 10 days of age, but was greater in the low weight line thereafter. Similar results were found for the GIT contents. Chicks from the high weight line were heavier, consumed more feed, utilized feed more efficiently and had a faster feed passage rate than the low weight line, even though they had a smaller relative GIT size.

Similar results were observed in a comparative study between broiler and layer chickens by Plavnik & Hurwitz (1982) who measured various organ weights as a percentage of body weight. The intestine was heavier in the broilers up to 4 weeks of age but thereafter this trend was reversed, even though both sexes of the broiler genotype grew to a heavier BM at a faster rate than the layer genotype. There was no difference in heart and liver weights between the two genotypes, which was similar to the results found by Dunnington & Siegel (1995), but the spleen, gizzard and pancreas were all heavier in the layers than the broilers.

Silversides *et al* (1997) studied the differences in organ weight and haematocrit value (packed cell volume measured in a blood sample), as an indicator of ascites, in 9 broiler genotypes. They observed differences in the relative weights of the heart, liver, lung and spleen, all of which were calculated as percentages of BM, between the 9 different genotypes. However, the haematocrit differences were more variable than those observed for the relative organ masses. Ascites was diagnosed in 34 to 53% of the birds that were necropsied, and there was no significant difference between genotypes.

However, there is some evidence that there are differences in key organ masses between different chicken genotypes (Nitsan *et al.*, 1991). The relative heart and liver weights, expressed as a ratio differed significantly between three chicken genotypes. The three genotypes were known to differ greatly in body weight comprising; a parent line (C) and two lines that had been selected for 30 generations

for either high (H) or low (L) BM at 8 weeks of age. Organ weights and digestive enzyme levels were measured in each genotype over the first 15 days post-hatch. There was a two-fold difference in BM between lines C and L that increased to approximately eight-fold by day 15. Line H remained intermediate at all times. Differences among lines were found for weight-at-hatching and for growth patterns, both absolute and relative to BM, of the heart, lungs, liver, pancreas, crop, proventriculus, gizzard, and individual segments of the small intestine (duodenum, jejunum and ileum). Although line C chicks initially had the largest crop, proventriculus, gizzard and small intestine, this ranking did not persist to 15 days. Genotype differences were also evident for the levels of digestive enzymes; trypsin, chymotrypsin and amylase, line H had a higher level of all of these enzymes in the pancreas and in the intestine for chymotrypsin, although the H line also had a larger amount of trypsin and amylase it was not throughout the 15 day trial. At hatching, both the relative and absolute masses of digestive organs were larger in C than in other genotypes, suggesting that these chicks had a greater capacity for feed intake, unless there are rate of passage differences. However, the relative weights of the GIT organs declined at about 8 days of age. These results show that gross BM did not reflect the allocation of resources to the growth and functional development of various organs with age. Since the study was only carried out over the first two weeks post-hatch, it is unclear whether these genotype differences would have continued with increasing age of the birds.

1.3.1.2 Comparison of Villi Structure in Chicken Genotypes

The chick's intestinal system is anatomically complete in the embryonic stage (Noy & Sklan, 1996). The rate of digestion and absorption of nutrients is dependent on the epithelial structure. Histological sections of the intestine and image analysis can be used to measure the intestinal structure. The morphometric indices conventionally measured are; the villus height, surface area, crypt depth and entrocyte migration (this is the time taken for the red blood cells to migrate from the crypt to the villus, which enables the determination of the time course of microvillus elongation), as these are an indication of any differences in the uptake of nutrients.

Smith *et al.* (1990) compared the villus structure and microvillus development of the small intestine in four-week-old chickens from three genotypes. These included; a commercial broiler genotype selected for fast growth, a genotype selected for fast growth only until 1972, and a genotype that had never been selected for increased growth rate. The differences in growth rates between highly selected, relaxed selected or unselected birds were found to be positively correlated with parallel changes in villus surface area determined by image analysis, i.e. the highly selected genotype that had the greatest growth rate also had the greatest surface area of villi. However, selection for growth did not change the density, dimensions or pattern of development of enterocyte microvilli. The microvilli that are present on the enterocyte surface further amplify the area of villus available for the digestion and absorption of nutrients. This study suggests that the GIT is not the limiting factor in the growth of the chicken. The broiler chicken grows faster not because it has larger organs but because the organs work at a higher rate. For example, a change in epithelial structure of the intestine which leads to increased rate of passage of food and a faster uptake of nutrients, thus allowing the bird to eat more. This is indicated by the intestinal ultra-structure increasing in parallel with the growth rate of the bird.

The morphometric indices of the intestinal structure of two genotypes of chicken, a heavy BM (H) and a light BM (L) genotype were compared (Uni *et al.,* 1995). Birds of the H genotype had faster growth rate and higher feed intake. Villi growth in the intestine of the young chick is stimulated by the presence of food. Access to feed notably increases villi height, whereas feed deprivation impedes villi growth (Uni *et al.,* 1995). The results of this study show that the duodenum had the greatest absorptive surface along the intestine, as indicated by the higher and denser villi compared with the jejunum, which in turn had higher and denser villi than the ileum. However, the increase in volume of villi with age was greater in the jejunum and ileum than in the duodenum. There were similarities in the rate of growth of the villi in the H and L genotypes of chicks, but the H genotype had larger villi and enterocytes than the L genotype. However, the overall length of the small intestine in the L genotype was 70% that of the H genotype. The differences in structure and volume of the different intestinal regions parallel the large differences in feed uptake between the two genotypes, that is, the H genotype of chicken had a greater feed

intake, which is in agreement with previously reported data (Smith *et al.*, 1990).

1.3.1.3 Intraspecific Comparison of Nutrient Transport Activities in Chickens

In a study by Jackson & Diamond (1995), the wet and dry organ masses, growth rate and food intake of the Red Jungle Fowl were measured on a weekly basis over a nine-week period. The organ masses measured were; the small intestine, liver, pancreas, kidneys, brain, heart, lungs, proventriculus, gizzard, caeca, large intestine, pectoral muscle and thigh muscle. Intestinal nutrient transporter activities were also measured using isotope markers of D-[^{3}H]glucose or L-[^{14}C]proline. Analysis of covariance (ANCOVA) was used to separate the effects of age and BM on organ mass. The liver and small intestine could not be analysed by ANCOVA as the interaction between age and BM was significant, instead the ratio of organ mass to BM as a function of age were plotted. Both of these relative masses declined with age. The BM accounted for much more of the variance of the relative masses of wet and dry organs, than did age. The visceral organ exhibiting the greatest relative growth rate (corrected for BM effects) was the caeca, which increased steeply with age. Weaker increases were seen for kidneys, pancreas and pectoral muscle and decreases with age were observed for lung, gizzard and thigh muscle. The proventriculus increased during the first week after hatch and then decreased thereafter. The nutrient uptake rates for D-glucose and L-proline in all regions of the small intestine declined with age. One exception was that L-proline increased with age in the duodenum. Digestive efficiency remained constant with age, suggesting that the age related increase in small intestinal mass (hence intestinal uptake capacities) keeps pace with increasing nutrient intake as the chick grows. Nitsan *et al.* (1991) contradicts these findings as their results showed a decline in the relative weights of the GIT organs after about day 8 post-hatch.

1.3.1.4 The Effect of Diet Comparisons in *Gallus gallus*

Havenstein *et al.* (1994a,b) compared a broiler genotype that has not been selected

for fast growth rate since 1957 (ACRBC) with a commercial broiler genotype from 1991 (AA). The rations fed were representative of the time the birds were developed, 1957 and 1991 respectively, in order to measure the relative influences of dietary changes on different traits. Birds were euthanized at 43, 57, 71 and 85 days of age. The shanks, feet, head, neck and preen gland were removed and discarded. The carcass components measured were the wings, saddle, legs, breast skin, *Pectoralis major*, *Pectoralis minor*, fat pad and rack (thoracic vertebrae and ribs overlying skin and muscle, the clavicle and the sternum). The mass of heart and lungs were measured at 71 and 85 days of age. The results showed a reduction in the growth rate of the AA genotype between days 42 to 84, when fed the 1991 diet compared to the 1957 diet. There was no significant difference in fat levels between the two genotypes when comparing them at market ages (42 days for AA genotype and 85 days for ACRBC genotype). When comparing total breast meat yield, the AA on the 1991 diet yielded approximately 3% more than the ACRBC on the 1957 diet for both sexes and across all ages. The meat yield of both years and sexes of birds on the 1991 diet averaged approximately 1.2% more than the same birds on 1957 diet, and females had slightly higher average breast yield (1%) than males. Heart and lung size as a percentage of live BM were lower in the AA than the ACRBC. Several studies therefore suggest that both heart and lung size have decreased with genetic selection for increased growth rate, though it must be noted that most of these studies used percentages of organ mass in their analysis (Nitsan *et al.*, 1991; Havenstein *et al.*, 1994 a,b).

1.3.1.5 Comparison of Different Feeding Regimes

Apart from comparing fast growing broiler genotypes with slower growing egg laying birds, other studies have assessed the effects of restrictive feeding on the size of organs in chickens. Zubair & Leeson (1994) examined the effect of early feed restriction on changes in the sizes of various digestive organs in broilers. Two groups of chickens were used; a control group with free access to food, and a restricted group, in which feed was restricted by 50% from day 6 to 12. This was followed by a realimentation period when all birds were provided with feed *ad*

libitum. Birds on restricted feeding had significantly better efficiency of utilization of metabolisable energy for weight gain compared with the control birds. The relative weights of digestive organs (especially that of the crop, proventriculus and gizzard), expressed as a percentage of BM, were significantly ($P \leq 0.01$) heavier for the restricted birds. The relative weights of liver and pancreas were significantly ($P \leq 0.05$) heavier in the restricted birds after 5 days of realimentation. Zubair & Leeson (1994) suggested that the enlargement of the liver observed during refeeding could be due to an increase in the activity of hepatic lipogenic enzymes during refeeding, following their suppression during earlier feed restriction. This enables the bird to exhibit a high rate of fat deposition when presented with an excess of diet. The enlargement of the pancreas during realimentation was probably a response to increased feed intake relative to body size, leading to a higher need for the production of digestive enzymes.

Unlike Zubair & Leeson (1994), Katanbaf *et al.* (1989) found no difference in relative liver weight between different feeding regimens, but did find a difference in relative digestive organ weights; these included the ileum, jejunum and duodenum. The weights and lengths of organs relative to BM, which were expressed as a ratio, were measured in a broiler breeder parent flock. There were four feeding regimens; *ad libitum* (AL), *ad libitum* but feed restricted daily (ALR), skip one day, and skip two days (SOD and STD, where the birds were given two or three times ALR allowance on Day 1 and not fed on the next 1 or 2 days, respectively). Birds fed *ad libitum* were heavier at all ages. Restricted feeding increased the relative weights and lengths of segments of the GIT and the pancreas, which may have been a response of the restricted fed bird to being temporarily starved. Therefore when the restricted fed bird was being fed it was attempting to utilise all the nutrients available to it from the diet, by increasing its GIT. In contrast, the relative weights of breast, leg, liver and heart were not affected by the feeding regimens. The relationships of most organs to total BM changed little after sexual maturity. However, the relative weights and lengths of GIT decreased, whereas breast and legs increased. The decrease in relative growth of the GIT components with age probably occurred because these organs underwent rapid growth during early posthatch development. Once the GIT has been established it is essential for the growth of other organs e.g.

skeletal muscle. The GIT then requires less nutrients for growth and instead supplies more nutrients for the building of the demand organs, like muscle.

Further evidence that feeding regime has an effect on the size or mass of the digestive organs was provided by Boa-Amponsem *et al.* (1991), where six groups of chickens were compared. Two meat lines known to differ in growth potential, were fed two diets, either a high (H) or low (L) nutrient diet in two feeding regimens; either daily *ad libitum* (D) or alternate day *ad libitum* (R). The faster growing line consumed more and utilized feed more efficiently than the slower growing line regardless of diet or feeding regime. The relative weights, expressed as percentages of BM, of heart, gizzard, small intestine, caeca and colon and lengths of the oesophagus, small intestine and shank were greater in the slower than the faster growing line regardles of diet or feeding regime. The opposite was found for relative weights of oesophagus, breast and leg, whereas the two genotypes had similar sized liver, lungs, proventriculus, abdominal fat pad and carcass lipid. Apart from relative leg weight and shank length, the diet had no influence on organ size. Birds fed on regimen R had larger relative weights of small intestine, GIT, gizzards, proventriculus, and longer small intestine than D by day 35, but had smaller breast, thigh, drumstick and abdominal fat pad. Line X feeding regimen interactions were noted for most traits; the faster growing line was influenced more by restricted feeding than the slower growing line.

Fontana *et al.* (1993) compared two broiler genotypes which were either given restricted access to feed at 4 days of age for 1 week or a control group which were fed *ad libitum* throughout the experiment. There were no significant differences in abdominal fat pad, liver and gizzard weights between the restricted and non-restricted birds, but there was a significantly heavier fat pad in the females compared to males. Early feed restriction had little effect on either organ weight, the level of fat deposition or carcass composition of broilers later in life. Therefore, early feed restriction does not appear to be effective in altering the size of organs or reducing abdominal and carcass fat in broilers.

Alterations to the feeding regime can induce significant changes in the internal

morphology of domestic chickens. This is the case whether the chickens are broiler breeders, layers or indeed broilers that have undergone different levels of genetic selection for performance. One thing is abundantly clear from these published studies, it is difficult to draw clear conclusions from these data. This is not only due to the fact that many of these studies differed considerably in their design but also because the method of analysing the data is fundamentally flawed. There has been a wide range of experiments studying the organ morphology of chickens. Comparison of these studies is not always appropriate as different genotypes or types of chicken and many different diets or feeding regimens are used or a combination of all of these criteria. In addition, many of these studies did not correctly account for the confounding effect of BM in their statistical analyses as discussed previously (Section 1.3). This not only makes study comparisons difficult but is a flawed way to analyse and present data of this nature. However, even accounting for these problems the data is equivocal on the organ allometry of chickens, and therefore, this is an area which warrants further investigation.

1.3.2 Organ Morphology in Other Species

Organ Morphology in Other Poultry Species

Body composition studies have been undertaken in various other birds and mammals. Toelle *et al.* (1991) and Oguz *et al.* (1996) investigated the carcass traits in both sexes of the Japanese quail. Oguz *et al.* (1996) compared one line which had been selected over 5 generations for high 4-week BM (S-line) and a second line which was the unselected base population (C-line). Toelle *et al.* (1991) used a line selected over 18 generations for high 4-week BM to study the genetic heritability estimates for different organs. The birds were euthanized at 42 days of age (Oguz *et al*, 1996) and 34 days of age (Toelle *et al.*, 1991) and organs removed and weighed. Oguz *et al.* (1996) analysed the data using analysis of variance (ANOVA) with line and sex as the main effects on the organ data, whereas Toelle *et al.* (1991) used ANCOVA with BM as the covariate. In Japanese quail females are heavier than males, but in the present study a sex difference was not found. Breast and thigh

mass, length and width were all greater in the S-line compared to the C-line, but there were no significant sex differences except for breast mass, which was greater in the males than the females (Oguz *et al.*, 1996). The mass of liver, gizzard, heart, testes, ovary and abdominal fat pad were greater in the S-line than those of the C-line birds, and again there were no significant differences in organ mass between sexes. However, there were significant differences between sexes with regard to abdominal fat mass. Thigh and breast mass were heavier in the S-line. However, thigh and breast meat yields (expressed as a proportion of the carcass mass) were similar in both lines and selection for 4-week BM did not change the yields of carcass parts. In quails selected for 18 generations of high BM there was a correlation between BM and abdominal fat pad (0.34), suggesting that selection for increased BM alone would lead to a bird with more abdominal fat (Toelle *et al.*, 1991). The positive and high genetic correlation among various muscle measurements indicates these traits can be used in the selection process.

Some studies have concentrated on the development of the supply organs, most specifically the GIT, as a possible limiting factor in the overall growth of the different poultry species. The intestinal mass of the turkey poult increased more rapidly than other body organs, reaching a peak by day 6 (Uni *et al.*, 1999). The size and morphology of the small intestine in turkey poults was determined from hatch to 12 days of age. The jejunum increased in mass more rapidly than either duodenum or ileum, and the duodenum increased little in length, whereas both jejunum and ileum increased twofold in length over the 12 days. The villus height and area increased several-fold in the jejunum and duodenum and less so in the ileum. It was suggested that villus size and area in poults were smaller and mucosal enzyme activity was lower than that found in broilers, which may help explain the initial slower growth rate in poults compared to broilers (Uni *et al.*, 1999).

Organ Morphology in Other Species Which Have Undergone Artificial Selection

Organ morphology in animals other than meat-type poultry has been widely investigated and these studies have concentrated on meat producing animals such as cattle, sheep, goats and pigs (Stamataris *et al.*, 1991; Mora *et al.*, 1996; Sainz &

Bentley, 1997; Kirkpatrick & Steen, 1999). Organ morphology in these species responds to alterations in both the quantity and the quality of the diet.

Feeding programs for beef animals often include changes in diet type and periods of feed limitation, and these in turn affect organ growth. Sainz & Bentley (1997) compared the effects of diet and intake on growth of visceral organs in beef steers that had been fed in two phases. During the growing phase, steers were fed either a high (C) or low (F) concentrate diet. Diet F was available *ad libitum* (FA), whereas, diet C was available either *ad libitum* (CA) or on a limited basis (CL). During the finishing phase, all steers received diet C either *ad lib.* or restricted to 70% of the intakes of CA steers. After slaughter the visceral organs dissected were; liver, heart, lungs, spleen, forestomachs (rumen, reticulum, omasum and abomasum), small and large intestines. The data was analysed using ANCOVA with BM as the covariate. The results showed that at the end of the growing phase CA cattle had the largest livers and CL the smallest livers and FA was intermediate. Empty intestine mass was greatest in FA and lowest in CL with CA as intermediate. At the end of the finishing phase the livers of the limited fed steers were smaller than the full-fed. The intestine mass was highest in the full fed control groups and lowest in the limited fed steers. There was no significant differences observed at the end of the growing phase in the mass of the heart, lungs and spleen but there were significant differences in the heart and spleen at the end of the finishing phase. The different visceral organs are responding to different limitations of the diet such as dietary fibre content or the amounts of absorbed nutrients from the feed.

Stamataris *et al.* (1991) studied the differences in organ mass in control and restricted fed pigs. Forty Landrace X Large White pigs were individually housed. The study consisted of two periods. Period 1 started at 6kg BM, when the pigs were allocated to three groups; an initial slaughter group (2 males and 2 females), and two feeding regimens *ad libitum* (AL) and restricted fed (R). The pigs on treatment R were offered 300 g/day of the same food as AL group throughout period 1 because this was about their intake at 6 kg BM, by holding the food allowance at this level the restriction becomes increasingly severe as the pigs increase in weight. When the pigs reached 12 kg BM, 8 pigs from AL and R groups were slaughtered. Period 2

started at 12 kg BM, when the remaining AL and R pigs were given free and continuous access to feed. When the pigs reached 24 kg BM they were slaughtered. All data analysis carried out was done on absolute organ masses. At 12 kg BM the R pigs were of quite different physical composition than the AL pigs. The R pigs had less gut fill, reflecting the lower rate of food intake prior to slaughter, lighter internal organs and skins, and heavier heads and carcasses. All of these differences were highly significant, but there were no significant differences between sexes. At 24 kg BM the R and AL pigs had very similar physical compositions. The R pigs had slightly more gut fill and lighter empty bodies. There was no significant effect of sex. The mass of the internal organs, with exception of the kidneys, skins and carcasses were similar between the two groups, the restricted fed pigs used compensatory or "catch up" growth to maximise their genetic potential. The restricted fed pigs were able to adjust their organ masses to be sufficient to process the allowance. Once they were given access to *ad libitum* feed, the mass of their organs rapidly increased to process the increased rate of intake.

The effect of length and severity of feed restriction on weight, carcass measurements and body composition of goats was investigated (Mora *et al.*, 1996). Twenty-four adult Nubian crossbred goats were used; half were feed restricted for 18 weeks (RP1) and the other half were restricted for 36 weeks (RP2). Both experiments had a stabilization period (SP) of 7 weeks, in which the goats were fed *ad libitum* which allowed them to reach and maintain a constant mass. At the end of SP, animals from each experiment were divided into six groups of four goats and assigned one of the following feed levels; FL1 (100%), FL2 and FL3 (80% and 60% of the previously observed consumption rate respectively). Animals were slaughtered at the end of the restriction period. The carcass and viscera masses were analysed as a percentage of slaughter mass. A linear regression was fitted to each FL within feed restriction period to evaluate changes in BM throughout time. Feeding level did not have significant effect on BM or carcass measurements during RP1 or RP2. In the case of the viscera, there were no differences between feeding levels in either restriction period, except the liver. Searle *et al.* (1972) stated that during prolonged periods of malnutrition, muscle, fat and bone tissues gradually return to their normal ratios, so this may have occurred in this study.

These data show that alterations to diet quality and quantity have an effect on organ morphology in those species that have undergone genetic selection for improved performance. However, this effect on organ morphology does not appear to be as evident for goats as for other species. This may be because goats have not been as intensively selected for improved growth rate as other agricultural animals such as cattle, sheep or pigs.

Organ Morphology in Other Species Which Have Undergone Natural Selection

Body composition has been extensively examined in a range of non-agricultural animals (Hammond & Diamond, 1992; Hammond, 1993; McDevitt & Speakman, 1994b; Borkowska 1995; Konarzewski & Diamond, 1995; Campbell & MacArthur, 1996). Many of these studies investigate how animals biologically respond or adapt to environmental stresses such as food shortages or extreme climatic changes or biological stresses such as reproduction (gestation and lactation). They compare the effects of natural selection on body composition as opposed to artificial genetic selection.

Gastro-intestinal tract morphology in various rodent species undergoes extensive seasonal changes (Borkowska, 1995; Hammond & Diamond, 1992). These seasonal changes are due to a combination of natural variation in temperature, photoperiod, feed availability and behaviour. Borkowska (1995) captured fifty-four striped field mice at different times of the year, spring, summer, autumn and late autumn. The effects of season and sex were determined by multivariate analysis of covariance with BM as the covariate. The results showed that there was a significant effect of season on the lengths of small intestine, caecum and large intestine in males. Gut length tended to decrease from spring to autumn. However this was not replicated in the females where gut parts did not significantly change in length. Seasonal effects on gut mass were also noted in the males, where stomach, caecum and small intestine decreased significantly in mass from spring to autumn. Large intestine mass did not vary throughout the year. There were no significant changes in wet mass of gut parts in females. Borkowska (1995) suggested that the decrease in gut

parameters might have been an effect of declining metabolic requirements and completion of reproduction in late autumn, rather than due to a stressful period because of low temperature or poor food quality. Therefore, these mice actively downsized their own metabolic machinery.

Hammond & Diamond (1992) examined the effect of reproduction on the size of different GIT components in mice. Mice were killed at the different stages of the reproduction cycle; first while the female mice were virgins, second the morning after parturition, third at peak lactation and lastly post-weaning. The results indicated that whole gut length, estimated wet mass, and estimated dry mass all varied with reproductive stage. Length increased by 5% from the virgin state to parturition, by a further 12% by peak lactation, and then remained at this length after weaning. Wet mass and dry mass of the GIT increased much more by 52% and 45%, respectively, up to parturition and by a further 43% and 46%, respectively, up to peak lactation. These changes in gut mass arose mainly from changes in size of the small intestine, which constitutes most of the gut. There was greater variation in small intestinal mass compared to length which reflects changes in intestinal thickness. These increases in intestinal mass were mainly due to increases in mucosal rather than serosal mass. As for the stomach, caecum and large intestine, only the caecum changed in length between the different reproductive stages. All three segments increased in wet mass at peak lactation over parturition or the virgin state, the increase being partly reversed after weaning for the stomach and caecum. These results show the plasticity of the components of the gut when placed under a metabolic stress, in this case reproduction.

Piersma *et al.* (1999) also indicated the adaptive value of changes in size in different organ and muscle groups. They studied the morphology of the red knot (*Calidris canutus islandica*) in relation to their migration. Their results indicated the changes that some organs go through in preparation for their migratory flight. Birds were sampled at five time points; at arrival in Iceland, two times during their subsequent refuelling, at departure and on return from the Arctic breeding grounds. Most increase in body mass shown over these time periods was due to an increase in fat storage, but lean body mass also increased. Over the first interval the masses of the

heart, liver and stomach increased, during the refuelling period the size of legs, intestine, liver and kidneys increased and stomach mass decreased. At departure the stomach decreased further, and the intestine, leg muscle and liver also decreased, but pectoral muscle and heart increased in size. These results indicate the importance of the leg muscles, intestine, liver and kidneys during the refuelling period the legs required for foraging and the intestine, liver and kidneys for nutrient extraction. Also the importance of the pectoral (flight) muscles and heart in preparation for the migratory flight. The kidneys also changed little before take-off, which suggests that they are required as much during flight as during refuelling. A change in organ size may not only change due to the increased workload, but may also be due to internal regulation through neuronal or hormonal factors (Piersma *et al.*, 1999).

All of the literature reviewed here clearly demonstrates that there is a tremendous amount of variability associated with various internal organs. That is, the mass of most organs can change in response to either endogenous or exogenous stimuli and that this change can occur over the short term as well as the long term. Factors that cause such changes can be dietary (such as increased levels of fibre or energy in the diet) or feed restriction or indeed they can be environmental, such as temperature or photoperiod. However, one of the most powerful stimuli for causing alterations in organ morphology is genetic selection - whether this is artificial as in the case of chickens, cattle and pigs or whether it is natural selection as in the case of all free-living animals.

1.4 Energy Expenditure in Animals

All animals require fuel for the maintenance of body systems including tissue growth and repair, chemical, osmotic, electrical and mechanical internal work and to perform work. In the absence of external work or the storage of chemical energy as fat, all the energy released during metabolic processes appears eventually as heat (Eckert, 1988). Therefore, by measuring the HP, either directly or indirectly (calorimetry), of an animal it is possible to determine its metabolic rate (MR).

Energy expenditure (EE) in animals has been widely investigated over the past century, generally using calorimetry, in order to investigate the energy effect of; food intake, exercise, disease and the environment on EE (Elia, 1992). The rate of EE is often measured in terms of oxygen consumption (VO_2) and is highly variable between species. It is influenced by many factors including; the cost of body maintenance, the cost of various behaviours, such as feeding, territorial defence, courting, and reproduction, and the minimal and maximal rates of metabolism (McNab, 1997).

Basal metabolic rate (BMR) is usually the largest component of total EE (~60-75% of the daily EE) (Elia, 1992). The BMR is the stable rate of energy metabolism measured in an organism in the zone of thermoneutrality when the individuals are postabsorptive (eliminating the effect of dietary-induced thermogensis (DIT)), adult (thereby eliminating the cost of growth), nonreproductive (eliminating the cost of pregnancy, lactation, egg formation, or incubation) and are not actively regulating body temperature, or being physically active (Kleiber, 1932; 1961 cited in McNab, 1997). True BMR cannot be measured in animals, only in compliant humans, since animals cannot be kept completely inactive during the monitoring process. Therefore the minimum MR recorded in animals is termed resting MR (RMR).

The measurement of metabolic rate can be used to calculate the energy requirements of an animal. Metabolic rate can also be used to investigate how temperature change, activity and other energetically expensive demands such as reproduction or lactation can affect the energy requirements of that animal. There are two main methods in which EE can be measured: direct calorimetry or indirect calorimetry. In the first method, the experimental animal is placed in a well-insulated respirometry chamber. The heat lost by the animal is determined from the rise in temperature of a known mass of water.

Indirect calorimetry measures the exchange of gases (respirometry), of an experimental animal within a sealed chamber. In respirometry, either the oxygen (O_2) consumption or the carbon dioxide (CO_2) production, or both are measured. In aerobic oxidation, the amount of heat produced is related to the quantity of oxygen

consumed. The oxygen consumption is calculated as a difference between the rate of oxygen flow into and out of the sealed chamber. Generally in this system the CO_2 is absorbed from the air stream before entering the oxygen analyser, as is water (H_2O).

Another indirect method is doubly labelled water (DLW), this is an expensive technique often used in the field to measure the free-living EE of an activity in an animal species. The stable isotopes deuterium (H^2) and oxygen (O^{18}) are injected into the experimental animal. At regular 24h intervals blood samples are removed and the isotope levels measured. As the animal uses energy it loses water and carbon dioxide through respiration. The decrease in deuterium levels gives an indication of how much oxygen is lost in the form of water. This allows the amount of O^{18} lost in carbon dioxide to be calculated. Consequently the levels of the isotopes decrease at a rate correlating to the amount of energy used (Lifson & McClintock, 1966).

Depending on the hypothesis being addressed, studies measuring metabolic rates use any one of these three different methods described above. Generally DLW is used in studies where the subject has to be in its natural environment responding to natural factors, such as food availability, and environmental conditions e.g. seasonal variation in T_a. In contrast, in most laboratory based studies indirect calorimetry is the preferred method used for determining relatively short-term, intensive bouts of MR.

1.5 Aims of the Current Project

The broiler industry loses an estimated one billion US dollars per year worldwide due to ascites. Ascites and other metabolic diseases are more prominent in today's broiler industry and are most likely to be due to the extreme selection the bird has undergone in the past 50 years. General selection traits such as increased growth rate, reduced FCR and reduced fat content have clearly affected the domestic chicken in many obvious ways such as increased body mass and increased breast meat yield, but what of the more hidden changes that have occurred? The alterations to the

supply tissues of the domestic chicken to produce this marked increase in muscle mass have had little investigation, and when they have been investigated quite often an inappropriate statistical analysis has been used. Also the balance between the growth of the supply and demand tissues, known as symmorphosis, is very important. It may be possible that a breakdown in symmorphosis may be a contributing factor to the development of metabolic disorders in the modern broiler. Thus the following research aims to evaluate these long term changes produced by genetic selection by measuring metabolic rates, both resting and peak (induced by cold exposure) and the growth and organ allometry of chickens from different genotypes which have undergone different levels of genetic selection.

The experimental work contained in this thesis has been divided into three parts. In Chapter 2, organ morphology and carcass composition were measured and related to overall energy demands, as determined MR, in three genotypes of chicken. The comparisons were made of the first fourteen days post-hatch when the difference in growth rate is most pronounced (Ricklefs, 1985). Chapter 3 addresses the effect of genetic selection on both the mass of the supply and demand organs, and carcass composition of three chicken genotypes, during the whole growth phase of each genotype. Finally, the use of near-infrared spectroscopy as a technique for predicting chicken carcass composition is reported in Chapter 4.

CHAPTER 2

2. THE RELATIONSHIP BETWEEN METABOLIC RATE AND ORGAN SIZE IN THREE GENOTYPES OF CHICKEN (*Gallus gallus domesticus*)

2.1 Introduction

2.1.1 Metabolic Rates in Chickens

Since the middle of the 20th century there has been much interest in the EE of the chicken. This is due to the great improvements in the growth rate of this animal in such a short period of time as well as the importance of the chicken as an agricultural animal and food product (Kibler & Brody, 1944; Berman & Snapir, 1964; van Kampen, 1974; Farrell & Swain, 1977; MacLeod *et al.*, 1980, 1988, 1993; Boshouwers & Nicaise, 1981; Johnson & Farrell, 1983; 1985; Meltzer, 1983; MacLeod, 1990; Jones, 1994; MacLeod & Waibel, 1997; Koh & MacLeod, 1999; Ohtani & Leeson, 2000).

The chicken, as a species, makes an interesting model to study for many reasons. Firstly, no other agricultural species, apart from the pig, has undergone the same level of intensive genetic selection. Secondly, no other wild species has shown such large, rapid changes in growth rate, which could only have occurred artificially. Thirdly, different genotypes of the same species exist, ranging from the chicken ancestor the Red Jungle Fowl to genotypes selected for different criteria, both muscle yield and reproductive output. Fourthly, the genetic selection is still progressing and resulting in significant phenotypic changes even today. Therefore comparisons between the broiler and its predecessor the Red Jungle Fowl (Jackson & Diamond, 1995; 1996) and its closest genetic relative, the layer chicken (Kuenzel & Kuenzel, 1977; Stewart & Muir, 1982; Pesti *et al.*, 1990; Visser & van Kampen, 1991) are of

great interest in characterising the effects of genetic selection on the chicken.

The ability of the chick, like all homeotherms, to adjust both HP and heat loss over a range of ambient temperatures, determines its capacity to achieve homeothermy (the ability to maintain a stable core body temperature despite changes in the ambient temperature) (Dietz, 1995). The amount of metabolically active tissues and the ability of these tissues to generate heat are the factors determining the capability of the chick to produce heat. The heat loss of the chick is determined by its insulative properties (plumage, surface-to-volume ratio and evaporative water loss) (Dietz, 1995). The important factors which determine the capacity of the chick to thermoregulate are; BM (which is correlated with the amount of metabolically active tissues, the surface-to-volume ratio and evaporative water loss); level of functional maturity (which is correlated with the heat generating ability of metabolically active tissues); and the plumage, thickness and permeability of the skin (Dietz, 1995).

The effects of genetic selection on MR under a variety of conditions has been investigated in chickens (Table 2.1). Kuenzel & Kuenzel (1977) found that fast-growing genotypes of domestic fowl have lower RMR than slow-growing ones; the RMR of broilers ranged from 23.5 J/g/hr (1.1 ml.O_2/g/hr) during the first week of life to 15.7 J/g/hr (0.8 ml.O_2/g/hr) at 8 weeks of age compared to 25.4 J/g/hr (1.2 ml.O_2/g/hr) to 21.8 J/g/hr (1.1 ml.O_2/g/hr) in a layer genotype of the same age. Visser & van Kampen (1991) also showed that by four weeks of age a faster growing broiler genotype had lower RMR than its slower growing counterpart. The MR ranged from 23.2 to 25.8 J/g/hr (3.0 to 7.2 Watts/$kg^{0.75}$) for the broiler genotype compared to a layer genotype 21.6 to 30.3 J/g/hr (2.7 to 6.4 Watts/$kg^{0.75}$) from 6 to 28 days of age. Since the birds were only taken to 4 weeks of age in the Visser & van Kampen (1991) study it is hard to compare the values from these two studies. Other comparisons, Jackson & Diamond (1996), have concluded that there was no significant difference in RMR between a broiler and its ancestor the Red Jungle Fowl once BM had been taken into account. Many published studies (Table 2.1) have shown the great variation within MR both between genotypes and between ages of the same genotype.

Table 2.1 Summary from the literature of resting metabolic rates determined in chickens.

[1]MR (J/g/hr)	Age (days)	Genotype	Condition	Source
22.4	21	Broiler	Birds kept on a continuous lighting regimen	Ohtani & Leeson (2000)
17.3	42	Broiler	Birds kept on a continuous lighting regimen	Ohtani & Leeson (2000)
15.1	56	Broiler	Birds kept on a continuous lighting regimen	Ohtani & Leeson (2000)
41.5	27	Broiler	Genotype selected for fast growth kept at 21°C	Jones *et al.* (1994)
25.8	28	Broiler	Measured at 25°C	Visser & van Kampen (1991)
30.3	28	Layer	Measured at 25°C	Visser & van Kampen (1991)
8.4	~252	Layer	Measured at peak production	Pesti *et al.* (1990)
10.7	~252	Layer	Measured at peak production	Pesti *et al.* (1990)
23.2	245	Layer	Birds kept on a continuous lighting regimen	MacLeod & Jewitt (1984)
12.4-16.4	296	Broiler breeder	Measurements recorded in three different chambers	Johnson & Farrell (1983)
53.8	12	Broiler	Measured at 30°C	MacLeod *et al.* (1980)
40.9	28	Broiler	Measured at 25°C	MacLeod *et al.* (1980)
20.6	50	Broiler	Measured at 20°C	MacLeod *et al.* (1980)
14.6	280	Broiler breeder	Measured at 20°C	MacLeod *et al.* (1980)
15.7	56	Broiler	Measured at 24°C	Kuenzel & Kuenzel (1977)
21.8	56	Layer	Measured at 24°C	Kuenzel & Kuenzel (1977)
18.5	46	Broiler	Birds were starved and measured at 22°C	Farrell & Swain (1977)
17.3	1272	Layer	Measured at 25°C	Van Kampen (1974)

Where; [1]MR - Metabolic rate measured in Joules per gram of body mass per hour.

However, many of these studies used different genotypes, at different ages and they also studied them in different years, therefore different amounts of genetic selection would have occurred. It is therefore hard to fully compare these studies.

The great advancements in genetic selection have led to an increased growth rate in the broiler chicken compared to its predecessor. As these advancements continue so do the possible changes in the broiler's EE. Due to the lack of up-to-date, comparable information on MR of the domestic fowl it is important to establish what effects have occurred on the animal due to its increased growth rate and the link between MR and organ morphology.

2.1.1.1 Environmental Effects on Metabolic Rates

Ohtani & Leeson (2000) studied the effect of intermittent lighting on HP of the broiler. Most broilers are reared in either continuous or nearly continuous lighting schedules in order to maximise food intake and growth rate. The experimental birds were kept on an intermittent lighting (IL) schedule and were compared to those kept in a continuous lighting (CL) schedule. The HP measurements were carried out when the chickens were 3, 6 and 8 weeks of age using an indirect open circuit calorimeter. The birds reared in the IL treatment had significantly larger mass specific HP at 3 and 8 weeks compared to the CL treatment, whose HP remained essentially unchanged throughout the measurement period. The IL chickens also had significantly higher HP during the light periods compared to the dark periods. The IL chickens had a tendency for a lower body weight gain during the early stages of this light treatment. However, during the subsequent weeks the body weight gain and food intake were significantly higher in IL chickens compared to CL chickens. Ohtani & Leeson (2000) concluded that the IL chickens might be more active in the light phase, causing overall an increase in HP.

Alternative lighting schedules have also been studied in laying hens (MacLeod *et al.*, 1988). A standard lighting regime of 14 hours light: 10 hours dark (14L:10D) was compared to two alternative lighting regimes, a fragmented (15 hours of

(13minL:47minD):9D) and an interrupted (2L:10D:2L:10D) lighting pattern. To determine whether there was a reduction in EE and activity when hours of illumination were reduced. They observed that MR per unit time was higher during the relatively short light periods of the alternative light regimes than during the unbroken control light periods. During the dark phase of the study day, MR was respectively 22% and 35% higher than the night MR in the two alternative regimes. They concluded that the MR was redistributed in hens placed on an alternative lighting schedule, in order to minimise the change in total EE. The MR in the light phase was greater in the alternative regimes, MR in the dark phase during the study day was higher than in night-time darkness and MR at night was about 6% higher in birds on alternative regimes. These changes in MR combined to counteract the effects of the reduced duration of lighting.

Feed restriction has also been shown to affect HP in broilers (Zubair & Leeson, 1994). Feed restricted birds had a lower BMR, measured by indirect calorimetry, when compared to *ad libitum* birds. Restriction of feed intake by 50% resulted in about 40% lowered daily HP. After 3 days of realimentation the MR of the feed restricted birds had risen to the same level as their full-fed counterparts. It was concluded that some of the nutrients that support growth compensation could possibly come from improved metabolic efficiency associated with maintaining a smaller BM. Feed restriction was also found to effect HP in laying hens (Koh & MacLeod, 1999). The HP of laying hens decreased significantly with decreasing food intake.

2.1.2 Metabolic Ceilings

A metabolic ceiling is the point reached when an animal cannot produce a further increase in MR or other performance indicator such as reproductive output in response to an increased energy demand. Such increased energy demands or energetic loads may be imposed due to an increase in levels of exercise, an increase in reproductive output (gestation and/or lactation) or indeed, in response to seasonal temperature acclimatisation. The inability to increase performance beyond a certain

point is described as having attained or reached a metabolic ceiling. These metabolic ceilings vary with respect to whether the increased energy demand applies to a relatively short period of time or is of a longer duration (Suarez, 1996). Much higher MR can be achieved over short bouts maximal or peak MR (PMR) (e.g. the energy required for a 200 m dash), because they are fuelled by consumption of carbohydrate stores such as glycogen, whereas, longer term exercise e.g. migration or overwintering costs are met by utilising fat stores (Hammond & Diamond, 1997). Long-term physical activity can produce sustained metabolic rates (SusMR), which can be defined as the highest time-averaged MR sustainable for prolonged periods (days or weeks) during which energy balance is maintained via increased food intake (Peterson *et al.*, 1990). The amount of energy that an animal holds in reserve, or that it is capable of producing in response to a given requirement is its metabolic scope (MS), and is calculated as a multiple of BMR or RMR. Metabolic scope values vary between 1.5 and 5 times RMR (Peterson *et al.*, 1990), with some extreme values approaching 7 (Hammond & Diamond, 1992). However MS varies enormously depending on species and the nature of the energy demand placed upon it (Weiner, 1992; Suarez, 1996). For example, Hammond & Diamond (1992) measured MR in virgin and lactating laboratory mice where the MS reached 7.2 times RMR. Larger values have been recorded for PMR compared to SusMR, probably due to PMR being a brief burst of activity. Hayes (1989) measured the thermoregulatory costs of the Deer Mouse. The mice were exposed to a cold thermal environment, a PMR of 15.4 times RMR was calculated in the month of December and 7.9-9.6 during the other time periods. Dawson & Dawson (1982) measured the metabolic response to cold in two small dasyurid marsupials and the PMR they calculated was 8-9 times RMR.

There are four hypotheses (reviewed by Weiner, 1992; Konarzewski & Diamond, 1994; Hammond & Diamond, 1997) which have been proposed to explain the physiological limitations that produce these metabolic ceilings.

First, metabolic ceilings might be imposed simply by food availability or abundance and not by any inherent property of an animal's body, for example its cardio-pulmonary or digestive system or skeletal muscle. That is, a simple lack of food may

limit an animals ability to perform in response to an additional energetic load. This hypothesis may hold true in the field, but most studies observing the effects of metabolic ceilings have taken place in a laboratory and generally the animal has had free and constant access to food and nutrients. Hammond & Diamond (1992) indicated that this hypothesis is unlikely to limit MR or performance of the animal. When lactating mice were cross-fostered with more than 14 pups to wean (16-26 pups), within four days the litter size would be reduced to 14 pups, by the death of excess pups. This result occurred in the presence of excess food, therefore food availability did not appear to be the limiting factor in this case.

Second, there might be a peripheral limitation in operation associated with the energy-consuming machinery of an animal's body that limits performance. For example, the energy budget might be limited during physical exercise by the work properties of skeletal muscle, or during lactation by the milk-producing capacity of the mammary glands or in the ability of an animal to produce heat during cold exposure. This hypothesis assumes that the energy-supplying organs can supply the energy but the peripheral organs cannot convert the energy to work in the form of exercise or HP (Else & Hulbert, 1985; McDevitt & Speakman, 1994a, b).

Third, there might be a central limitation operating on the ability of an animal to perform, which is associated with the energy-supplying machinery in the animal's body. The limitation could, for example, occur in the gut's capacity to digest and absorb food, or the liver's capacity to process the absorbed food, or the heart's capacity to pump oxygen around the body (Konarzewski *et al.*, 1989). This hypothesis assumes that muscles could work faster if only the rest of the body could supply nutrients and oxygen, and remove wastes faster (Konarzewski *et al.*, 1989; Weiner, 1992; Hammond & Diamond, 1992; McDevitt & Speakman, 1994a, b; Koteja, 1996b, c).

The fourth hypothesis assumes that all structures of an animals body are precisely tuned to each other, so that the functional capacity of a structure does not exceed that of any other. This balancing process is termed symmorphosis (Taylor & Weibel, 1981; Weibel, *et al.*, 1991). For example, the maximum rate at which the gut can

absorb nutrients, the lungs absorb enough oxygen to allow the nutrients to burn aerobically, and the muscles ability to convert the nutrients to energy in the form of work, may have evolved to operate at the same level (Taylor & Weibel, 1981; Weibel, *et al.*, 1991; Dudley & Gans, 1991). Therefore, the limit in this hypothesis is not restricted to one area of an animal's body, but suggests that all of the animal's physiological systems are limiting in some way.

Supporting evidence for these physiological limitations and the processes by which they are produced has been widely investigated in a range of avian and mammalian species (Westerterp *et al.*, 1986; Koteja, 1986; Konarzewski & Diamond, 1994; Reynolds *et al.*, 1999). These investigations usually take the form of studying an animal's metabolic response to an additional physiological stress above that normally encountered. The stress usually comes in one of three forms; exercise (Westerterp *et al.*, 1986; Hayes *et al.*, 1992), temperature challenge (too hot or too cold) (Konarzewski & Diamond, 1994; McDevitt & Speakman, 1994a, b; Koteja, 1995, 1996c), reproduction (Genoud & Vogel, 1990; Kenagy *et al.*, 1990; Hayes *et al.*, 1992; Hammond & Diamond, 1992; Poppitt *et al.*, 1994; Speakman & McQueenie, 1996; Koteja, 1996b), or indeed a combination of any of these (Hammond *et al.*, 1994; Hinchcliff *et al.*, 1997; Reynolds *et al.*, 1999).

However, there is another biological stress which has not been widely investigated before and this is the effect of genetic selection on an animal. For example the genetic selection for fast growth rate in broiler chickens may have placed a biological load on that species. This makes the broiler chicken an ideal model to test the limitation theories.

2.1.2.1 Exercise as a Physiological Stress

Westerterp *et al.* (1986) investigated the effect of extreme sustainable exercise in humans, by measuring the EE in one of the world's most demanding cycle races, the Tour de France. The EE's of four cyclists were measured using the DLW technique and these were compared with simultaneous measurements of energy intake and BM.

Assuming maximum performance was reached in the cycle race, the energetic ceiling or MS of the performance in these highly trained athletes was 4-5 times BMR. This effort was comparable with that of some other animals, such as small mammals and birds (Peterson *et al.*, 1990; Bryant & Tatner, 1991). However, the results indicated that there was a discrepancy between the DLW and energy intake as measured using food record technique. The measurements of energy intake in this study are based on the somewhat flawed assumption that the subjects can and will accurately recall what they have eaten. Also there are limitations due to the variation in composition among different examples of the same item of food. The value from food intake was therefore likely to be an underestimate. The values given by the DLW technique may also be subject to error due to the fact that high levels of EE at high temperatures caused increased levels of evaporative water loss. Reynolds *et al.* (1999) also investigated EE in humans climbing Mount Everest as an example of extreme physical exercise combined with cold exposure. As in the Tour De France cyclists, there was chronic under-reporting of dietary energy intake, especially by those subjects who reached the highest altitudes. This under-reporting may be due in part to diminished cognition or to a preferential focus on survival (Reynolds *et al.*, 1999). The EE averaged 2.5-3.0 times sea level resting EE. The resting EE was measured rather than true BMR because the subjects were not in a post-absorptive state during measurements.

Maximal metabolic rates induced by exercise have been measured in small mammals such as mice (Hayes *et al.*, 1992). The maximal oxygen consumption (VO_2max) was measured using treadmill exercise. The results showed that BMR, measured in an open circuit respirometer, averaged 38.5 ml O_2 h^{-1} compared to a mean VO_2max of 248 ml O_2 h^{-1}, producing a PMR of 6 times BMR. The effect of both exercise and cold on MR has been investigated in other, larger animals; for example Hinchcliff *et al.* (1997) observed the EE of Alaskan sled dogs during a 490 km race using DLW. The dogs completed the 70 hour race at an average speed of 7 km/h at ambient temperatures of -35 to -10 °C. Total EE was 47.1 MJ/d in the racing dogs compared to 10.5 MJ/d in a sedentary group of dogs, producing a MS of 4.5.

The results of these and other investigations indicate that there are definite metabolic

limits in operation when exercise is used as the additional energetic load, both in humans and other animals, but these studies give no indication as to where the site of the physiological limit occurs in the animal, i.e., in what organ or system.

2.1.2.2 Temperature as a Physiological Stress

One of the easiest models used to assess metabolic ceilings in animals is that of a sustained temperature challenge. There has been much research on the effect of temperature change on MR and metabolic ceilings in rodent species (Konarzewski & Diamond, 1994; McDevitt & Speakman, 1994a, b; Koteja, 1995, 1996c) and bird species (Marsh & Wickler, 1982; Koteja, 1986; Bech, 1991).

Food intake in laboratory mice increased when they were exposed to cold temperatures, without corresponding changes in the digestive efficiency (Konarzewski & Diamond, 1994). It was concluded that the MS for the cold exposed mice (4.7 x RMR) was much lower than the value measured in lactating mice in the same laboratory (7.2 x RMR) (Hammond & Diamond, 1992). Food intake was 100% higher and the small intestinal mass 21% greater in lactating mice compared to cold-exposed mice. Cold-exposed mice were unable to survive between -10 and -15 °C, even in the presence of unlimited food, indicating that these mice had reached their metabolic ceiling. They concluded that the gut did not limit SusMR. A possible interpretation is that the ceiling was imposed by the capacity of the peripheral, heat-producing tissues expending energy, such as brown adipose tissue (BAT) or skeletal muscle. The alternative explanation as to why the lactating mice appeared to have a higher MS than the cold-exposed mice was that a portion of the extra nutrients ingested during lactation were exported unmetabolised into milk. These extra nutrients therefore constituted no work load on the kidneys, respiratory or transport systems, whereas all nutrients were metabolised by the cold-exposed animals to produce heat which did constitute such a workload. McDevitt & Speakman (1994b) also concluded that gut size was not a limit to SusMR in their study of short-tailed field voles that had been exposed to other prolonged or short-term low temperatures.

There was a strong correlation between the maximum rate of energy assimilation and the mass of the small intestine in laboratory mice exposed to temperatures as low as -18 °C (Koteja, 1996c). Initially Koteja (1996c) concluded that the results obtained from this investigation indicated that the energy budgets of mice are limited centrally by the capacity of the gut, particularly by the process of food digestion and/or absorption in the small intestine (Weiner, 1992). However, there was also a high correlation between the maximum cold induced rate of energy assimilation and the mass of the liver and kidney. Therefore, energy budgets could be limited centrally not only by the gut but also by the maximum rate of one of the many processes in the liver or by the capacity of the kidneys to remove waste produced during metabolism. Therefore, it is likely that even if the capacity of the small intestine is a bottleneck for energy flow through the animal, the capacities of other organs involved in food digestion and whole-organism metabolism seem to be similar, which is evidence that symmorphosis is taking place (Koteja, 1996c).

The effect of temperature on MR has also been investigated in some avian species (Koteja, 1986). When house sparrows were exposed to low temperatures of -2 to 12°C for approximately 2 hours the maximum cold induced oxygen consumption was approximately 5.2 times RMR. Koteja (1986) hypothesised that the energy budgets in cold-exposed birds may be centrally limited by the pulmonary diffusing capacity (the lungs ability to transfer oxygen from air to blood). If this hypothesis was correct then the oxygen consumption of the bird should have increased when exposed to an atmosphere with increased concentrations of oxygen. The values of oxygen consumption in birds from normal (21%) and increased oxygen concentrations (33%) were very similar, and consequently the hypothesis did not hold.

2.1.2.3 Reproduction as a Physiological Stress

Reproduction is an energetically expensive process, be it during gestation or lactation (in mammalian species) or brooding eggs or feeding young (in avian

species). The total metabolic costs of gestation comprise of three main components: the products of conception, the foetus, placenta, enlarged uterus, and mammary tissue, the costs of maintaining these new tissues, and the energy deposited in maternal fat stores. The major energetic costs of lactation, seen in shrews for example, are the further deposition and maintenance of mammary tissue plus the cost of milk synthesis (Poppitt *et al.*, 1994). Many studies have investigated the energy costs of reproduction, (Randolph *et al.*, 1977; Millar, 1978; 1979; Mattingly & McClure, 1982; Glazier, 1985; Genoud & Vogel, 1990; Kenagy *et al.*, 1990; Hammond & Diamond, 1992; Poppitt *et al.*, 1994; Speakman & McQueenie, 1996; Koteja, 1996b; Derting & Austin, 1998). Most of these studies have involved small mammals such as mice, shrews and voles, due to their short gestation and lactation periods. Also, the level of the physiological stress on the animal can be altered simply by manipulating the number of young in the litter during lactation.

Some animals do not seem as responsive to the demand of reproduction as others. Derting & Austin (1998) examined the effect of lactation on gut size and MR in the pine vole (*Microtus pinetorum*). They showed that the pine vole only had moderately higher MR, but not significantly, during lactation when compared to control females. The length, but not mass, of the total gut and wet masses of the stomach and caecum were significantly greater in the lactating females. There was also no significant increase in the mass of the mucosa in any portion of the small intestine. Therefore the ability of the pine voles to digest and assimilate large amounts of food nutrients is likely to be low. The low rates of energy required for lactation may be associated with the low responsiveness of the gut to increases in energy demands and food ingestion.

In contrast, Hammond & Diamond (1992) compared the MR and gross morphology of mice as virgins, during parturition, peak lactation and post weaning states. They manipulated the litter size by varying the number of pups weaned by the mother to either 4, 8, or 14 pups in a litter. They observed an increase in the length and mass of the small intestine and the mass of the stomach, caecum and large intestine until peak lactation and then a decrease at post weaning. The food intake of the mother showed a similar pattern reaching a maximum at peak lactation. There was much

evidence to suggest that the mice approached a ceiling in their food intake and SusMR. As the litter size increased, the food intake per pup at peak lactation decreased. The mass of an individual pup at peak lactation declined with increased litter size, whereas pup mass at birth did not vary over the range of natural litter size. When the litter size was manipulated beyond 14 pups to as many as 26 pups, within 4 days litter size had declined to 14 pups by the death of excess pups. Hammond & Diamond (1992) concluded that these deaths were due to nutrition per pup becoming increasingly inadequate with increasing litter size. There was no significant difference in small intestinal mass among mothers with different litter sizes, although there was a 2.5-fold intestinal hypertrophy during lactation, which may represent a ceiling on the mouse intestinal growth. The increase in intestinal nutrient uptake capacity resulting from the hypertrophy failed to keep pace with the increase in food intake at peak lactation. This suggests that a further increase in food intake at this point would have saturated the intestine's ability to absorb the additional nutrients. The SusMR observed at peak lactation was 7.2 times RMR, indicating an upper limit had been reached. All of these considerations suggest that mice with 14 pups at peak lactation are near the upper limit of their ability to ingest food, absorb nutrients and metabolise these nutrients, to produce milk, to expand intestinal mass and uptake capacity, or a combination of any of these limits. This is in agreement with Speakman & McQueenie (1996), who stated that maximal SusMR reflected a link between the structure of the alimentary tract, maximal daily food assimilation and RMR. The results showed that RMR of virgin mice was significantly lower than that of either gestating or lactating mice. As the mice progressed through reproduction, their increasing food requirements were beyond the capacity of their GIT to supply the nutrients to the animal. This led to hypertrophy of the alimentary tract and other associated organs, with a consequent increase in RMR.

The evidence to date, using exercise, thermal or reproductive load, suggests that the sustainable metabolic scope (SusMS) is not usually greater than 7 and PMR is not greater than 15. The evidence varies but the most likely hypothesis indicates that there is a central limit to metabolic ceilings, although some evidence indicates that the MS may vary depending on the nature of the energetic load used, the age of the subject and indeed in which species it is investigated.

2.1.2.4 Artificial Selection as a Physiological Stress

There is one type of energetic load that has not been fully evaluated with regard to its effect on metabolic ceilings, and that is increased growth rate. The chicken is an ideal model in which to test metabolic ceilings because of the range of genotypes found within this species.

Artificial genetic selection is another form of metabolic load that can be placed on an animal. The improvement of growth rate is just one of the many selection criteria which are used. Rodents, pigs and chickens make good models for this area of research due to their short reproductive cycle and ease of breeding.

The growth of an animal is easy to manipulate by selective breeding due the large additive genetic variation within the trait. This manipulation has been realised by long-term selection in laboratory mice. Some of the growth parameters that have been selected for are: body mass, total protein amount in the carcass and endurance fitness (Klein *et al.*, 1999). Klein *et al.* (1999) investigated the influence these selection criteria would have on the energy balance and HP of the different genotypes of laboratory mice. The four genotypes of mice investigated in this study were: DU-6P, selected for total protein amount in the carcass, DU-6, selected for BM, DU-6+LB, selected for endurance fitness measured by treadmill activity, and DU-K, an unselected, randomly bred control. The RMR measurements were carried out in both a fasted (12 hours of feed withdrawl) and postprandial state (1 hour of feed withdrawl). The measurements were taken at 32-36 and 46-49 days of age in both fasted and postprandial, and 48-52 in only the fasted state. The DU-6+LB group had a lower feed intake prior to the RMR measurements that could explain the lower RMR of those mice at 32-36 days of age. Otherwise, there was no difference in RMR between the three selected genotypes of mice in either the fasted or postprandial state in all the different age groups. The RMR in the control line was higher by 10-15% compared to the selected groups. Klein *et al.* (1999) stated that the lack of a difference in RMR between the selected genotypes could have been due to the measuring time not being long enough.

Noblet *et al.* (1998) examined the effect of breed on HP in four groups of pigs. The breeds examined were Meishan castrates (MC), Large White castrates (LWC), Large White males (LWM) and Piétrain males (PM) between 20 and 60 kg live weight. Due to the genetic selection undergone by these pigs the MC breed could be considered as fat-type pigs and the other three groups as lean-type pigs. Heat production during fasting (FHP) and during activity (AHP) was measured in the different pig genotypes. The FHP and AHP were lowest in the MC breed whereas the LWC had the greatest FHP and the PM the greatest AHP. When the total HP was calculated, the MC breed had the lowest and the LWC breed had the highest. These differences between the fat MC and the lean LWC, LWM and PM are accompanied by a higher proportion of total HP associated with FHP in the lean animals. These results indicate that there is a large variance in EE between different breeds of pig which have been selected for either fat or lean growth, indicating that artificial selection can be used as an alternative metabolic load to test the limitation theories.

The comparison of metabolism of different genotypes has also been carried out in chickens. Jackson & Diamond (1996) compared the MR and daily energy assimilation (DEA) of the broiler with its ancestor the Red Jungle Fowl. Due to the great differences in body mass between these two genotypes at comparable ages, all comparisons were made on log-transformed data and the effects of body mass were removed. The DEA which was calculated from the total food intake, the measured food energy content and the measured digestive efficiency of the animal and the RMR of each individual bird were measured. The DEA was significantly greater in the broilers than in equal-sized jungle fowl, but the RMR of the two genotypes were statistically indistinguishable. The increased DEA indicates that the broilers have a bigger energy budget largely because of the higher costs of growth and possibly higher activity costs, such as digestion.

When the fasting metabolic rate (FMR) of two layer breeds of chicken were measured, significant differences in their MR were reported (Damme *et al.* 1986). The breeds used in this study were Rhode Island Red (RIR), Light Sussex (SS) and their reciprocal crosses. The breeds were classed as medium-heavy and were a

similar age when the measurements were taken. The difference in FMR between the pure breeds was 0.8 kJ/hr during the lighted periods and 1.5 kJ/hr during the dark periods. They concluded that the difference in FMR between the pure breeds shown, could be due to a lower maintenance cost per unit of body weight in the SS breed. Despite the criteria used for genetic selection, improved growth rate or reduced fat content, the results of these studies indicate that differences in MR are inherent to the breed of animal being studied.

The results of these studies indicate that physiological ceilings do exist but there is still some doubt as to how these ceilings occur in the animal, either centrally, peripherally or by another physiological limit.

In the present study metabolic and morphological comparisons were carried out during the first two weeks of life, when the difference in post-hatch growth rate is most pronounced (Ricklefs, 1985). In this comparison, a higher MR, both resting and peak, in the broiler chicken was expected, due to their comparatively faster growth rate. The PMR was measured to give an indication of the MS that the different genotypes had, i.e. reaching their maximal MR after an energetic load was placed on them. The relative contribution of the different organs to both BM and metabolism was also of interest, to help explain why these ceilings exist and where they occur physiologically.

2.2 Materials and Methods

Three genotypes of chicken were compared. These comprised of a fast growing commercial broiler genotype (Ross 308, FB, n=45); a broiler that had been selected for fast growth rate until 1972 at which point the selection was discontinued (Ross 1972, SB, n=20). Both broiler genotypes were of mixed sex and were obtained on the day of hatching from Ross Breeders Limited. The broilers were compared with a layer genotype which had not been selected for fast growth rate, but which had been selected for lighter BM and improved reproductive performance criteria (Euribrid

HISEX, L, n=51). Only males were used and these were obtained from a commercial supplier (Farm Fresh Hatchery, Preston). The number of birds removed per treatment was determined by the number of birds that could be processed each day throughout the experimental period. All day-old chicks were wing-banded and each genotype raised in separate brooders within the same pen. They were reared on wood shaving litter at a stocking density not exceeding 34 kg/m^2 (FAWC, 1992) with a 23 h light and 1 h dark lighting programme. All chicks were fed a commercial broiler starter diet containing 22% crude protein and with a ME 12.75 of MJ/kg. Water and feed were available *ad libitum*.

Chicks were serially removed at regular age intervals from 0-14 days for determination of MR and morphology. The day before the metabolic measurements were due to be taken, the selected chicks were individually weighed (± 0.1 g), to estimate the incremental increases in BM. Apart from the metabolic costs of growth, one of the main difficulties of measuring MR in hatchlings is that they are not in a post-absorptive state, due to the catabolism of yolk reserves (Klaassen *et al.*, 1987). Moreover, the necessary time required to achieve a post-absorptive state is likely to cause metabolic depression. It is impossible to measure the rates of metabolism in chickens under conditions that would meet most of the criteria for BMR. It was therefore decided not to fast the chickens during determinations of MR and refer to the measurements as RMR.

To determine RMR, pairs of chickens from the same genotype were transferred from a holding pen and placed individually in two simultaneously monitored metabolic chambers. These chambers were in an open-circuit respirometry system (Figure 2.1), that was used to measure VO_2. The chicks had no access to feed or water during the measurement. Air temperature was held constant (± 0.2 °C) by placing the chicks in a metabolic chamber kept in an ambient temperature (T_a) control cabinet (INL-40IN-010, Gallenkamp). This was adjusted to the chickens thermoneutral zone (28 – 32 °C, Jackson & Diamond, 1995). Before reaching the chambers the dried air passed first through mass flow controllers (Tylan General, Torrance, CA and ERG-100, Warsaw, Poland) and then through a copper coil to equalise temperatures. Air leaving the chambers passed first through a silica gel column to remove water. The

air was sub-sampled at the rate of 100 ml/min controlled with a wet flowmeter (Alexander Wright, UK) redried, scrubbed of CO_2 using calcium carbonate and monitored every 1 sec by a Servomex 1100 oxygen analyser interfaced to a microcomputer (Figure 2.1). A sub-sample of air was passed through the oxygen analyser and the excess air passed through the outlet tube, this was controlled by a valve on the outlet tube. Determinations of RMR began with a 30 min equilibration period and a 2 hour measurement period. Depending on the size and the age of the chicken, metabolic chambers of different volumes (1.1 or 2.3 litres) were used and the flow rates varied between 400 and 900 ml/min. The RMR was taken to be the lowest 4 min value that did not change in O_2 concentration.

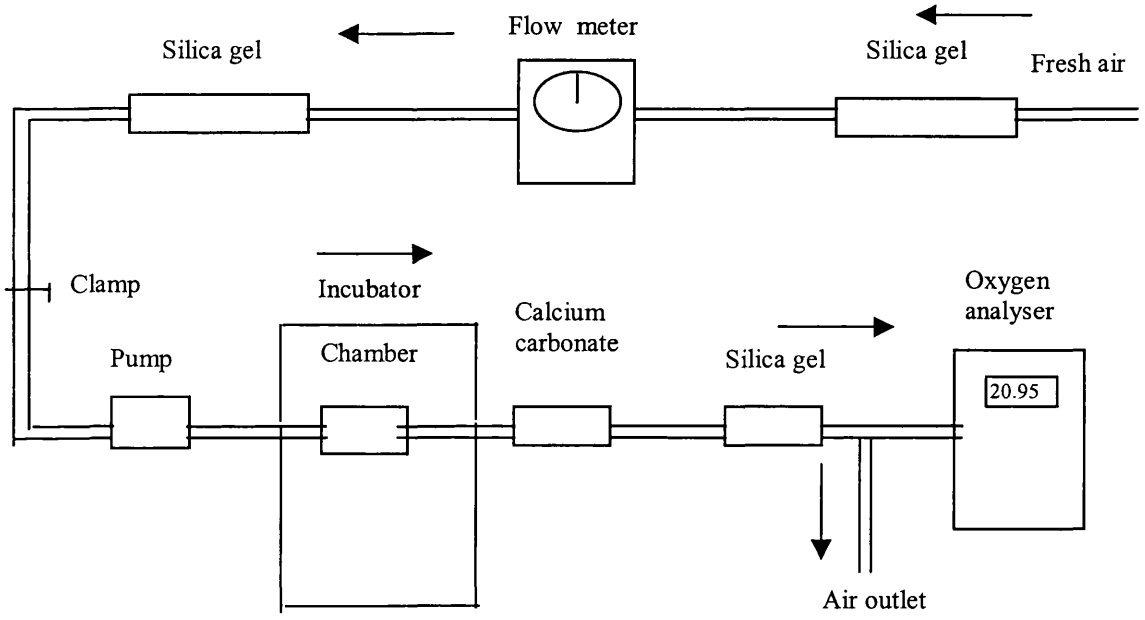


Figure 2.1 Diagram of open-circuit respirometry system.

After RMR measurements, PMR was measured only in chicks between 1-6 days of age and in those that had a measurement of RMR already determined. Due to limitations of the apparatus, specifically the flow control, there was an inability to measure peak metabolism beyond these ages. Depending on BM, the flow rate for the PMR measurement was set between 500 and 900 ml/min. The T_a in the chamber was lowered at a rate of 0.5 °C/min to between 0 and -7.0 °C. It was assumed that

PMR was reached when VO_2 began to decline with decreasing T_a. The measured PMR was defined as the highest VO_2 averaged over 2 min. After determination of MR, the core body temperature was measured (± 0.1 °C) rectally with a 2 cm long thermocouple probe attached to a Squirrel Data Logger. In all cases the bird's body temperature had dropped by at least 3 °C. The birds were killed by cervical dislocation and weighed. The wet masses of the intestine, caeca, gizzard, heart, liver, brain, pectoral muscle (*Pectoralis major, Pectoralis minor* and *supracoracoideus*) and all the leg muscle surrounding the femur and tibiotarsus were measured. Any organs remaining were combined with the skeleton and feathers, and referred to as the carcass. All tissues were dried to a constant mass in an oven at 70 °C. The dry organ masses were added to the dry carcass value to obtain the total dry mass of the whole bird. Water content of muscle was calculated as [wet mass - dry mass].

All MR data was analysed using the Sable Systems DATACAN V software. The computer software calculated VO_2 rates using the following equation from Hill (1972).

$$V_{O_2} = (1 - FI_{CO_2})\, VI \frac{F^1I_{O_2} - F^1E_{O_2}}{1 - F^1E_{O_2}}$$

Where V_{O_2} is the oxygen consumption of the animal in volume of dry oxygen at standard temperature and pressure (STP) per unit time, $F^1E_{O_2}$ is the volume fractional concentration of oxygen in dry, CO_2-free outlet gas, VI is the volume of dry ambient air flowing into the animal chamber per unit time, corrected to STP, $F^1I_{O_2}$ is the volume fractional concentration of oxygen in dry, CO_2-free inlet air and FI_{CO_2} is the volume fractional concentration of CO_2 in dry inlet air.

After finishing the morphometric measurements all the body parts of individual birds were re-combined and milled. The whole carcasses were then analysed in duplicate for ash using a muffle furnace, fat was determined using the Soxtec solvent

extraction method, nitrogen was determined using a colorimetric method and crude protein (CP) was calculated. For full descriptions of these methods of analyses refer to Appendix 1.

2.3 Data Analysis and Statistics

When all of the data were analysed together, significant interactions between all possible combinations of genotype, BM and age were evident in both the metabolic and morphological measurements. An attempt was made to analyse the data using a curvilinear model, but due the number of significant terms and interactions it was extremely difficult to interpret the results. These interactions also persisted despite attempts to minimise them with various transformations. To remove the effect that the interactions might have on the interpretation of the results the birds were divided into two groups; those weighing less than 80 g (this included 22 and 33 chicks from the FB and L genotypes, respectively) and those weighing more than 80 g (23, 20 and 17 chicks of the FB, SB and L genotypes, respectively), and analysed each group separately. Due to the limitations of equipment and time we were only able to measure VO_2 in birds weighing more than 80 g within the SB genotype. Therefore no PMR was measured within the SB genotype.

This division was arrived at by applying the computer programme described by Konarzewski *et al.* (1989). The programme is based on the least-squares method and fits the best one- or two-segmented linear models to a data set. The division of the data also had a biological basis, the relationship between BM and organ mass was generally biphasic with the inflection point at a BM of about 80 g (Figure 2.2 illustrates the biphasic relationship using the wet intestine and liver masses). This division of the data enabled complete statistical analysis of PMR because this parameter was only measured in birds weighing less than 80 g. Furthermore, it has been shown that most of the supply organs are developed by 8 to 10 days posthatch, and thus they can begin to support the growth of the demand tissues (Katanbaf *et al.*, 1988a cited in Dunnington & Siegel, 1995). This initial fast development of the

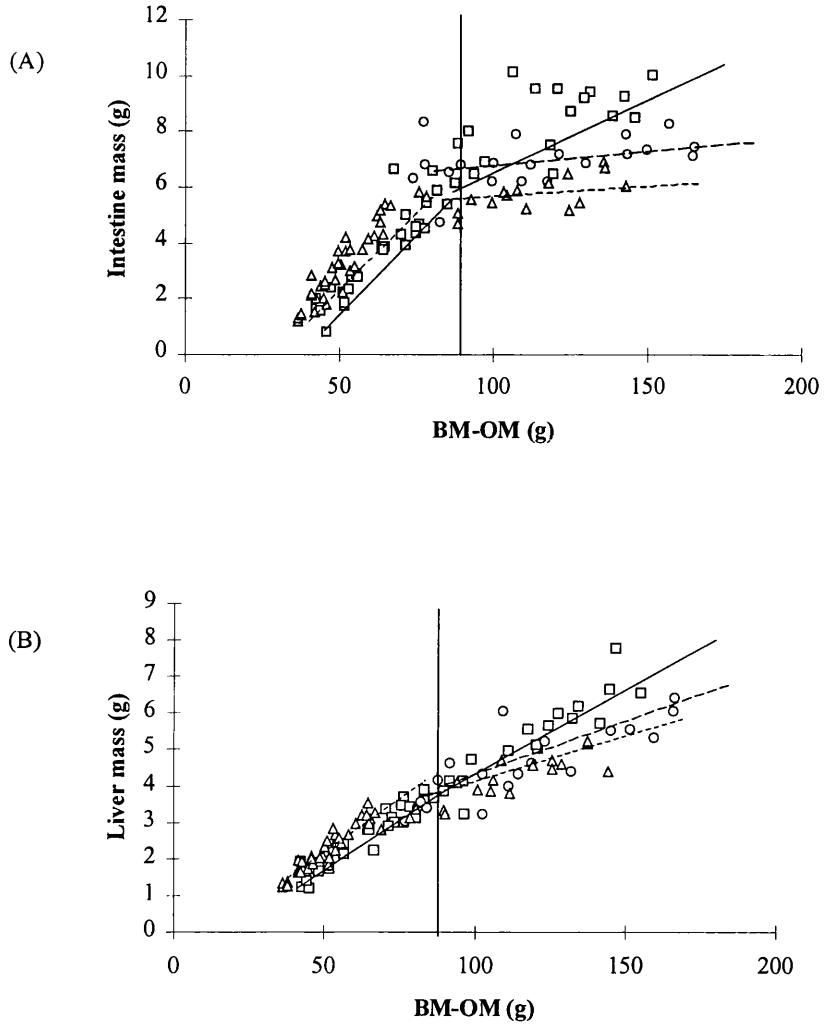


Figure 2.2 The relationship between (A) wet intestine and (B) wet liver mass and corrected body mass in three genotypes of chicken; fast broiler (□, whole line), slow broiler (O, wide dashed line) and layer (Δ, narrow dashed line). The data shown indicates the general biphasic relationship between organ mass and body mass, with an inflection point at ~80 g, which is shown on the graphs as a vertical line at 80 g corrected body mass.

organs of the digestive system may be the first part of the biphasic relationship that can be seen in the current data. The second part of the biphasic relationship could be the initial growth of the demand organs.

To avoid the possibility of spurious autocorrelation, the mass of the organ under consideration was subtracted from BM (BM-OM). Due to the possibility of fat or water content confounding the results, the organ mass was subtracted from lean body mass (LBM-OM) for wet organ mass data and the dry mass of the organ was subtracted from the total dry weight of the bird (DM-OM) for dry organ mass data before each computation (Christians, 1999).

Differences between genotypes were tested statistically by analysis of covariance (ANCOVA) with BM and age as covariates. Each analysis began by looking for gender effects within the FB and SB genotypes. No sex-related differences were detected and therefore gender was omitted as a main effect in the between-genotype comparisons. Two people carried out the dissections; this was accounted for in the statistical model by including a factor labelled "dissector". The analyses started with a full model, which incorporated all possible main effects, covariates and interaction terms using Genstat 5 (Genstat 5, release 4.1 for windows (Lawes Agricultural Trust, Rothamsted Experimental Station));

$$Y = g_i + d_j + gd_{ij} + a_iBM + b_jBM + ab_{ij}BM + c_iAge + f_jAge + cf_{ij}Age + e$$

Where Y = the response variable, g_i = the intercept for genotype i, d_j = the intercept for dissector j, gd_{ij} = the intercept for genotype i by dissector j interaction, a_i = slope for genotype i with BM, b_j = slope for dissector j with BM, ab_{ij} = slope for genotype i by dissector j interaction with BM, c_i = slope for genotype i with age, f_j = slope for dissector j with age, cf_{ij} = slope for genotype i by dissector j interaction with age, e = residual error. For full Genstat programme see Appendix 2.

Dissector and its interactions were removed from the model when metabolic rates, metabolic scope, growth increment and proximate analysis data were analysed. Once it was known what terms were significant, a predict line was then entered into

the model at the point where it was only taking the significant effects into account. Therefore, the adjusted means produced only included the significant terms. Producing adjusted means was only possible when no interaction term was shown to be significant, therefore no interaction was occurring between the genotypes and BM or age. The growth and MR data are presented as adjusted means from ANCOVA analysis ± S.E.M. (standard error of the mean). Unless stated otherwise, means that differed at the 5% level were considered statistically significant.

Where significant interactions were revealed in the ANCOVA model, which occurred mainly with the organ masses, another method of analysis was used, a predictive model. The organ masses were analysed using a stepwise regression model. The significant terms generated from the stepwise regression were then entered into a prediction model in Genstat.

To analyse the relationship between RMR and PMR linear regressions were first conducted of RMR and PMR on BM and age using Minitab (Minitab 10.5 Xtra, Minitab Inc., Birmingham, UK). After determining which of these variables were statistically significant as independent variables ($P < 0.05$), the residuals were used for further analysis. The residuals are measures of RMR and PMR after controlling for the effects of BM and age. The residuals from the regression of RMR against the residuals from regression of PMR were corrected and plotted. Also similar analyses were conducted to look for the relationship between metabolic rates and organ masses. Statistical analyses were performed using linear regression and Pearson correlations in Minitab.

2.4 Results

2.4.1 Absolute Data

The absolute values of the individual organ masses at different BM's give an indication of what effect genotype has had on these organs (Table 2.2).

Table 2.2 The mean absolute wet mass (g) of each organ, for each of the three genotypes of chicken at three body mass intervals.

Organ	Genotype	Body mass (g)		
		40-50	80-90	130-140
Pectoral	[1]FB	0.75	3.06	9.92
	[2]SB	-	3.82	9.65
	[3]L	0.63	5.17	8.98
Legs	FB	4.42	8.25	13.14
	SB	-	7.16	13.34
	L	3.66	7.16	12.43
Intestine	FB	1.69	5.42	9.17
	SB	-	6.55	6.85
	L	2.21	5.74	5.72
Caeca	FB	0.27	0.68	1.12
	SB	-	0.66	1.07
	L	0.29	0.61	0.75
Gizzard	FB	2.53	3.91	6.12
	SB	-	4.71	6.23
	L	3.45	5.08	5.77
Liver	FB	1.51	3.54	5.82
	SB	-	3.34	4.38
	L	1.83	3.24	4.56
Heart	FB	0.302	0.65	0.99
	SB	-	0.61	1.07
	L	0.34	0.61	1.03
Brain	FB	0.96	1.22	1.38
	SB	-	1.23	1.42
	L	1.03	1.24	1.58

Where [1]fast broiler, [2] slow broiler and [3] layer

However, these values cannot be compared statistically, since the BM and age of the birds would not have been taken into account. However, many of the effects of

genotype that are seen in the absolute data did not change when the organs were analysed relative to BM and age.

2.4.2 Body Mass and Growth

During this study the daily BM's of all the birds were not measured, but the BM of the subject birds were measured the day before their metabolic measurements were taken. The increase in BM from this day to the day the measurements were taken, for each bird, has been called the daily BM increment. At a BM of less than 80 g, genotype had a significant effect on the daily BM increment ($F_{1,38} = 16.78$, $P \leq 0.001$, Figure 2.3; age was a significant covariate) with FB birds growing faster (mean corrected for age = 8.71 ± 1.02 g/day) than L birds (mean = 3.78 ± 0.62 g/day).

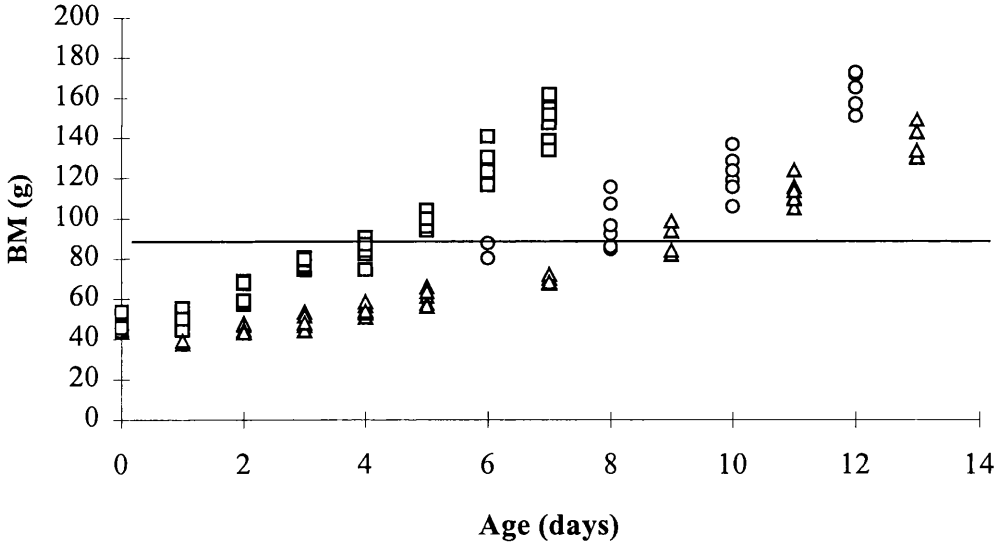


Figure 2.3 The relationship between body mass and age in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ). A solid, horizontal line divides the data into two subsets of birds, those weighing less, and more than 80 g.

Likewise, among birds weighing more than 80 g, a significant difference between genotype was shown ($F_{2,42} = 35.42$, $P \leq 0.001$, there was no significant covariate). There was also a significant interaction between age X genotype, this interaction

indicated that each genotype had a similar initial BM but that it subsequently increased at different rates for each genotype. The mean BM increment was 34.24 ± 2.15 g/day, 10.10 ± 0.93 g/day and 8.75 ± 1.50 g/day for FB, SB and L chickens, respectively.

2.4.3 Metabolic Rates

There was no between-genotype difference in RMR for chicks below 80 g when BM was used as a covariate ($F_{1,48}$ = 0.12, P > 0.727, BM and age were significant covariates). Also there was no between-genotype differences in RMR among heavier birds, BM>80 g ($F_{2,50}$ = 1.02, P > 0.368, BM and age were significant covariates). Hence RMR was not different between the three genotypes.

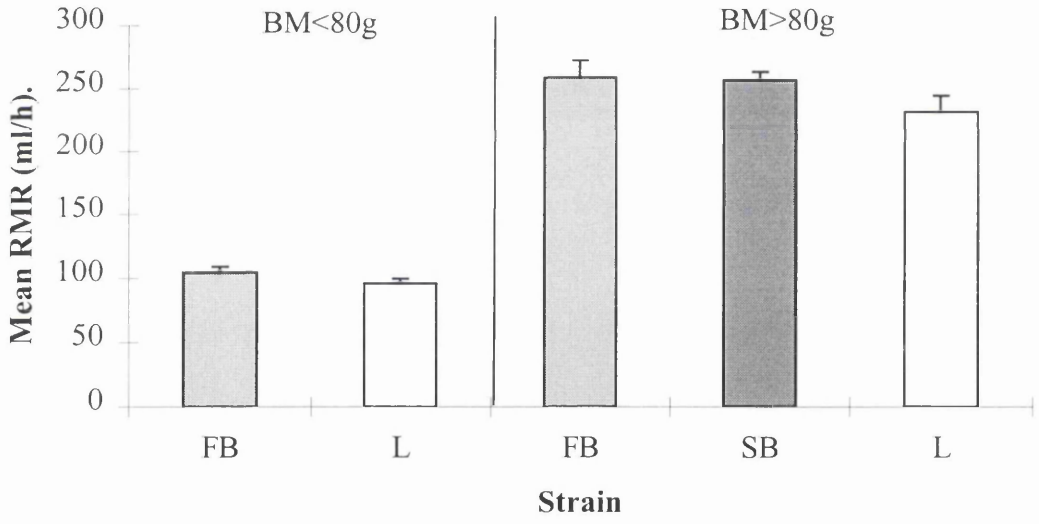


Figure 2.4 Mean (± SEM) resting metabolic rate (RMR) adjusted for lean body mass (LBM) and age for; fast broiler (grey), slow broiler (black) and layer (white) genotypes of chicken, below (<80 g) and above (>80 g) 80 g body mass.

Body mass was corrected for fat content, to generate lean body mass (LBM), which was then used as a covariate in the model, replacing BM (Figure 2.4). There was still no between-genotype difference in RMR evident for chickens below 80 g ($F_{1,48}$ =1.21, P > 0.276; LBM and age were significant covariates), whereas, for the chickens above 80 g the genotype difference became significant ($F_{2,50}$ = 4.59, P ≤

0.05; LBM and age were significant covariates). Mean RMR was 258.72 ± 13.73 ml O_2/h, 256.77 ± 6.76 ml O_2/h and 231.05 ± 13.08 ml O_2/h for FB, SB and L, respectively.

In contrast to RMR, there was a significant genotype difference in PMR (Figure 2.5), when both BM ($F_{1,47}$ = 14.40, P ≤ 0.001; BM and age were significant covariates) and LBM ($F_{1,47}$ = 27.95, P ≤ 0.001; LBM and age were significant covariates) were used as covariates in the analysis. The mean PMR was 289.75 ± 11.07 ml O_2/h and 204.69 ± 5.57 ml O_2/h for FB and L chickens, respectively. The mean PMR only differed slightly when LBM replaced BM as the covariate; 292.48 ± 10.18 ml O_2/h and 201.20 ± 4.95 ml O_2/h for the FB and L chickens, respectively.

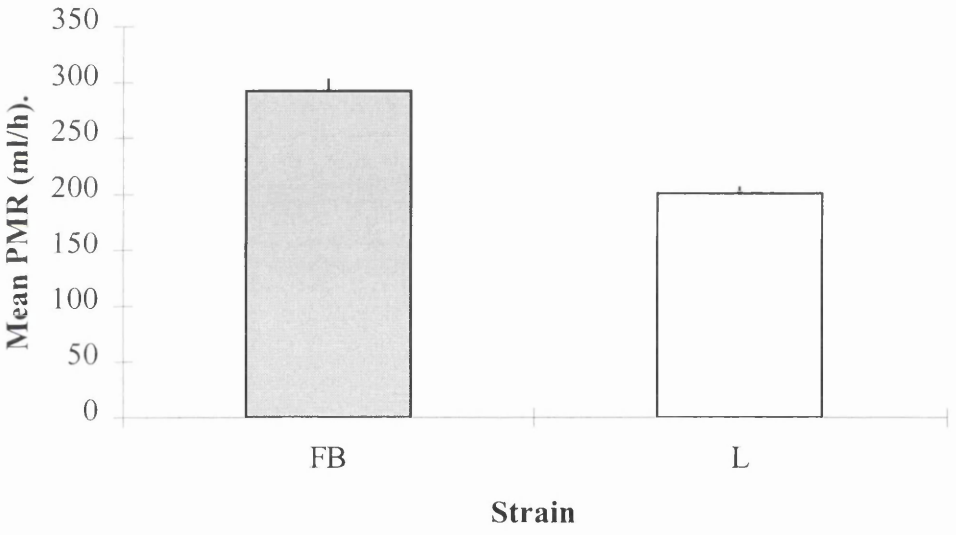


Figure 2.5 The mean (± SEM) peak metabolic rate (PMR) adjusted for lean body mass and age for fast broiler (FB) and layer (L) genotypes of chicken below 80 g.

2.4.4 Relationship Between Resting and Peak Metabolic Rates

The effects of BM and age (Figure 2.6) confounded the relationship between RMR and PMR. To determine what the relationship was, residuals from a linear regression equation controlling for the effects of age and BM were computed, in the pooled data set of chickens. Pooling the data enabled all of the between-genotype variation in

RMR and PMR resulting from artificial selection to be used. A significant, positive correlation between RMR and PMR corrected for BM and age was evident (R^2 = 0.332, $P \leq 0.05$; Figure 2.7A). The same relationship was then searched for within each genotype. A significant, positive correlation between RMR and PMR in the L chicks was shown (R^2 = 0.483, $P \leq 0.05$; Figure 2.7C), but not in the FB genotype alone (Figure 2.7B). The analysis was then repeated replacing BM with LBM. The same significant correlations between RMR and PMR for the pooled data set ($P \leq 0.001$) and L chickens ($P \leq 0.01$) were evident and no correlation in the FB chickens was evident.

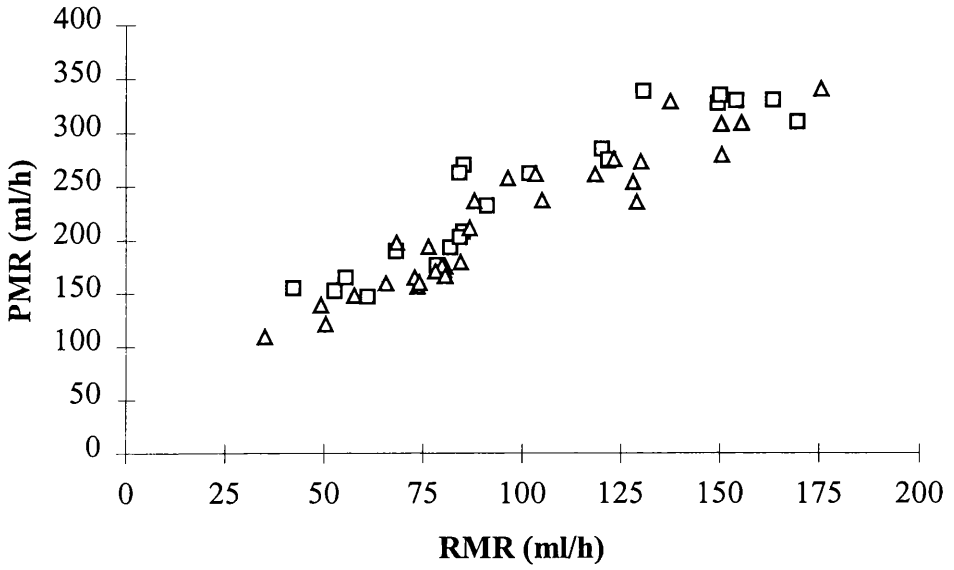
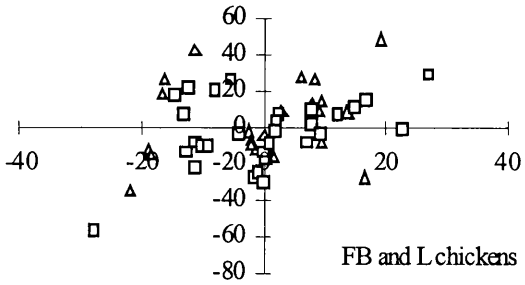
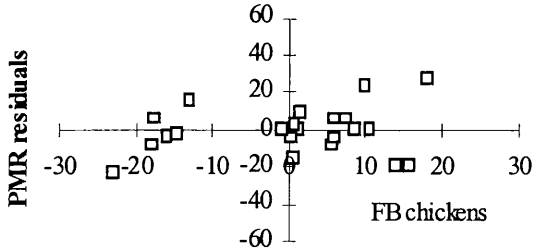


Figure 2.6 The relationship between resting (RMR) and peak metabolic rate (PMR) in fast broilers (□) and layer (Δ) genotypes. The data are absolute RMR and PMR for each individual chick.

(A)



(B)



(C)

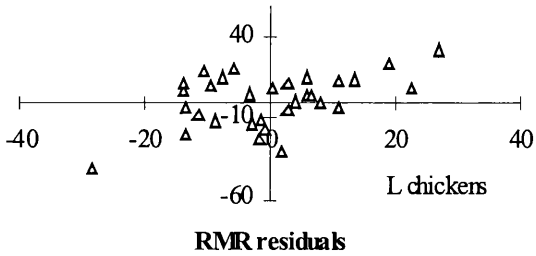


Figure 2.7 The residuals of peak metabolic rate (PMR) on body mass and age plotted against the residuals of resting metabolic rate (RMR) on body mass and age for; (A) fast broiler (FB,□) and layer (L, Δ) genotypes pooled, (B) fast broiler only and (C) layer only.

2.4.5 Metabolic Scope

There was a significant effect of genotype on metabolic scope (ratio of PMR to RMR), the FB had a greater MS compared to the L genotype ($F_{1,46} = 6.72$, $P \leq 0.05$; age was a significant covariate). The analysis was repeated replacing BM with LBM as a covariate as was done for the metabolic rate measurements, the same genotype result occurred ($F_{1,46} = 5.47$, $P \leq 0.05$; age was a significant covariate). The mean RMR's, not adjusted for BM or age, were 26.64 ± 1.15 and 28.75 ± 1.02 J/g/hr and the PMR's were 65.31 ± 1.65 and 64.89 ± 1.51 J/g/hr for FB and L chickens, respectively. The unadjusted mean metabolic scope was 2.5 and 2.3 times RMR for FB and L chickens, respectively.

Definitions of Interaction Terms

Within the following organ morphology and carcass composition results there were many significant interactions. Explanations for each of these interaction terms are described below. To simplify the explanations, the term BM was used, but BM-OM, LBM-OM or DM-OM depending on the covariate being used in the model for that particular organ could replace this.

The BM X age interaction indicates that the organ mass at the initial ages of the birds was similar but as age and BM increased differences in the organ mass began to develop. The BM X genotype interaction indicates that each genotype had a similar initial organ mass but as BM increased the organ mass subsequently increased at a different rate for each genotype. The age X genotype interaction indicates that each genotype had a similar initial organ mass but as age increased the organ mass subsequently increased at a different rate for each genotype. The age X dissector interaction indicates that the initial organ mass for each dissector was similar but as age increased a difference in organ mass developed between the different dissectors. The BM X dissector interaction indicates that the initial organ mass for each dissector was similar but as BM increased a difference in organ mass dissected by different operators became apparent. The genotype X dissector interaction indicates

that there was a significant dissector effect within each genotype for organ mass. As stated previously there were two people carrying out the dissections within this trial. There was often consistent error between these two dissectors in the removal of certain organs.

2.4.6 Muscle Growth

At BM of less than 80 g, there was a significant difference in relative pectoral muscle mass between genotypes ($F_{1,45}$ = 10.04, P ≤ 0.01; BM-OM and age were significant covariates, Figure 2.8A) with FB birds having heavier muscles than L birds. There was also a significant BM-OM X age interaction.

The pectoral muscles of the FB chickens had significantly more water than those of the L genotype ($F_{1,49}$ = 8.59, P ≤ 0.001; BM-OM and age were significant covariates, Figure 2.8B), and there were significant interactions between BM-OM X age and BM-OM X genotype. Dry pectoral muscle mass in the FB chickens was significantly higher than that of the L genotype ($F_{1,45}$ = 5.56, P ≤ 0.05; DM-OM and age were significant covariates, Figure 2.8C).

In contrast, at BM above 80 g there was no significant difference in pectoral masses between genotypes ($F_{2,45}$ = 3.16, P > 0.052; BM-OM and age were significant covariates, Figure 2.8A), but there was a significant age X genotype interaction (P ≤ 0.01). There was a significant dissector ($F_{1,45}$ = 12.04, P ≤ 0.001) and age X dissector effect observed. The SB genotype had larger pectoral muscle mass than the L genotype.

There was no significant effect of genotype on water content of pectoral muscle ($F_{2,50}$ = 2.69, P > 0.078; BM-OM and age were significant covariates, Figure 2.8B) but there was a significant age X genotype interaction. The FB chickens had significantly more water in their pectoral muscles compared to the SB or L chickens. There was no difference in dry pectoral mass between genotypes ($F_{2,45}$ = 0.96, P > 0.392; BM-OM and age were significant covariates, Figure 2.8C), but there was a

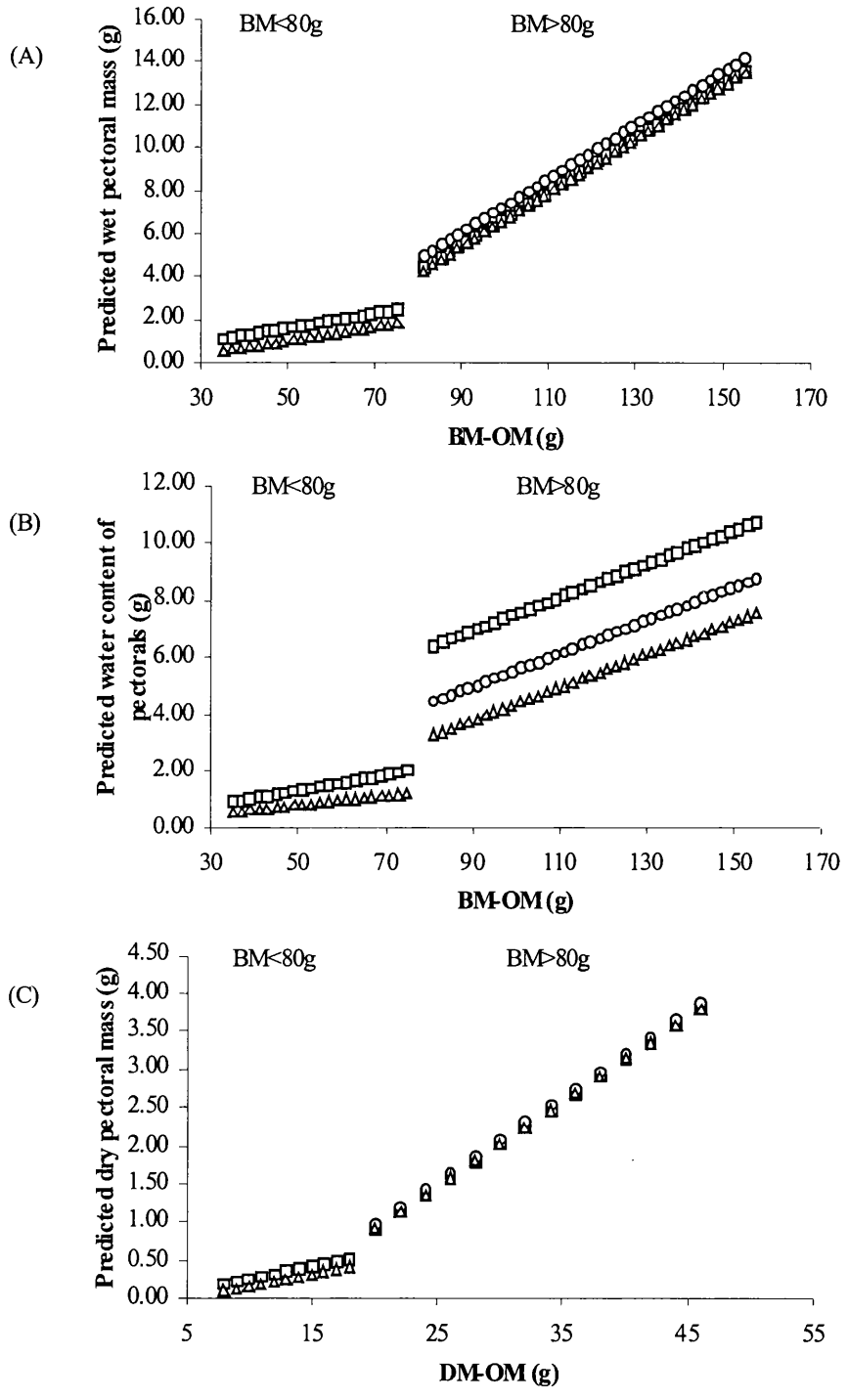


Figure 2.8 The relationship between corrected body mass and pectoral muscle mass in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ), where (A) is the wet pectoral muscle mass, (B) the water content of this muscle and (C) the dry pectoral muscle mass. The predicted values are shown for each genotype below and above 80 g.

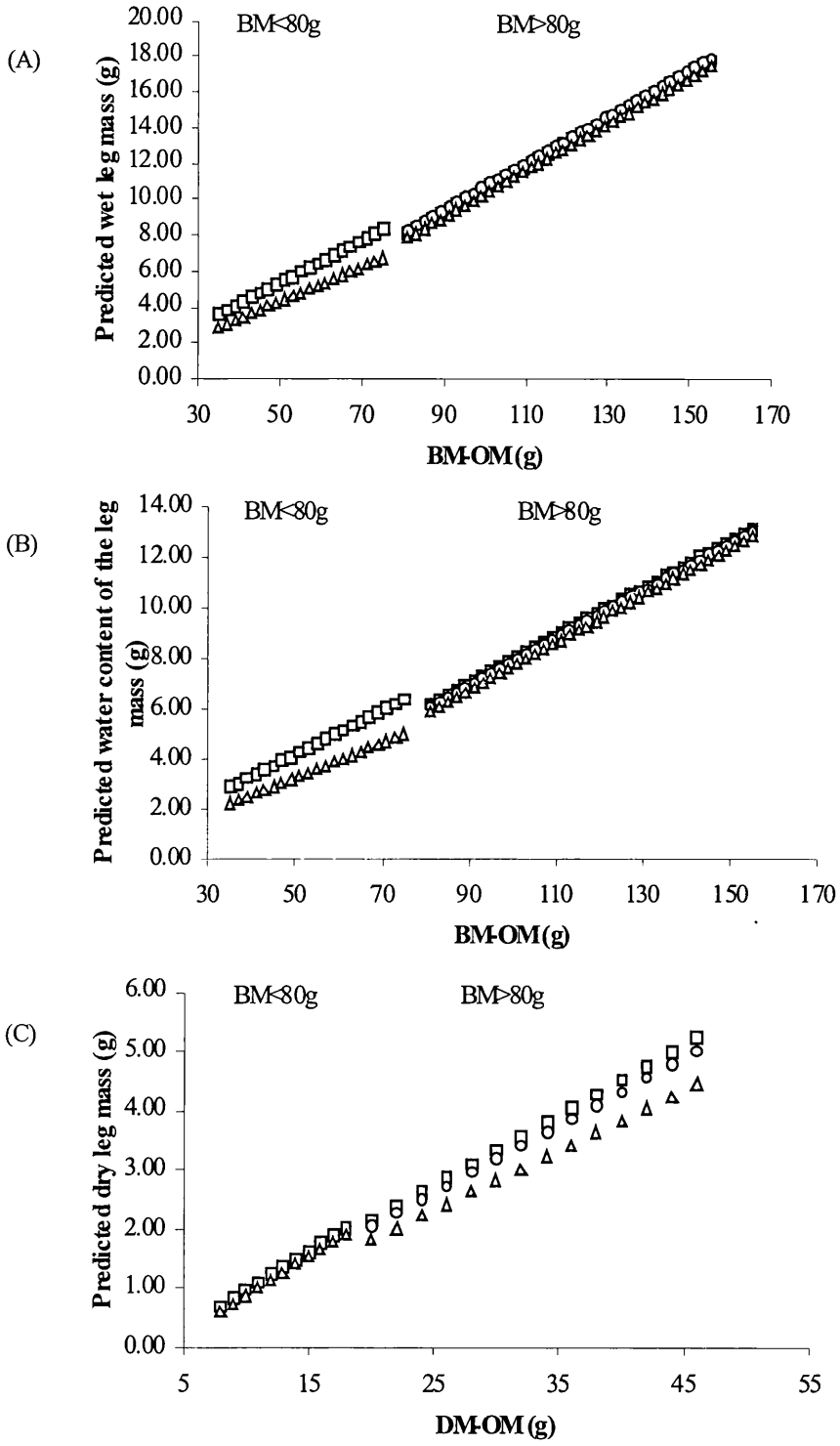


Figure 2.9 The relationship between corrected body mass and leg muscle mass in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ), where (A) is the wet leg muscle mass, (B) the water content of this muscle and (C)the dry leg muscle mass. The predicted values are shown for each genotype below and above 80 g.

significant DM-OM X age and dissector effect.

The comparison of leg muscle in chickens below 80 g revealed significant effects of genotype ($F_{1,45}$ = 8.99, P ≤ 0.01; BM-OM and age were significant covariates) and dissector ($F_{1,45}$ = 17.28, P ≤ 0.001). There were also significant interactions between age X genotype and BM-OM X dissector. The predicted leg muscle mass was greater in the FB compared to the L chickens (Figure 2.9A).

A similar effect of genotype was evident for the water content of the leg muscles ($F_{2,50}$ = 6.18, P ≤ 0.05; BM-OM and age were significant covariates, Figure 2.9B). The predicted values indicated that the FB chickens had significantly more water in the leg muscles than those from chickens of the L genotype (Figure 2.9B). There were also significant interactions between age X genotype and BM-OM X genotype. The dry leg muscle in the FB chickens was greater than that of the L chickens ($F_{1,45}$ = 4.98, P ≤ 0.05; DM-OM and age were significant covariates, Figure 2.9C) and there were also significant dissector and genotype X dissector effects.

There was also a significant effect of genotype on leg muscle mass in chickens above 80 g ($F_{2,45}$ = 3.37, P≤0.05; BM-OM and age were significant covariates, Figure 2.9A) and again there was a significant age X genotype interaction. The predicted leg muscle mass was indistinguishable between genotypes.

In chickens above 80 g, there was a significant effect of genotype on dry leg muscle mass ($F_{2,45}$ = 6.01, P ≤ 0.01; DM-OM and age were significant covariates), FB had larger dry leg muscle than SB or L genotype birds (Figure 2.9C). No such genotype difference could be seen in the water content of the leg muscle ($F_{2,50}$ = 3.08, P > 0.055; BM-OM and age were significant covariates, Figure 2.9B).

2.4.7 Organ Mass

2.4.7.1 Masses of the GIT Organs

Wet intestine mass was significantly affected by genotype, both in chickens below 80 g ($F_{1,45} = 4.20$, $P \leq 0.05$; BM-OM and age were significant covariates) and in the heavier chickens ($F_{2,45} = 3.75$, $P \leq 0.05$; BM-OM was a significant covariate). There were significant effects of dissector and age X genotype at both weight ranges, while a significant BM-OM X age interaction was found only in the heavier chickens. The predicted means revealed that at BM less than 80 g, the intestine in the FB genotype weighed more than the intestine mass from the L genotype (Figure 2.2A and 2.10A). A similar pattern occurred in the heavier chickens, where the intestine mass of the FB genotype was greater than that of the SB and L genotype (Figure 2.10A).

The replacement of BM-OM with LBM-OM caused no change in the significant effect of genotype on wet intestine mass in birds below ($F_{1,45} = 8.68$, $P \leq 0.01$, LBM-OM and age were significant covariates) and above 80 g ($F_{2,45} = 6.18$, $P \leq 0.01$, LBM-OM was a significant covariate). There was a significant dissector effect and age X genotype interaction in both weight ranges and a LBM-OM X age interaction in the birds above 80 g. The predicted intestine mass relative to LBM was greater in the FB compared to the L genotypes of chicken in the lower weight range of BM and the FB genotype was greater than the L with the SB chickens being intermediate in the higher weight range of BM (Figure 2.10B).

In chickens below 80 g the dry intestine mass was not affected by genotype but there was a significant age X genotype interaction ($F_{1,45} = 11.34$, $P \leq 0.01$; DM-OM and age were significant covariates). There was also a significant effect of dissector on dry intestine mass ($F_{1,45} = 6.56$, $P \leq 0.05$). The predicted dry intestine mass was greater in the FB than the L genotypes of chicken (Figure 2.10C). The intestine mass of heavier chickens was significantly affected by genotype ($F_{2,45} = 4.22$, $P \leq 0.05$; DM-OM was a significant covariate) and there were significant dissector, DM-OM X

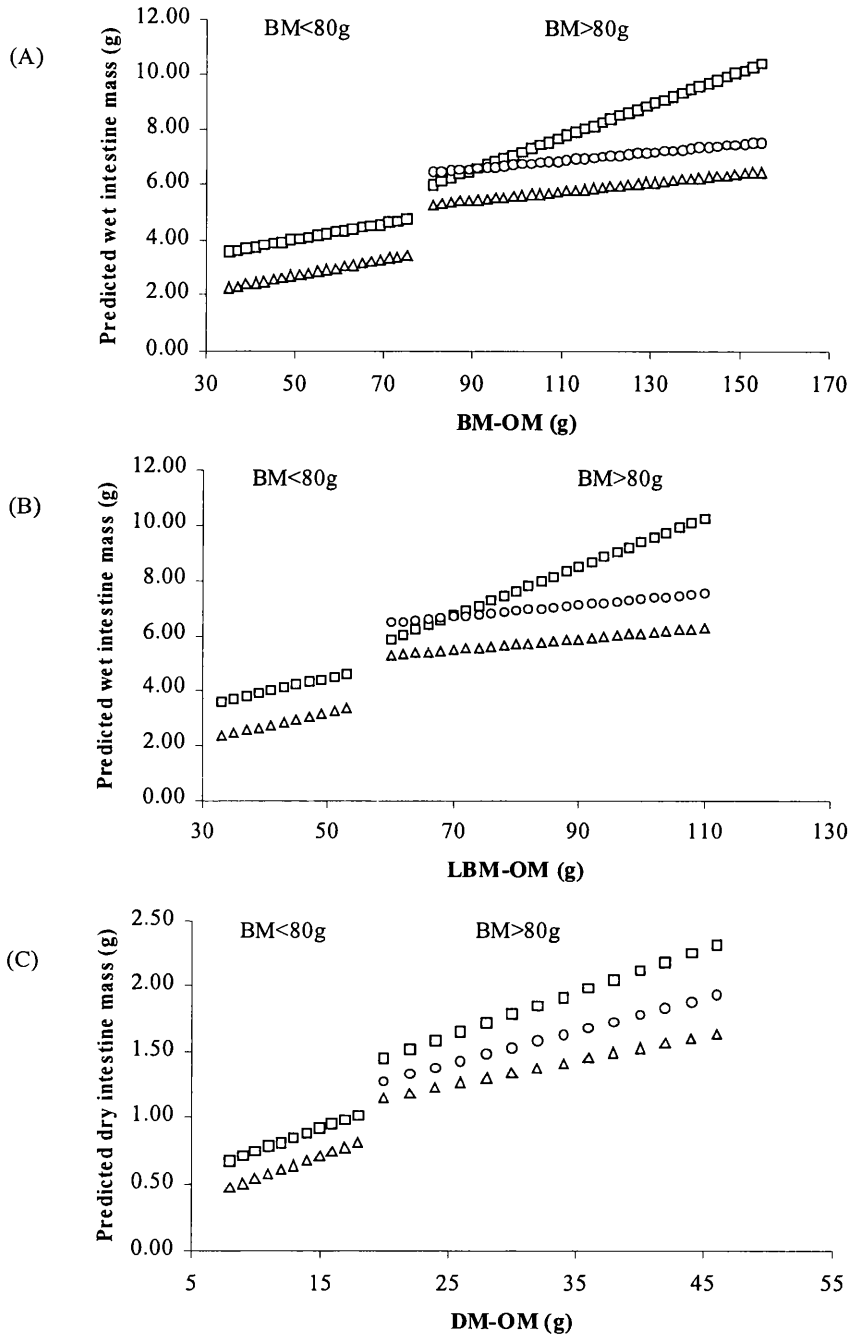


Figure 2.10 The relationship between corrected body mass and intestine mass in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ). Predicted intestine mass was adjusted for age, dissector and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM). The predicted values are shown for each genotype below and above 80 g.

age and age X genotype interactions. The predicted dry intestine mass in chickens above 80 g was heavier in the FB genotype compared to the SB or L genotype (Figure 2.10C).

There was a significant effect of genotype on wet caeca mass in chickens below 80 g ($F_{1,45}$ = 5.89, P ≤ 0.05; age was a significant covariate, and a significant BM-OM X age). Predicted caeca mass was greater in the FB than the L chickens below 80 g (Figure 2.11A). Genotype had no influence on caeca mass at BM above 80 g ($F_{2,45}$ = 2.30, P > 0.112; age is a significant covariate), but there was a significant dissector effect (Figure 2.11A).

There was a significant effect of genotype on wet caeca mass relative to LBM ($F_{1,45}$ = 15.17, P≤0.001, LBM-OM and age were significant covariates) in chickens weighing less than 80 g. There were also significant dissector (P ≤ 0.001), age X genotype and age X dissector interactions. The predicted caeca mass was greater in the FB chickens compared to the L chickens relative to LBM in birds below 80 g (Figure 2.11B). In the heavier chickens there was no significant effect of genotype, but LBM-OM was a significant covariate. There was a significant dissector effect.

There was a significant effect of genotype on dry caeca mass relative to DM in chickens below 80 g ($F_{1,45}$ = 9.54, P ≤ 0.01; DM-OM and age were significant covariates and there were significant dissector, age X genotype and age X dissector effects) and in those above 80 g ($F_{2,45}$ = 3.46, P ≤ 0.05; DM-OM and age were significant covariates and dissector had a significant effect). Predicted dry caeca mass in birds below 80 g was indistinguishable for FB and L genotype chickens. The pattern observed in the chickens above 80 g was that the caeca mass of the FB genotype was greater than both SB and L genotypes (Figure 2.11C).

In chickens below 80 g there was a significant effect of genotype on wet gizzard mass relative to BM ($F_{1,45}$ = 5.89, P ≤ 0.05; age was a significant covariate), and a significant BM-OM X age interaction. The predicted wet gizzard mass was greater in the L than in the FB genotype of chicken. There was no significant effect of

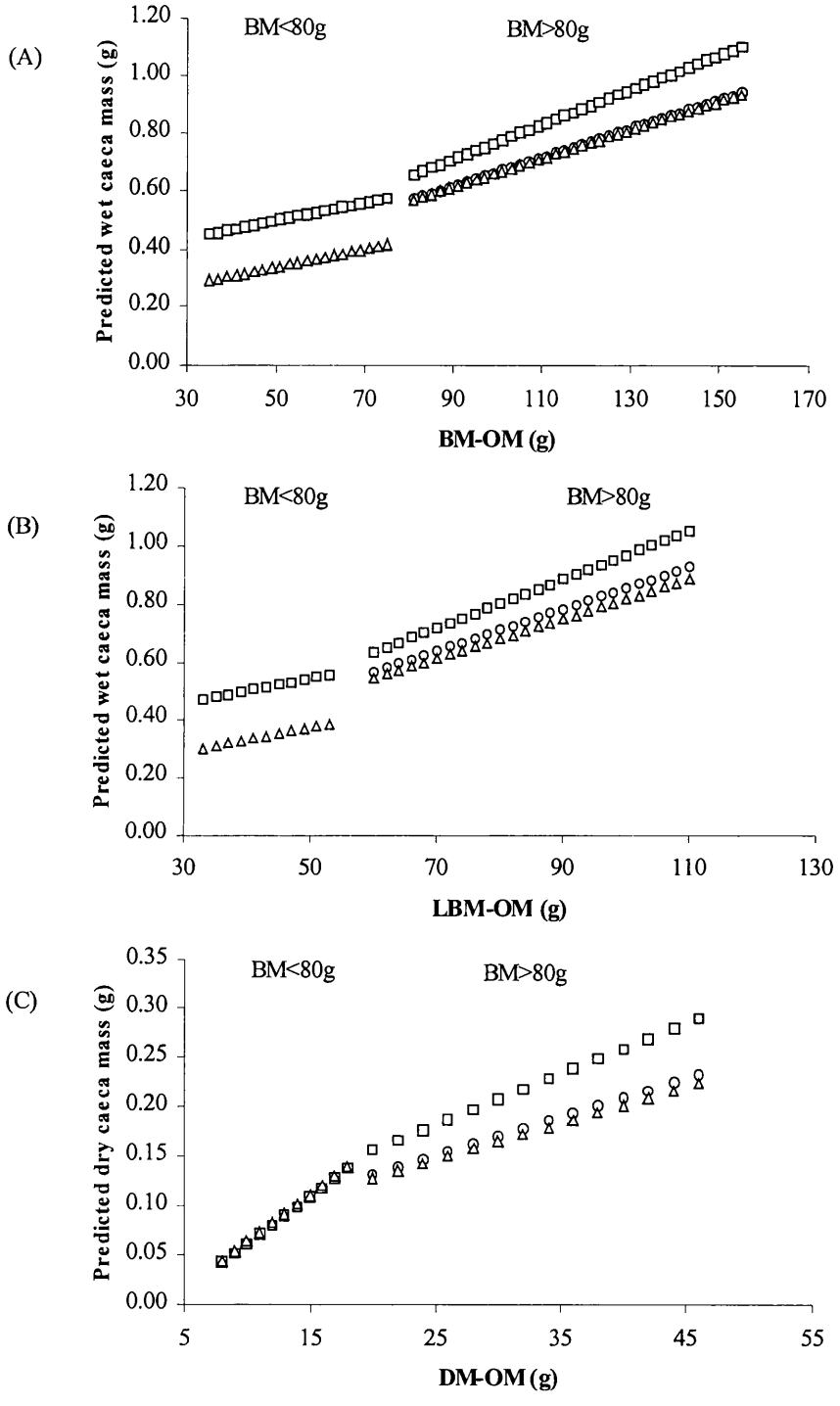


Figure 2.11 The relationship between corrected body mass and caeca mass in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ). Predicted caeca mass was adjusted for age, dissector and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM). The predicted values are shown for each genotype below and above 80 g.

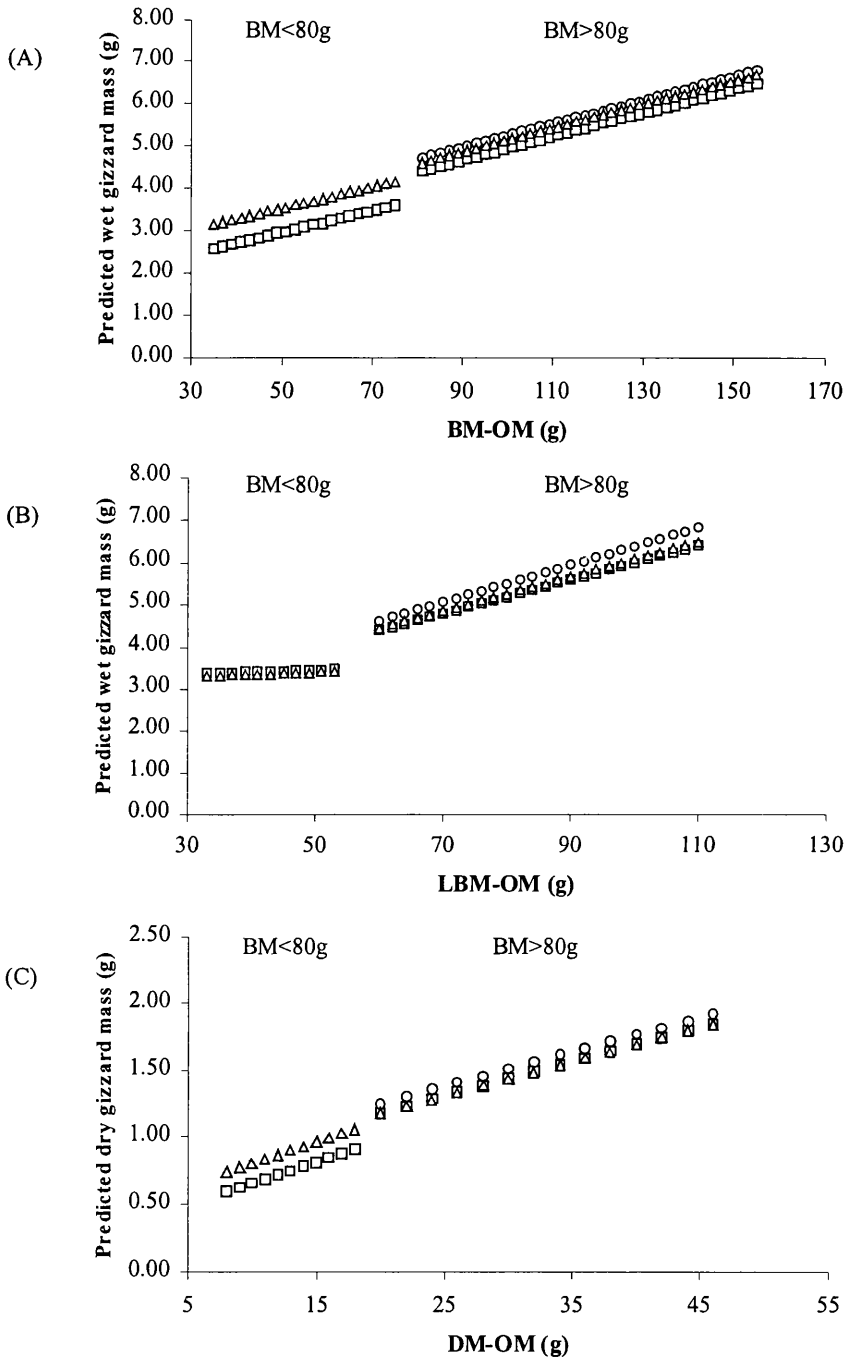


Figure 2.12 The relationship between corrected body mass and gizzard mass in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ). Predicted gizzard mass was adjusted for age, dissector and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM). The predicted values are shown for each genotype below and above 80 g.

genotype in the heavier chickens ($F_{2,45}$ = 1.36, P > 0.268; BM-OM and age were significant covariates and there was a significant dissector and genotype X dissector effect) (Figure 2.12A).

The replacement of BM-OM with LBM-OM as a covariate resulted in a significant effect of genotype on gizzard mass ($F_{1,45}$ = 6.87, P ≤ 0.05, age was a significant covariate and there was a LBM-OM X age interaction). Predicted gizzard mass relative to LBM was greater in the FB genotype compared to the L genotype (Figure 2.12B). There was also a significant effect of genotype in the heavier chickens ($F_{2,45}$ = 3.71, P ≤ 0.05, LBM-OM and age were significant covariates), and significant dissector and age X dissector interaction. The predicted gizzard mass relative to LBM was greater in the SB chickens compared to the FB and L chickens (Figure 2.12B).

Dry gizzard mass was significantly affected by genotype in chickens below 80 g ($F_{1,45}$ = 6.05, P ≤ 0.05; age was a significant covariate), and there was a significant DM-OM X age interaction. Predicted dry gizzard mass was greater in the L genotype than in the FB (Figure 2.12C). In chickens above 80 g there was no significant effect of genotype on dry gizzard mass ($F_{2,45}$ = 1.64, P > 0.205; DM-OM and age were significant covariates). There were also significant dissector, DM-OM X age, genotype X dissector and age X dissector effects (Figure 2.18).

2.4.7.2 The Masses of Key Support Organs

There was no significant effect of genotype on wet liver mass at a BM either below 80 g ($F_{1,55}$ = 0.01, P > 0.907; BM-OM and age were significant covariates), or in birds above 80 g ($F_{2,45}$ = 0.81, P > 0.453; BM-OM and age were significant covariates) although there was a significant interaction between BM-OM and age (Figure 2.2B and 2.13A).

In the chickens weighing less than 80 g, there was no significant effect of genotype on wet liver mass relative to LBM, but LBM-OM and age were significant covariates

(Figure 2.13B). In the heavier weight range of chicken there was no evidence of a effect of genotype on liver mass ($F_{2,45}$ = 0.95, P > 0.394, LBM-OM and age were significant covariates). There was a significant LBM-OM X age interaction.

There was also no significant effect of genotype on dry liver mass relative to DM, for the lighter chickens ($F_{1,45}$ = 0.16, P > 0.695; DM-OM and age were significant covariates) or for the chickens above 80 g ($F_{2,45}$ = 0.28, P > 0.759; DM-OM was a significant covariate), there was also a significant dissector and DM-OM X age interaction (Figure 2.13C).

In chickens below 80 g there was a significant effect of genotype on wet heart mass ($F_{1,45}$ = 4.68, P ≤ 0.05; BM-OM and age were significant covariates) and there was a significant dissector effect. Predicted wet heart mass was indistinguishable for the chickens below 80g (Figure 2.14A). There was also a significant effect of genotype on heart mass for the heavier chickens ($F_{2,45}$ = 6.64, P ≤ 0.01; BM-OM and age were significant covariates, and a significant dissector and BM-OM X age affect). Heart mass was heavier in the L genotype compared to the SB and FB genotype (Figure 2.14A).

No significant effect of genotype on wet heart mass relative to LBM was observed in chickens below 80 g ($F_{1,45}$ = 0.00, P > 0.973, LBM-OM and age were significant covariates). There were significant dissector and LBM-OM X genotype interaction (Figure 2.14B). In the heavier chickens, there was also no significant effect of genotype on heart mass, but LBM-OM and age were significant covariates. There were also significant dissector and LBM-OM X age interaction (Figure 2.14B).

The dry heart mass relative to DM in chickens below 80 g was not significantly affected by genotype but there was a significant age X genotype interaction ($F_{1,45}$ = 7.41, P ≤ 0.01; DM-OM and age were significant covariates). There was also a significant dissector and genotype X dissector effect observed. The predicted heart mass was greater in the L chickens compared to the FB chickens. In the heavier chickens, there was no affect of genotype on dry heart mass ($F_{2,45}$ = 0.68, P > 0.513;

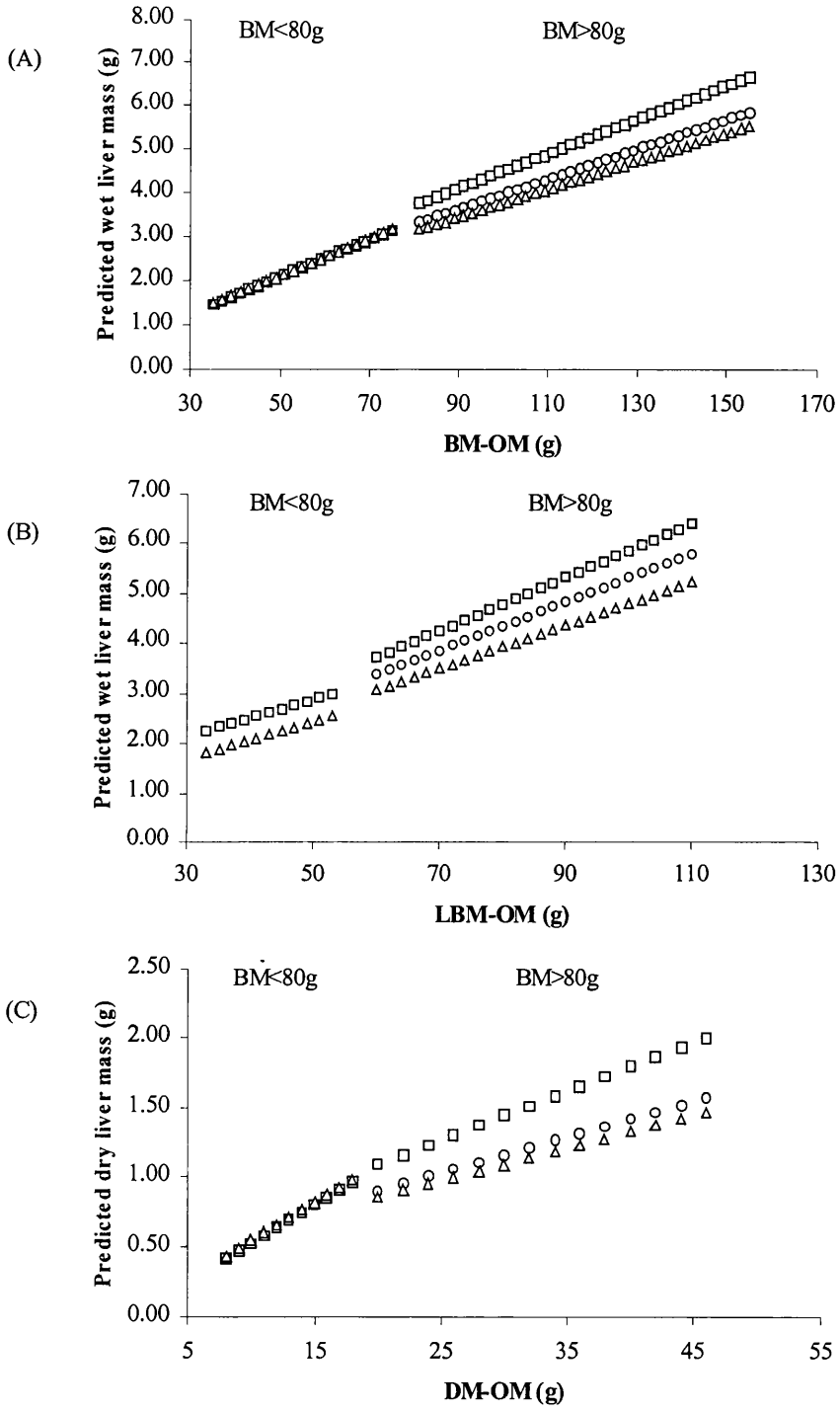


Figure 2.13 The relationship between corrected body mass and liver mass in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ). Predicted liver mass was adjusted for age, dissector and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM). Predicted values are shown for each genotype below and above 80 g.

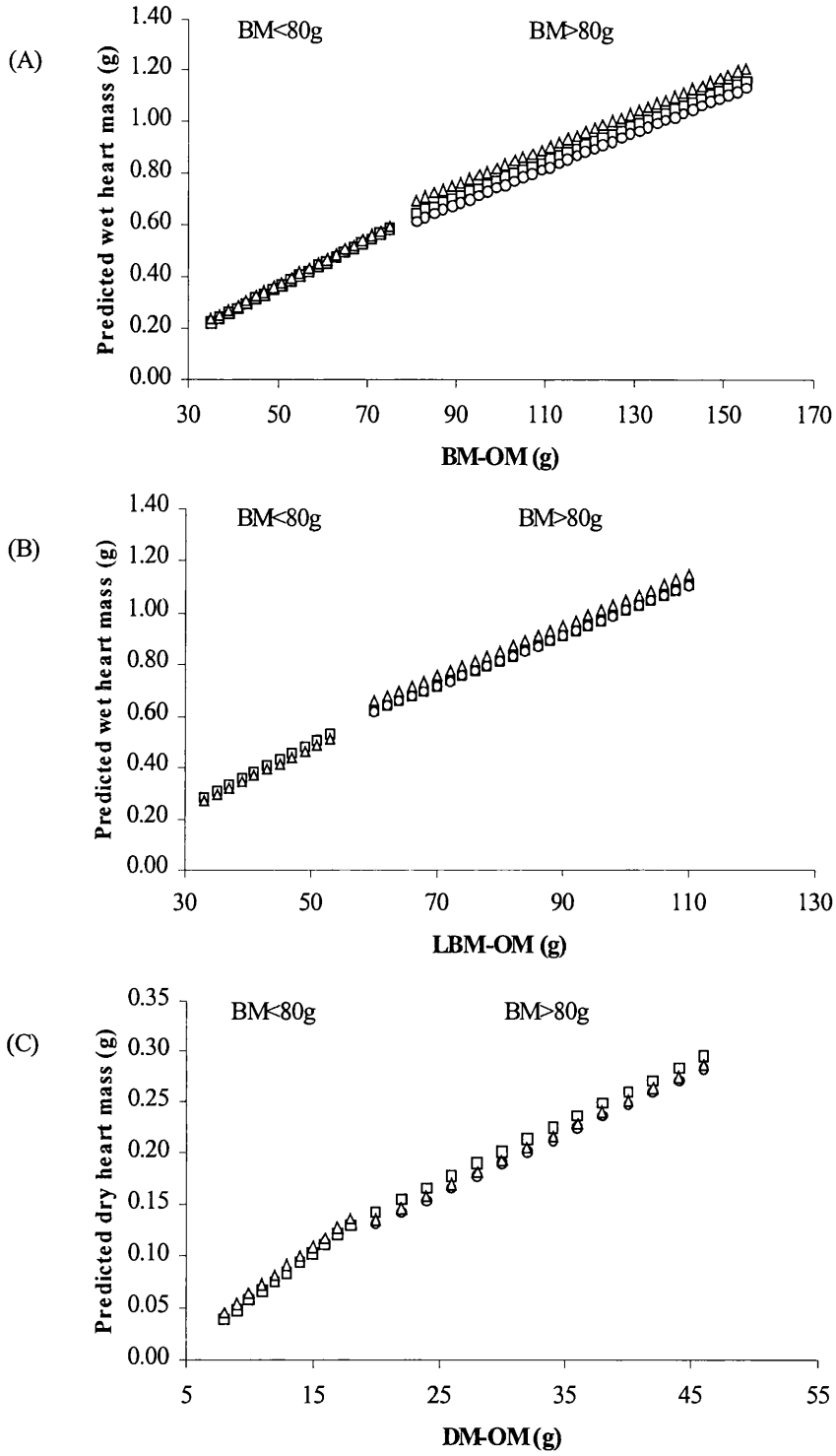


Figure 2.14 The relationship between corrected body mass and heart mass in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ). Predicted heart mass was adjusted for age, dissector and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM). The predicted values are shown for each genotype below and above 80 g.

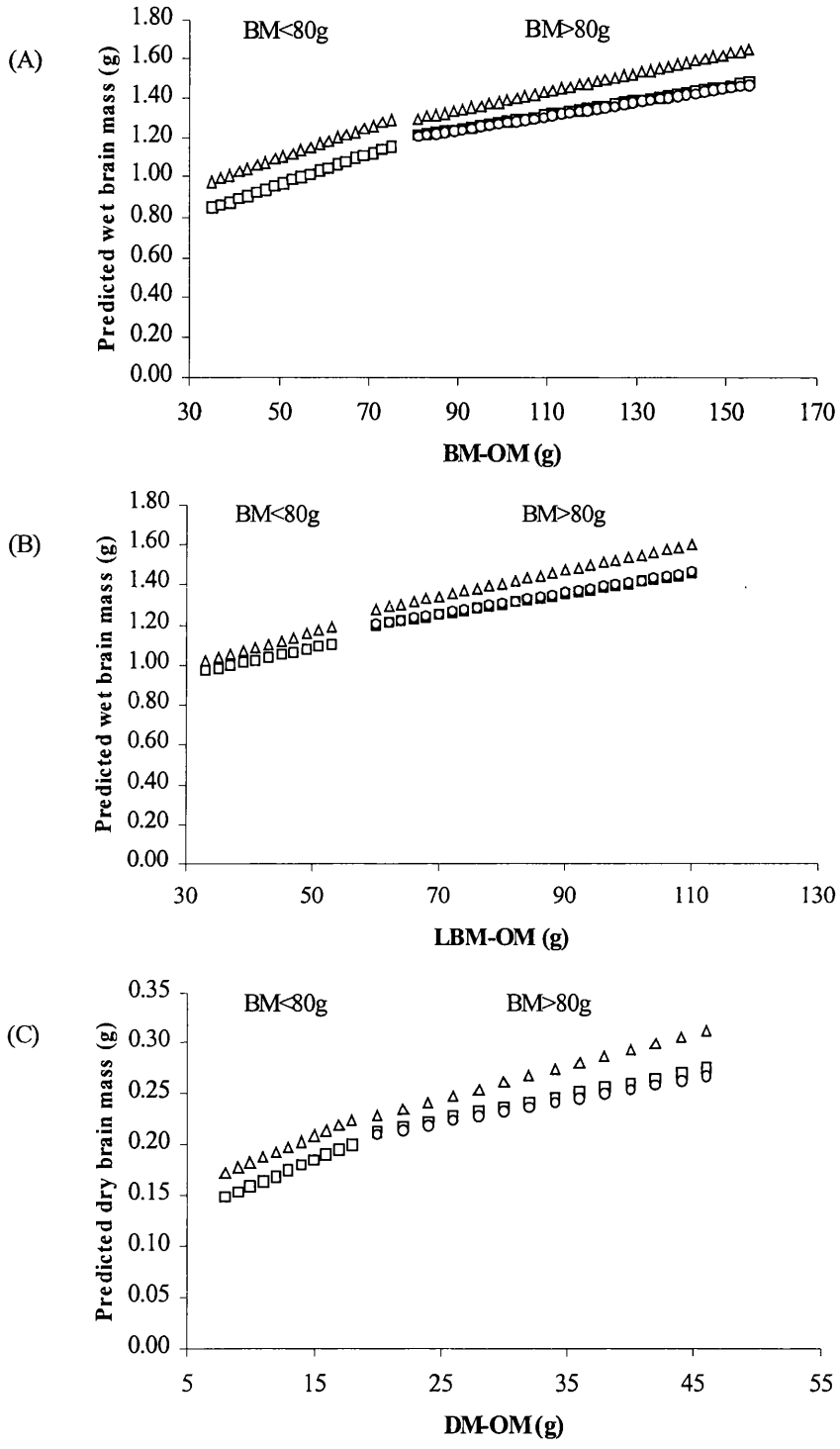


Figure 2.15 The relationship between corrected body mass and brain mass in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ). Predicted brain mass was adjusted for age, dissector and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM). The predicted values are shown for each genotype below and above 80 g.

DM-OM and age were significant covariates, and a significant DM-OM X age effect, Figure 2.14C).

There was a significant effect of genotype on wet brain mass in chickens below 80 g ($F_{1,45}$ = 7.18, P ≤0.01; BM-OM and age were significant covariates), and there was also a significant genotype X dissector interaction. Predicted brain mass was heavier in the L genotype compared to the FB genotype (Figure 2.15A). The brain mass of heavier chickens was also significantly affected by genotype ($F_{2,45}$ = 10.77, P ≤ 0.001; BM-OM and age were significant covariates). Predicted wet brain mass relative to BM was greater in the L compared to the FB and SB genotypes (Figure 2.15A).

There was no significant effect of genotype on lean wet brain mass ($F_{1,45}$ = 2.99, P > 0.091, LBM-OM and age were significant covariates, there was also a significant genotype X dissector interaction). In contrast, in the heavier range of chickens there was a significant effect of genotype ($F_{2,45}$ = 8.44, P ≤ 0.001, LBM-OM and age were significant covariates). The predicted brain mass relative to LBM was greater in the L genotype compared to the FB or SB genotypes (Figure 2.15B).

The dry brain mass of chickens below 80 g was significantly affected by genotype ($F_{1,45}$ =12.64, P ≤ 0.001; DM-OM and age were significant covariates). There were also significant genotype X dissector and DM-OM X dissector interactions. Predicted dry brain mass was greater in the L genotype compared to the FB genotype (Figure 2.15C). The genotype difference observed in dry brain mass relative to DM in the lighter chickens was also apparent in the heavier chickens ($F_{2,45}$ = 17.43, P ≤ 0.001; DM-OM and age were significant covariates), where predicted dry brain mass was greater in the L genotype compared to the FB or SB genotypes (Figure 2.15C).

2.4.8 Relationship Between Organ Mass and Metabolic Rate

The relationship between metabolic rate and the different organ masses was analysed

using the same statistics as described for the relationship between RMR and PMR. The residuals were computed from the linear regressions of the MR's controlling for BM and age by placing these covariates in the model. This was repeated for each organ mass separately, placing age, BM-OM and dissector in the regression model. The regressions were then repeated replacing BM-OM with LBM-OM. Correlations were performed on both sets of residuals against the residuals produced from the MR's.

2.4.8.1 Relationship Between Organ Mass and Resting Metabolic Rate

For the pooled data and the L genotype alone from all chickens weighing less than 80g, using BM-OM as a variable, there were no significant correlations (Table 2.3). However, there were significant correlations between RMR and the masses of caeca and heart in the FB chickens (Table 2.3).

When LBM-OM was used as a variable in the analysis, to remove the effects of body fat, different correlations between RMR and organ mass became evident. In the birds weighing less than 80 g there was a significant, positive correlations between RMR and both leg muscle and liver mass in the pooled data set (Table 2.4). Resting MR was significantly, positively correlated with pectoral muscle mass, but significantly, negatively correlated with caeca mass in the FB chickens. Heart mass was no longer significantly correlated with RMR within the FB when LBM was used as a variable. Both leg muscle and heart mass were significantly, positively correlated with RMR in the L chickens (Table 2.4).

For chickens weighing more than 80 g, where BM-OM was the variable used in the regressions, there were no significant correlations within the pooled data set (Table 2.5). Within-genotype analysis revealed no correlations in the FB chickens. There was a significant, negative correlation between RMR and caeca mass, and a significant, positive correlation with heart mass evident in the SB chickens. Within the L genotype there was a significant, negative correlation between RMR and brain mass (Table 2.5).

Table 2.3 The correlation between resting metabolic rate (RMR) and organ masses relative to body mass in fast broilers (FB), layers (L) and these two genotypes combined to produce a pooled data set, for chickens weighing less than 80 g. The data are correlation coefficients based on the residuals from regression equations of organ mass and RMR. The regression equations were produced using age, corrected body mass and dissector as the main explanatories. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

Organ	[1]Pooled data	[2]FB	[3]L
Pectoral muscle	0.009	0.148	0.006
	(NS)	(NS)	(NS)
Leg muscle	0.114	0.019	0.286
	(NS)	(NS)	(NS)
Intestine	-0.052	-0.401	0.154
	(NS)	(NS)	(NS)
Caeca	-0.072	-0.574	0.005
	(NS)	(≤0.01)	(NS)
Gizzard	-0.089	-0.263	-0.104
	(NS)	(NS)	(NS)
Liver	0.181	0.333	0.022
	(NS)	(NS)	(NS)
Heart	0.263	0.423	0.340
	(NS)	(≤0.05)	(NS)
Brain	0.024	0.120	-0.124
	(NS)	(NS)	(NS)

Where; [1]Pooled data-data from the fast broilers and layers combined, [2]FB-fast broiler, [3]L-layer.

When these analyses were repeated using LBM-OM as a variable, a significant, positive correlation was evident between RMR and liver mass and a significant, negative correlation with brain mass within the pooled data set among birds heavier than 80 g (Table 2.6). Separate analyses for the individual genotypes revealed significant, positive correlations between RMR and heart mass in the FB chickens, and for leg muscle and heart mass in the SB chickens. There was a significant,

negative relationship between RMR and leg muscle mass in the L chickens (Table 2.6).

Table 2.4 The correlation between resting metabolic rate (RMR) and organ masses relative to lean body mass in fast broilers (FB), layers (L) and these two genotypes combined to produce a pooled data set, for chickens weighing less than 80 g. The data are correlation coefficients based on the residuals from regression equations of organ mass and RMR. The regression equations were produced using age, corrected lean body mass and dissector as the main explanatories. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

Organ	[1]Pooled data	[2]FB	[3]L
Pectoral muscle	0.173	0.447	0.041
	(NS)	(≤0.05)	(NS)
Leg muscle	0.286	0.120	0.377
	(≤0.05)	(NS)	(≤0.05)
Intestine	-0.209	0.068	0.218
	(NS)	(NS)	(NS)
Caeca	0.001	-0.443	0.196
	(NS)	(≤0.05)	(NS)
Gizzard	-0.186	-0.182	-0.010
	(NS)	(NS)	(NS)
Liver	0.387	0.192	0.234
	(≤0.01)	(NS)	(NS)
Heart	0.018	0.242	0.468
	(NS)	(NS)	(≤0.01)
Brain	0.079	0.153	-0.170
	(NS)	(NS)	(NS)

Where; [1]Pooled data-data from the fast broilers and layers combined, [2]FB-fast broiler, [3]L-layer.

Table 2.5 The correlation between resting metabolic rate (RMR) and organ masses relative to body mass in fast broilers (FB), slow broilers (SB), layers (L) and these three genotypes combined to produce a pooled data set, for chickens weighing more than 80 g. The data are correlation coefficients based on the residuals from regression equations of organ mass and RMR. The regression equations were produced using age, corrected body mass and dissector as the main explanatories. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

Organ	[1]Pooled data	[2]FB	[3]SB	[4]L
Pectoral muscle	-0.110	-0.144	-0.186	0.006
	(NS)	(NS)	(NS)	(NS)
Leg muscle	-0.044	-0.255	0.265	0.005
	(NS)	(NS)	(NS)	(NS)
Intestine	0.084	-0.152	-0.229	0.077
	(NS)	(NS)	(NS)	(NS)
Caeca	-0.040	0.034	-0.471	-0.094
	(NS)	(NS)	(≤0.05)	(NS)
Gizzard	-0.027	-0.313	0.032	-0.378
	(NS)	(NS)	(NS)	(NS)
Liver	0.187	0.216	0.169	0.265
	(NS)	(NS)	(NS)	(NS)
Heart	0.184	0.392	0.529	-0.291
	(NS)	(NS)	(≤0.05)	(NS)
Brain	-0.211	-0.181	-0.029	-0.501
	(NS)	(NS)	(NS)	(≤0.05)

Where; [1]Pooled data-data from the fast broilers, slow broilers and layers combined, [2]FB-fast broiler, [3]SB-slow broiler, [4]L-layer.

Table 2.6 The correlation between resting metabolic rate (RMR) and organ masses relative to lean body mass in fast broilers (FB), slow broilers (SB), layers (L) and these three genotypes combined to produce a pooled data set, for chickens weighing more than 80 g. The data are correlation coefficients based on the residuals from regression equations of organ mass and RMR. The regression equations were produced using age, corrected lean body mass and dissector as the main explanatories. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

Organ	[1]Pooled data	[2]FB	[3]SB	[4]L
Pectoral muscle	0.167	0.359	0.310	0.477
	(NS)	(NS)	(NS)	(NS)
Leg muscle	0.212	0.037	0.460	0.522
	(NS)	(NS)	(≤0.05)	(≤0.05)
Intestine	0.026	0.087	-0.203	0.477
	(NS)	(NS)	(NS)	(NS)
Caeca	-0.001	0.110	-0.398	-0.023
	(NS)	(NS)	(NS)	(NS)
Gizzard	-0.047	0.246	0.059	-0.055
	(NS)	(NS)	(NS)	(NS)
Liver	0.356	0.120	0.235	0.467
	(≤0.01)	(NS)	(NS)	(NS)
Heart	-0.089	0.701	0.517	0.263
	(NS)	(≤0.001)	(≤0.05)	(NS)
Brain	-0.301	0.356	0.110	-0.274
	(≤0.05)	(NS)	(NS)	(NS)

Where; [1]Pooled data-data from the fast broilers, slow broilers and layers combined, [2]FB-fast broiler, [3]SB-slow broiler, [4]L-layer.

Table 2.7 The correlation between peak metabolic rate (PMR) and organ masses relative to body mass in fast broilers (FB), layers (L) and these two genotypes combined to produce a pooled data set, for chickens weighing more than 80 g. The data are correlation coefficients based on the residuals from regression equations of organ mass and PMR. The regression equations were produced using age, corrected body mass and dissector as the main explanatories. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

Organ	[1]Pooled data	[2]FB	[3]L
Pectoral muscle	0.038	-0.669	0.135
	(NS)	(≤0.001)	(NS)
Leg muscle	0.175	-0.238	0.330
	(NS)	(NS)	(NS)
Intestine	0.301	-0.324	0.333
	(≤0.05)	(NS)	(NS)
Caeca	0.347	0.002	-0.103
	(≤0.05)	(NS)	(NS)
Gizzard	-0.168	-0.109	-0.094
	(NS)	(NS)	(NS)
Liver	0.052	0.165	-0.054
	(NS)	(NS)	(NS)
Heart	0.091	0.359	0.264
	(NS)	(NS)	(NS)
Brain	-0.256	-0.113	-0.179
	(NS)	(NS)	(NS)

Where; [1]Pooled data-data from the fast broilers and layers combined, [2]FB-fast broiler, [3]L-layer.

Table 2.8 The correlation between peak metabolic rate (PMR) and organ masses relative to lean body mass in fast broilers (FB), layers (L) and these two genotypes combined to produce a pooled data set, for chickens weighing more than 80 g. The data are correlation coefficients based on the residuals from regression equations of organ mass and PMR. The regression equations were produced using age, corrected lean body mass and dissector as the main explanatories. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

Organ	[1]Pooled data	[2]FB	[3]L
Pectoral muscle	0.209	-0.395	0.160
	(NS)	(NS)	(NS)
Leg muscle	0.319	-0.212	0.381
	(≤0.05)	(NS)	(≤0.05)
Intestine	0.020	0.018	0.351
	(NS)	(NS)	(≤0.05)
Caeca	0.344	0.150	-0.017
	(≤0.05)	(NS)	(NS)
Gizzard	-0.274	-0.133	0.000
	(≤0.05)	(NS)	(NS)
Liver	0.306	-0.003	0.100
	(≤0.05)	(NS)	(NS)
Heart	-0.060	0.222	0.358
	(NS)	(NS)	(≤0.05)
Brain	-0.158	-0.113	-0.159
	(NS)	(NS)	(NS)

Where; [1]Pooled data-data from the fast broilers and layers combined, [2]FB-fast broiler, [3]L-layer.

2.4.8.2 Relationship Between Organ Mass and Peak Metabolic Rate

The analysis of PMR and organ mass where BM-OM was used as a variable, revealed significant, positive correlations between PMR and both intestine and caeca mass in the pooled data set (Table 2.7). Although there was no significant

correlation for pectoral muscle mass in the pooled data, there was a highly significant, negative correlation between PMR and pectoral muscle mass in the FB chickens. There were no correlation in the L genotype (Table 2.7).The comparisons were repeated using LBM-OM as a variable, and significant, positive correlations were evident between PMR and leg muscle, caeca and liver mass in the pooled data set. There was also a significant, negative relationship between PMR and gizzard mass in the pooled data set (Table 2.8). The within-genotype analysis revealed no correlations between PMR and organ mass in the FB chickens. There was significant, positive correlations between PMR and each of leg muscle, intestine and heart mass in the L chickens (Table 2.8).

2.4.8.3 Relationship Between Metabolic Rates and Muscle Water Content

When the data were pooled to include each genotype, there was no relationship between RMR and the water content of either the pectoral or leg muscle mass, at either weight range, where BM-OM was used as the variable in the regression analysis. This lack of relationship was also evident for the individual genotypes.

There was also no relationship between PMR and the water content of either pectoral and leg muscle mass, for the pooled data set and the L chickens, but there was a significant, negative correlation between PMR and the water content of pectoral muscles in the FB chickens.

2.4.9 Carcass Chemical Composition

At BM's less than 80 g, there were significant differences between genotypes in relative carcass fat (g) ($F_{1,49} = 5.00$, $P \leq 0.01$; BM and age were significant covariates and there was a significant BM X age interaction) but no differences were shown in CP (g) ($F_{1,49} = 3.50$, $P > 0.67$; BM and age were significant covariates) and ash content (g) ($F_{1,49} = 0.30$, $P > 0.587$; BM and age were significant covariate and there was a BM X genotype). The FB genotype had a greater fat content than the L

genotype, but the predictive model for ash content was indistinguishable for the chickens below 80 g.

Table 2.9 The correlation between resting metabolic rate (RMR) and organ masses relative to body mass in fast broilers (FB), layers (L) and these two genotypes combined to produce a pooled data set, for chickens weighing less than 80 g. The data are correlation coefficients based on the residuals from regression equations of carcass composition and RMR. The regression equations were produced using age and body mass as the main explanatories. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

Carcass component	[1]Pooled data	[2]FB	[3]L
Crude protein	-0.268	-0.565	0.149
	(≤0.05)	(≤0.01)	(NS)
Fat	0.093	0.402	-0.145
	(NS)	(NS)	(NS)
Ash	-0.094	-0.071	0.198
	(NS)	(NS)	(NS)

Where; [1]Pooled data-data from the fast broilers and layers combined, [2]FB-fast broiler, [3]L-layer.

At BM's above 80 g a between-genotype difference was evident for relative CP (g) ($F_{2,50} = 8.89$, $P \leq 0.001$), fat (g) ($F_{2,50} = 13.82$, $P \leq 0.001$) and ash content (g) ($F_{2,50} = 5.68$, $P \leq 0.01$) of the carcasses and in all cases BM and age were significant covariates. There was also an age X genotype interaction for fat and ash content. The SB genotype had a greater fat content than the FB or L genotypes and the L chickens had a greater ash content than the FB or SB chickens. The FB and L genotypes had a larger relative CP content compared to the SB genotype (Figure 2.16A-C).

The relationship between MR and the carcass composition of the chicken was analysed as before, using BM as the variable in the regression analysis of the carcass composition. In the chickens weighing less than 80 g a significant, negative correlation was evident between RMR and absolute CP content in both the pooled data set and in the FB chickens (Table 2.9). When these analyses were repeated for

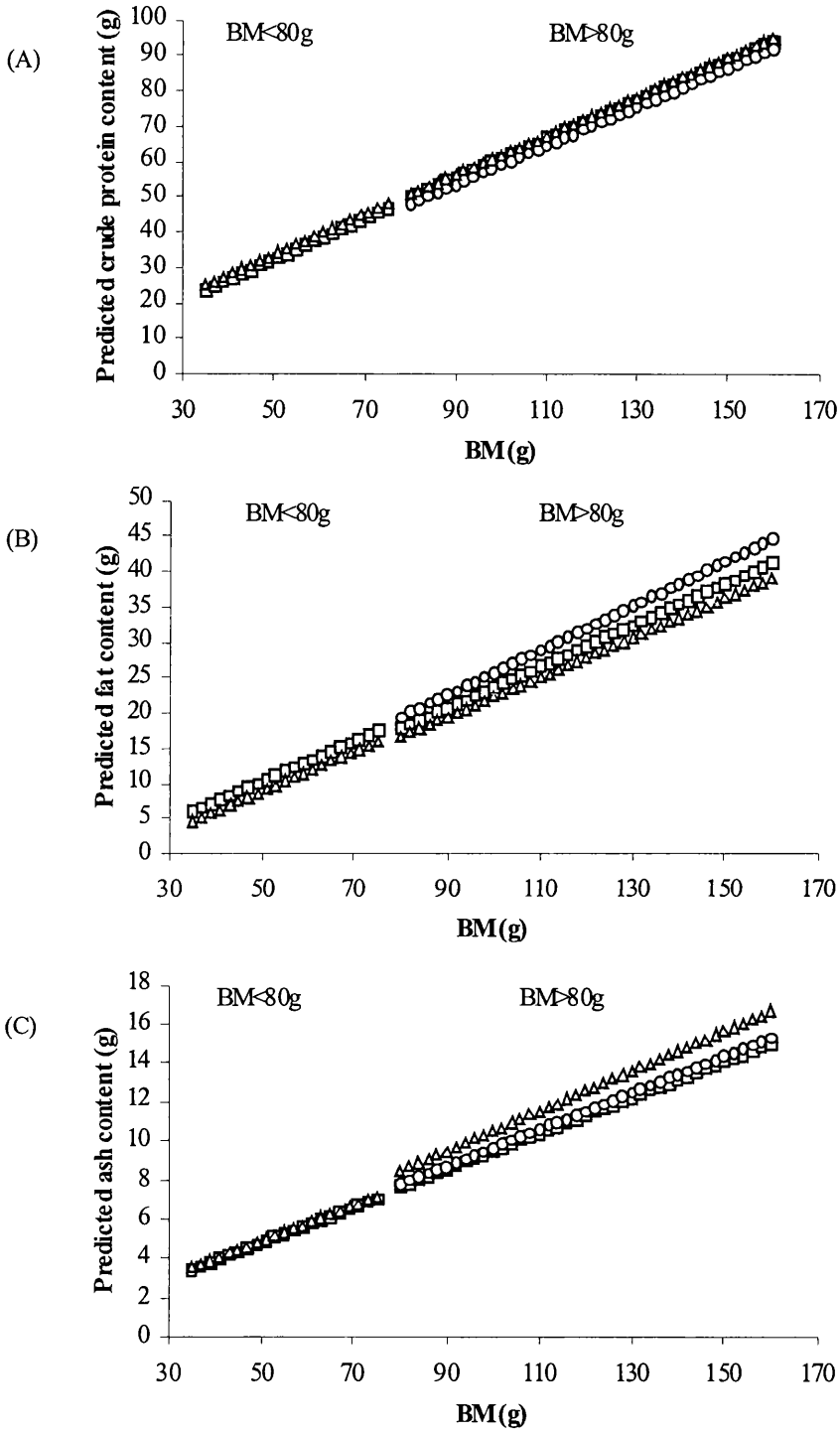


Figure 2.16 The relationship between body mass and carcass composition in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ). Predicted absolute (A) crude protein, (B) fat and (C) ash mass were adjusted for body mass (BM) and age. The predicted values shown are for each genotype below and above 80 g.

chickens weighing more than 80g, no significant correlations within the pooled data set were evident, also no correlations were shown in the individual genotypes (Table 2.10).

Table 2.10 The correlation between peak metabolic rate (PMR) and organ masses relative to body mass in fast broilers (FB), slow broilers (SB), layers (L) and these three genotypes combined to produce a pooled data set, for chickens weighing more than 80 g. The data are correlation coefficients based on the residuals from regression equations of carcass composition and PMR. The regression equations were produced using age and body mass as the main explanatories. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

Carcass composition	[1]Pooled data	[2]FB	[3]SB	[4]L
Crude protein	-0.122	0.246	0.024	-0.189
	(NS)	(NS)	(NS)	(NS)
Fat	0.087	-0.197	-0.131	-0.067
	(NS)	(NS)	(NS)	(NS)
Ash	-0.185	-0.090	0.065	-0.141
	(NS)	(NS)	(NS)	(NS)

Where; [1]Pooled data-data from the fast broilers, slow broilers and layers combined, [2]FB-fast broiler, [3]SB-slow broiler, [4]L-layer.

In the pooled data set and the separate analyses for the individual genotypes revealed no significant correlations between PMR and absolute CP, fat or ash content of the chickens.

2.5 Discussion

2.5.1 Effect of Genotype on Metabolic Rates

The growth increment and BM of the chickens were, as expected, greater in the FB

genotype compared to the L genotype with the SB genotype being intermediate. Adult RMR and chick RMR include costs such as; digestion, assimilation, processing and storage (i.e., specific dynamic action), but chicks have the additional costs of growth (i.e., biosynthesis) (Dietz & Drent, 1997). Despite broilers having a significantly higher mature body mass and therefore an increased growth rate, there was no corresponding elevation in RMR to support this increased mass. Resting MR determined in this study was 26.64 ± 1.15 J/g/hr and 28.75 ± 1.02 J/g/hr in broilers and layer chickens respectively, which were similar to RMR previously determined (Table 2.1). Jackson & Diamond (1996) compared RMR in a modern broiler chicken with that of its ancestor, the Red Jungle Fowl, and also found the two genotypes were statistically indistinguishable. However, Scott *et al.* (1996) stated that the metabolic output of fat was very low in relation to other tissues, such as breast muscle or liver, therefore LBM should be used as a covariate when analysing MR data rather than BM to correct for the mass of the bird. When the BM covariate was replaced with LBM in the current analysis, RMR was observed to be greater in the FB chickens at the heavier weight range compared to the L chickens. As one had expected, suggesting there was an energetic cost to maintaining larger muscle mass, since the leg and breast muscle mass made up the largest part of LBM. This was in complete contrast to Kuenzel & Kuenzel (1977) and Visser & van Kampen (1991), who found that a faster growing broiler genotype had lower RMR than its slower growing counterpart, once BM was taken into account.

Peak MR in contrast to RMR, was greater for the broiler compared to the layer genotype, when either BM or LBM were used as covariates. This is almost certainly attributable to the fact that the broilers had a greater muscle mass, and it is muscle that is used during shivering thermogenesis (ST) to produce heat in response to cold exposure. At hatching, the leg muscles of the chicken are generally larger than the breast muscle and probably contribute more to ST (Dietz *et al.*, 1997). The significantly larger leg muscle mass shown here in the broiler chickens at immediately post-hatch, is due to the genetic selection for greater muscle mass and is indicative of their potential to produce heat by ST. Visser & van Kampen (1991) also measured PMR by lowering the temperature below thermoneutrality (to 20 °C), but only in neonates weighing approximately 45 g. No significant difference in

PMR between broiler (50.0 J/g/hr, 13.89 mW/g) and layer (50.5 J/g/hr, 14.03 mW/g) chick was found. There was also no difference in MS (2.1 and 2.3) between the two genotypes.

Within the range of BM's analysed in this study there was a significant positive correlation between the residuals of RMR and the residuals of PMR in both the pooled data and the layer chickens. These results indicate that RMR is a fixed ratio of PMR, that is, an animal with a low RMR will have a low PMR, and vice versa. Hayes (1989) reported similar findings in deer mice, where residuals of VO_{2max} elicited by treadmill exercise were significantly, positively related to RMR residuals. Also, Bozinovic (1992) indicated partial correlations between RMR and MMR within 29 species of rodent after the effects of BM were removed. In contrast, Meerlo *et al.* (1997) showed no correlation between the residuals of BMR and the residuals of daily EE in field voles that varied in reproductive status. Similarly, Konarzewski & Diamond (1994) reported no correlation between the residuals of RMR and daily energy intake (DEI) in the laboratory mouse. Meerlo *et al.* (1997) stated that the daily EE could be sub-maximal and liable to large fluctuations, and that this could also be true of DEI. Therefore the lack of a correlation between RMR and performance may be due to daily EE, or DEI, not being a maximal or sustained energy metabolism.

The mean metabolic scope was significantly higher for the broiler genotype (2.5) compared to the layer genotype (2.3). These were consistent with reviews by Peterson *et al.* (1990), Weiner (1992) and Suarez (1996), who stated that the metabolic scope of an animal could only range between 1.5 and 5, and the actual value of this factor was species specific. Bech *et al.* (1991) also showed a low MS (1.6 times RMR) in newly hatched Antarctic petrel (*Thalassoca antarctica*) when compromised by a low thermal environment. This low value was possibly due to the chicks requiring parental brooding to maintain body temperature whilst they were in the process of growing and developing. Bech *et al.* (1991) concluded that the ability of these birds to breed in a cold environment does not necessarily imply a great thermogenic capacity of newly hatched chicks. The MS shown in the current study is fairly low compared to other studies (Table 2.11), perhaps because the

Table 2.11 Summary of metabolic scopes in various mammal and bird species taken from the literature.

Species	[1]MS	Metabolic Load	Source
Mammals			
Peromyscus maniculatus	15.4	Winter season and low altitude	Hayes (1989)
Peromyscus maniculatus	8.2	Summer season and high altitude	Hayes (1989)
Peromyscus maniculatus	7.7	Cold exposure	Koteja (1996b)
Notomys cervinus	6.1	Cold exposure	Dawson & Dawson (1982)
Pseudomys gracilicaudatus	3.8	Cold exposure	Dawson & Dawson (1982)
Mus musculus	7.2	Peak lactation	Hammond & Diamond (1992)
Mus musculus	4.7	Cold exposure	Konarzewski & Diamond (1994)
Mus domesticus	6.4	Exercise	Hayes *et al.* (1992)
Microtus agrestis	2.9	DEE	Meerlo *et al.* (1997)
Apodemus flavicollis	3.7	Cold exposure	Koteja (1995)
Marsuipials			
Planigale gilesi	8.2	Cold exposure	Dawson & Dawson (1982)
Dasyuroides byrnei	8.8	Cold exposure	Dawson & Dawson (1982)
Birds			
Carpodacus mexicanus	6.3	Winter season	O'Connor (1995)
Carpodacus mexicanus	4.8	Late spring season	O'Connor (1995)
Phalacrocorax aristotelis	1.6	Cold exposure	Bech & Østnes (1999)
Calidris canutus	1.5	Cold exposure	Piersma *et al.* (1995)
Agelaius phoeniceus	2.3	Cold exposure	Olson (1992)
Passer domesticus	5.2	Cold exposure	Koteja (1986)
Thalassoica antarctica	1.6	Cold exposure	Bech *et al.* (1991)

Where; [1]Metabolic scope was calculated; from sustained metabolic rate, peak metabolic rate, or daily energy expenditure divided by resting or basal metabolic rate.

measurements were made in chicks and not adults, i.e. the growth of the chick used a large proportion of energy. The larger metabolic scope found in the broilers of this study could again be attributed to their greater muscle mass and their ability to increase their PMR when required.

Metabolic scope is species specific and can vary widely (Table 2.11). Metabolic scopes tend to be slightly larger in mammals, both eutherian and marsupial, compared to bird species. Most of the mammals that have been previously studied are from the order rodentia. The species within this order and certain other mammals possess a unique thermogenic capacity produced by BAT. Brown adipose tissue is generally deposited in the neck or between the shoulders of the animal. It contains large amounts of mitochondria. These mitochondria are required for the oxidation of fat, therefore a large number of mitochondria increases the potential oxidation of fat to produce heat (Eckert *et al.* 1988). It has been reported that the mass of BAT, which is the principle site of nonshivering thermogenesis (NST), increases when an animal is exposed to cold to allow increased NST (McDevitt & Speakman, 1994b). Bird species do not possess BAT and this could explain why their MS are slightly lower than the mammalian species because they are relying on only muscle to generate heat, rather than both BAT and muscle for ST and NST in mammals (Table 2.11).

2.5.2 Organ Development Within Different Genotypes

Muscle mass, both pectoral and leg, and the water content or level of maturation of these tissues were greater in the FB compared to the L genotype of chicken. The exception was in chickens above 80 g, where the leg muscle mass was indistinguishable between genotypes and there was no difference in the water content of this muscle. The mass and level of maturation of the muscles were significant in determining how much heat was produced when the bird was challenged with cold. In this study, the FB chickens had greater PMR's compared to those of the L genotype, which was despite the lack of maturation of the FB chickens muscles. However, the results in the present study suggest that even if the their muscle was

less mature and produced relatively less heat per unit mass, this effect was offset by their overall higher muscle mass.

The organ mass results in this study were examined in three ways, firstly corrected BM was used as the covariate in the model, secondly due to the possibility of fat content confounding the results corrected LBM was used as a covariate. Thirdly the results of the dry organ masses using corrected DM (the mass of the whole body dried) as the covariate were examined.

Several components of the GIT were examined and these varied in their relation to genetic selection. The effect of genotype on the mass of the sections of the GIT was not consistent between the different genotypes. For example, the intestine mass was larger in the FB genotype, but this was reversed for the gizzard mass. The gizzard mass was larger in the L chickens below 80 g and a genotype difference was not evident in birds above 80 g. These were the results no matter which covariate was used, except for the analysis of gizzard mass in birds using LBM-OM as a covariate. The results of the caeca mass were very variable depending on which covariate was used. In birds below 80 g the caeca mass was larger in the FB chickens compared to the L chickens when BM-OM or LBM-OM were used as the covariate, but when BM-OM was replaced with DM-OM the predictive model was unable to pick out the genotype difference. In birds above 80g no significant genotype difference was shown when BM-OM or LBM-OM were used as the covariate, but when this was replaced with DM-OM, the FB chickens had larger caeca mass than either the SB or L chickens.

The selection for fast growth in many genotypes of poultry typically results in a marked increase in the relative size of digestive organs (Jackson & Diamond 1996; Mahgna & Nir, 1996). The results of the present study are in partial agreement with this, since the faster growing genotype of chicken (FB) had a larger intestine mass than the slower growing genotypes (SB or L). Although mass is not a measure of the capacity of an organ, it would be fair to assume that a large organ would be more likely to have a large capacity. Therefore, the larger intestine mass shown in this study could indicate a larger capacity to absorb nutrients from the digestive tract.

However, some studies have contradicted this, reporting that faster growing genotypes of chicken have relatively smaller GIT organs than their slower growing counterparts (Mitchell & Smith 1991; Boa-Amponsem *et al.* 1991). The difference in these results cannot simply be attributed to the difference in genotype but may also be due in part to the different methods of statistical analysis used on the morphology data. (See section 3.3.2).

The smaller gizzard mass observed in the FB genotype in the current study could possibly be due to the improved nutritional value of the feed that is now used for broilers. These results are consistent with that reported by Nitsan *et al.* (1991) and Plavnik & Hurwitz (1982) who showed that relative gizzard mass was consistently larger in layer chickens when compared to a broiler genotype. The results of the present study indicate that the size of the caeca may not be limiting for the chicken, since no clear effect of genotype was evident. This is despite improvements in the nutritive quality of feed, including the use of endogenous enzymes and pro-biotics, which suggest that smaller caeca could be just as effective.

The results for the other supply organs denote that the brain was larger in the L genotype, except when LBM-OM was used as a covariate in birds below 80 g and that there was no difference in liver mass within the different genotypes of chicken. The lack of genotype difference for the liver and the larger brain mass in the L genotype was similar to the findings of Jackson & Diamond (1996). The lack of increase in liver mass is somewhat surprising in view of the fact that the intestine mass increased in the FB genotype. Since a larger intestine would be expected to absorb more nutrients, then one would expect a larger liver would also be required to deal with the by-products and metabolism of increased nutrients processed by the larger intestine. An explanation as to why the genotypes had a similar relative liver mass could be a response to the higher protein and energy content of the broiler type diet that was being fed to them. Jackson & Diamond (1996) also found no difference in heart mass when they compared a broiler chicken to its ancestor the Red Jungle Fowl. However, the effect of genotype on heart mass in the present study appears to be very dependent on the covariate used in the analysis, making it very difficult to clearly identify which genotype had a larger heart mass. When LBM-OM was used

as a covariate there was no difference between the genotypes, but when DM-OM was used the L genotype had a larger heart (birds below 80 g) or there was no difference between the genotypes (birds above 80 g). Since the heart is a very muscular organ and the water content of the organ could have been confounding the results, the dry organ mass appears to be a good indication of the actual heart mass. This lack of an effect of genotype on relative dry heart mass indicates the future limitations of the cardio-pulmonary system. Since it is the cardio-pulmonary system that is the starting point of most metabolic disorders, ascites and SDS.

2.5.3 Relationship Between Metabolic Rate and Organ Morphology

Most supply organs are key metabolic organs, they are not increasing in mass in line with increasing BM, and therefore there is a lower energy cost to maintaining these tissues. This may explain why RMR was lower than expected in the broiler genotype, when BM was used as a covariate. Since the broiler industry use lean tissue mass as a selection criteria, it would therefore be more accurate to use LBM as a covariate in the analysis. When LBM was used the FB genotype had a larger RMR compared to the L genotype probably due to the larger lean tissue mass in the FB genotype.

Konarzewski & Diamond (1994) reported significant correlations between RMR residuals and the residuals of kidney and heart mass in the laboratory mouse, when lean fresh body mass was used as an independent variable in the regression model. These findings were supported by Meerlo *et al.* (1997) who also showed a significant correlation between the residuals of RMR and residuals of dry heart mass relative to LBM, but not with dry kidney mass relative to LBM. The results from the present study indicate that correlations did exist between both RMR and PMR and some organ masses. Heart mass correlated well with MR, as did other organs. Heart mass was significantly correlated to RMR in the FB chickens below 80 g and the SB chickens above 80 g, when BM-OM was used as the independent variable, and in the L chickens below 80 g and in the FB and SB chickens above 80g, when LBM-OM was used as the variable. It appeared from the correlation results that the use of BM-

OM as the independent variable was masking some of results and confounding the interpretation of those results. Therefore only the correlations produced using LBM-OM as the independent variable were compared.

There were significant correlations between RMR and other organ masses in chickens with a BM lower than 80 g. The leg muscle and liver mass were significantly, positively correlated with RMR in the pooled data set. The pectoral muscle within the FB chickens and leg muscle within the L chickens were both significantly, positively correlated with RMR. The L genotype also showed a significant, negative correlation with caeca mass. These results imply that an increase in RMR would cause an increase in pectoral muscle mass in the FB genotype and an increase in leg muscle mass and a decrease in caeca mass in the L genotype. Therefore the continued genetic selection for increased pectoral muscle mass in the modern broiler will also cause an increase in the chickens RMR and therefore higher energy costs.

In the chickens weighing more than 80 g there was a significant, positive correlation between RMR and liver mass and a negative correlation with brain mass in the pooled data set. There were significant, positive correlations between RMR and leg muscle within the SB and L chickens and between RMR and heart mass within the FB and SB chickens. The results of the heavier chickens imply that an increase in RMR would cause a reciprocal increase in leg muscle in the SB and L chickens and an increase in heart mass of the FB and SB chickens. The broiler industry are continually trying to keep its energy costs down, therefore they may select birds with lower energy costs and therefore lower RMR, in turn this may, potentially be selecting for modern broilers with a lower heart mass.

There were significant, positive correlations between PMR and leg muscle, caeca and liver mass and a negative correlation with gizzard mass in the pooled data set. The leg muscle, intestine and heart masses were all significantly, positively correlationed with PMR in the L genotype. These results imply that as PMR increases so does the leg muscle, intestines and heart masses of the L genotype. There were no correlations between PMR and organ masses evident in the FB

genotype.

From these residual correlations it is evident that a number of organs, both support and demand tissues, were good predictors for RMR and PMR, but unfortunately none of these results appeared consistently within individual genotypes as well as the pooled data set. The relation between BMR or RMR, and the size of metabolically active organs such as the heart occur in mammals as well as birds, both between and within species (Meerlo *et al.*, 1997). As previously stated, the FB chickens were able to achieve a higher PMR than the L genotype even though their heart mass was in fact smaller when the effects of BM-OM were removed. Although this result is reversed when fat was removed from BM, where a greater heart mass in the FB chickens compared to the L chickens was evident. Also, the heart mass correlated with both RMR and PMR, but only within individual genotypes and not within the pooled data set. These results indicate that although the heart was smaller, when the effects of BM were removed, its ability to deliver oxygen to the other tissues has not been impaired during the selection for fast growth rate in the modern broiler, since a larger PMR was achieved.

2.5.4 Carcass Chemical Composition Within the Whole Chicken

The fat content (g) of the chickens below 80 g was greater in the FB genotype compared to the other two genotypes, whilst in birds greater than 80 g the SB chickens had more fat than the other two genotypes. The ash content (g) was greater in the L chickens and there was a significant effect of genotype shown in the CP content (g) of the chickens above 80 g, with the FB and L genotypes having a larger CP content compared to the SB genotype. Due to the larger muscle mass, specifically the pectoral muscle, that it possesses one would expect the FB genotype to have a larger CP content, since one of the main selection criteria for broilers is for an increase in breast muscle yield, which is also a main source of protein within the bird. It may be possible that this selection for increased breast meat yield that has occurred may not have increased the protein content of this muscle but instead it may simply be an increase in water content that has increased the pectoral muscle mass.

Also the fat content was shown to be lower in the FB genotype when compared to the SB genotype in the heavier range of chickens. Again this is consistent with the selection criteria used in the broiler breeding industry, that is, the selection for leaner chickens. The difference in ash content between the L and FB genotypess indicates that the selection criteria used for broilers has indirectly reduced the ash content of these birds. It is unlikely that ash content has increased in the layer genotype through genetic selection, due to the high incidence of osteoporosis in laying hens. Osteoporosis is defined as a decrease in the amount of structural bone, the matrix which is normally mineralised, resulting in bone fragility (Bishop *et al.*, 2000; Cransberg *et al.*, 2001). The lower ash content of the FB chickens observed in the present study would imply there was a reduced mineral content of their skeleton. This result may help to explain the increased occurrence of leg disorders in the modern broiler.

None of the carcass chemical components correlated well with either RMR or PMR, at either weight range, except between RMR and CP in the pooled data and the FB genotype in birds below 80 g. This indicates a lack of relationship between metabolic rates and the fat and ash content of the chicken carcass, whereas CP appears to be a good predictor of RMR. This result was as expected because of the relationship between PMR and muscle mass and the link to ST previously described. It is not surprising that CP correlates well with RMR since a large proportion of CP is made up from the muscle mass, and there would be a higher energy cost to maintaining this large muscle mass.

2.5.5 Metabolic Ceilings-Where is the Limitation in the Chicken?

When a metabolic load is placed on an animal, for example the artificial selection for increased growth rate, the animal must respond by increasing the digestive and absorption capacity of its GIT to increase the quantity of nutrients required to cope with this load. This then would cause an increase in the heart capacity it would increase its cardiac output in order to deliver more nutrients and oxygen to the peripheral tissues (Konarzewski & Diamond, 1994). In addition there would be an

increase in the capacity of the liver to breakdown and metabolise nutrients into usable products. There would also be an increase in muscle production from the protein metabolised by the liver for the animal to increase its growth potential. If any of these points in the system fail to increase in line with the demands placed on them, then the metabolic load may have caused a ceiling to have been reached.

The present study observed differences between the broiler chicken that have been artificially selected for fast growth rate and those which had not, specifically when the birds were placed under a second short-term thermal load, in the form of cold exposure. The FB chickens were able to increase their maximal metabolic output more than the L chickens to cope with this energetic load. The birds reached a PMR fairly rapidly and only produced a MS of 2.5 and 2.3 times RMR. Since the FB birds could not manage to produce a further increase in their metabolic output there must have been a limitation within one of the animal's systems.

Four hypotheses have been proposed for why this limitation may occur. The first hypothesis proposed that any limitation in ability to perform was due to a lack of food availability. This hypothesis is more relevant and more likely to occur in animals in a wild population as opposed to laboratory animals or livestock, which are generally fed *ad libitum*. The birds in the present study had full and constant access to feed during the trial except when they were placed into the metabolic chamber. Therefore, this hypothesis does not apply to this study.

The second hypothesis states that symmorphosis exists. Symmorphosis is maintained when there is parallel growth between the support and demand tissues within an animal (Taylor & Weibel, 1981; Weibel, *et al.*, 1991; Dudley & Gans, 1991). The results of the present study indicate that there was a breakdown in symmorphosis, because although the muscle was larger in the broiler genotype not all of the support organs increased in parallel to this. For example the brain, gizzard, liver and heart were not shown to be larger in the FB chickens when compared to the other two genotypes. Due to these key metabolic organs not increasing in parallel with the growth of the broiler genotype, it can be concluded that a breakdown in symmorphosis has occurred, therefore this hypothesis has not been supported by this

study.

The third hypothesis proposed that a peripheral limitation occurs within the animal. This hypothesis assumes that the support organs can supply the energy but the demand organs cannot convert the energy to work (Else & Hulbert, 1985; McDevitt & Speakman, 1994a, b). If the peripheral limitation had occurred one would have expected very little difference between the RMR and PMR results and PMR would not vary between the different genotypes of chicken, because the production of heat would be limited by the muscles mass. As can be seen from the results this is not the case, there was a significant difference in PMR between the FB and L genotypes of chicken, therefore it is unlikely that the peripheral limitation hypothesis has not been supported by this study.

The fourth hypothesis proposed that a central limitation might exist within the animal. This hypothesis assumes that muscles could work faster or grow more or grow more quickly if only the rest of the body, i.e. the support organs, could supply nutrients and oxygen, and remove wastes faster (Konarzewski *et al.*, 1989; Weiner, 1992; Hammond & Diamond, 1992; McDevitt & Speakman, 1994a, b; Koteja, 1996b, c). In the present study there were differences between genotypes in the support organ masses. For example, the intestine was larger in the FB genotype, but the gizzard, brain, heart and liver were either smaller in the FB chickens or not significantly different between the genotypes. Due to the gizzard, brain, heart and liver clearly not having increased in mass along with the increase in muscle mass during the genetic selection process of the broiler chicken, the central limitation hypothesis appears to have been supported by this study.

2.5.6 Summary

There has been intensive genetic selection of broiler chickens for increased breast muscle as well as increased growth rate, decreased fat and a decrease in eviscera mass (Pollock, 1997; Le Bihan-Duval *et al.*, 1998). Consequently, the broiler chicken is a larger bird than its slower growing counterpart, the layer. However

there was not a corresponding increase in the FB's RMR, as was expected. However, it did have a larger PMR and consequently a larger MS compared to the layer genotype. This larger PMR could be attributed to the greater relative muscle mass of the broiler, which is used primarily for heat production during ST. Due to the broilers ability to increase its MR when a cold-induced metabolic load was placed on it, implies that the supply tissues were unable to increase their activity to cope with this energetic load. Therefore this suggested that it was the supply organs that were the limiting factor i.e. the central limitation hypothesis.

This genetic selection has also had a profound effect on the chicken's organ morphology in the first two weeks of life, specifically the selected broiler has sacrificed the size of its brain and gizzard and its overall ash content to enable the increase in muscle mass. This confirms that a breakdown in symmorphosis has occurred. The continuing genetic selection of the broiler chicken will only increase this breakdown in symmorphosis leading to an increased susceptibility to metabolic disorders and potentially future improved performance.

CHAPTER 3

3. ORGAN MORPHOLOGY AND CARCASS PERFORMANCE IN THREE GENOTYPES OF CHICKEN

3.1 Introduction

In the past five decades there has been powerful selection for rapid growth, low feed conversion and greater meat yield in the broiler industry, producing a chicken that grows twice as fast as its 1950's predecessor. Thus, the time taken by the bird to reach a slaughter weight of 2kg has been halved over the last 40 years (Gyles, 1989). According to Havenstein (1994 a,b), genetic selection accounts for approximately 80% of this increased performance of modern broilers and nutrition only accounts for 20%. Selection for high body mass is accomplished by elevating the level of, and prolonging the length of, the period of maximal growth early in development (Ricklefs, 1985; Nitsan *et al.*, 1991). An unforeseen consequence of this intensive genetic selection for improved performance in broilers has been the association with growing mortality due to metabolic disorders (Groves, 1997). These metabolic disorders, such as ascites, SDS and leg disorders have had a very important impact, both on bird welfare and the economy of the poultry industry. Before 1980 these conditions tended to occur largely as a result of gross nutritional disorders or because of extremes of the rearing environment, like high altitude. In addition, as non-infectious diseases, some have underlying morphological defects that give rise to the clinical symptoms of the syndrome in question, as seen in ascites (Nicholson, 1998). These morphological defects will in turn lead to an increased incidence of metabolic disorders that may in turn represent a constraint for further improvements in performance in the future.

The growth of the broiler chicken has been widely investigated and some studies have concentrated on the development of the organs that supply nutrients or oxygen

to the body, such as the GIT or the cardio-pulmonary system (Katanbaf *et al.*, 1988b; Nitsan *et al.*, 1991; Nir *et al.*, 1993). The GIT has been implicated as a limiting factor in both food intake and subsequent growth in broiler chickens (Mahagna & Nir, 1996). Plavnik & Hurwitz (1982) suggested that the heart and liver generally remain a constant proportion of BM when comparing a fast growing broiler and a slower growing Leghorn cross. Initially, the intestine was heavier in the faster growing genotype but at 5 weeks of age this trend was reversed and the intestine was lower in the fast growing birds. Dunnington & Siegel (1995) found similar results for heart and liver mass when comparing broilers with high and low 8-week body mass. However, these responses have not been elicited consistently by other researchers. Nitsan *et al.* (1991) observed no differences in heart and liver weights relative to BM between three genotypes that differed greatly in body weight, but found a significant effect of genotype in the relative masses of all the different components of the GIT. A key factor which may contribute to the lack of consistency between comparative studies is the inappropriate statistical analysis of morphology data that is commonly used (Katanbaf *et al.*, 1988b; Nitsan *et al.*, 1991; Mitchell & Smith, 1991; Mahagna & Nir, 1996). Organ masses are often expressed in relative terms either as a proportion or a percentage of BM. The use of such ratios is inappropriate as they fail to eliminate the contribution of the organ masses themselves to BM, and may thus invalidate statistical analysis (Poehlman & Toth, 1995; Packard & Boardman, 1999). Since most physiological and life-history traits do not scale as a fixed proportion of BM, it is more appropriate for this mass dependence to be taken into account when making genotype comparisons by using either multiple regression or ANCOVA (Christians, 1999).

The balance in growth and capacity between the different systems of an animal's body has been described as the process of "symmorphosis" (Weibel & Taylor, 1981; Weibel, *et al.*, 1991). This effectively describes how, under normal conditions, the masses of supply tissues, such as the heart, gut or liver, increase in proportion to the masses of demand tissues, like skeletal muscle. If, however, there is no increase in the mass of supply organs in parallel with an increase in the mass of demand organs, then a breakdown in symmorphosis has occurred. It may be possible that such a breakdown in symmorphosis may be a contributing factor to the development of

metabolic disorders in the modern broiler.

The objective of the present study was to investigate if there has been a breakdown in symmorphosis in the modern broiler chicken and whether this represents a potential limit to improvements in performance in the future. A lack of balance between demand and supply tissues may also represent a potential limit to future increases in muscle yield. In order to quantify this, the post-hatch development of a range of key support organs and muscle in three genotypes of chicken, which have undergone different rates of genetic selection for growth rate, were compared over a relatively long timespan.

3.2 Materials and Methods

A total of 600 newly hatched male and female chicks from three genotypes of chicken (*Gallus gallus domesticus*) were compared. These genotypes had been subjected to differential rates of selection for fast growth rate, FCR and meat yield and comprised of firstly, a modern commercial broiler (Ross 308, FB, n=100). The second genotype was, a broiler that had been selected for fast growth rate until 1972 at which point selection was discontinued, a relaxed selected broiler (Ross 1972, SB, n=100). Thirdly, a layer chicken which has not been selected for fast growth rate, but has been selected for lighter BM and reproductive performance criteria (Euribrid HISEX, L, n=400). Each of these genotypes was reared in sequential order at different times of the year, the FB for 6 weeks, then the SB for 8 weeks and then the L for 16 weeks. The chicks were individually wing-tagged and reared as separated genotypes and sexes in pens on wood-shavings litter at a stocking density not exceeding 34 kg/m^2 for broilers and 17 kg/m^2 for layers as stated in the Farm Animal Welfare Council's recommendations (FAWC, 1992; FAWC, 1997). Each genotype was grown following the breeding company recommendations for environmental temperature and lighting regimen for that genotype. The photoperiods used were 23L:1D for the two broiler genotypes and 8L:16D for the replacement pullets.

All three genotypes were fed a standard current commercial broiler ration (22.8-19.8 % protein and 12.7-13.5 MJ/kg energy content). In order to ensure that any inter-genotype differences in morphology were not simply due to differences in the energy and protein contents of the diet, the layer chickens were split into two groups and fed two different rations. One group was fed the standard broiler ration and the second group was fed commercial replacement pullet ration (20.5-16.4 % protein and 11.5-11.8 MJ/kg energy content) (Table 3.1). Water and food were available *ad libitum.* For full dietary specifications refer to Appendix 3.

Table 3.1 The duration of each phase of feeding programme (days) used for each genotype.

Genotype	Starter ration	Grower ration	Finisher ration
[1]FB	0-7	7-21	21-42
[2]SB	0-21	21-35	35-56
[3]L	0-35	35-77	77-112

Where; [1]fast broiler, [2] slow broiler and [3] layer

Each genotype was grown to just beyond the point of maximum growth rate, namely at 6, 8 and 16 weeks for the FB, SB and L genotypes respectively. This maximum growth rate was determined by weekly weighing of all the birds. Throughout this time, a random sub-sample (n = 4 to 8) of chicks from each genotype were removed at weekly intervals for serial morphological measurements (FB, n=51, SB, n=64 and L, n=144). The number of birds removed per treatment was limited to the number of birds that could be processed each dissection day throughout the experimental period. Birds were killed by cervical dislocation and weighed to the nearest 0.1 g. The sex of the bird was confirmed by gonadal inspection. The wet masses of the gastrointestinal tract (gizzard, intestine and caeca) heart, liver, brain, lungs, pectoral muscle (*Pectoralis major, Pectoralis minor* and *supracoracoideus*) and leg muscle (surrounding the femur and tibiotarsus) were taken. Any organs remaining were combined with the skeleton and feathers, and referred to as the carcass. All tissues were then dried to a constant mass in an oven at 70 °C. The dry organ masses were added to the dry carcass value to obtain the total dry mass of the whole bird. The

water content of muscle was calculated as [wet mass - dry mass]. The masses of pectoral and leg muscles were combined to give total muscle mass, and the masses of all the individual organs were combined to give total organ mass, the ratio between these two is described by M:O.

When the morphometric measurements for all the body parts of each individual bird were finished, these tissues were re-combined and milled to a heterogenous mixture. The carcasses were then analysed in duplicate for ash, fat and for nitrogen from which was calculated the crude protein content. The proximate analysis methods used are outlined in Appendix 1.

3.3 Statistical Methodology

In this section the method of analysis of absolute data are described, the uses and problems encountered when analysing relative data are discussed, and finally, the models used in the analysis of relative data in the present study are described in full.

3.3.1 Analysis of Absolute Data

The data for absolute mass of tissues and organs are presented as uncorrected means ± standard error of the mean (SEM). The BM's were split into 500g intervals and the mean of each organ mass at each interval was calculated. The first BM interval (0-500 g) and the final BM interval (2000-2500 g) were analysed using one way analysis of variance (ANOVA), with organ mass being the response and genotype the factor in the analysis (Minitab 10.5 Xtra, Minitab Inc., Birmingham, UK), this analysis was repeated using sex as the factor. The genotypes and sexes which were statistically different were compared using the Tukey matrices produced from these analyses. Due to the data in these groups being very unbalanced and the variation in BM and age not being taken into account within the analyses, they are simply an indication of what relationships have occurred within the different organs.

3.3.2 The Analysis of Morphological Data and How to Control for Body Mass

Most researchers commonly compute percentages or size-specific indices in an attempt to remove the effects of BM from physiological data (Packard & Boardman, 1999). Unfortunately, such ratios seldom eliminate the influence of BM on a physiological response and these ratios can introduce major problems with respect to statistical analysis and interpretation of the data.

Table 3.2 Hypothetical data used to illustrate the use of analysis of covariance instead of ratios or percentages when analysing physiological data.

Group 1		Group 2	
Body Mass	Physiological variable	Body Mass	Physiological variable
245	80	345	170
250	80	355	170
260	100	360	195
270	90	370	185
290	130	395	220
300	120	405	210
310	140	420	230
320	140	430	230
330	160	450	250

Packard & Boardman (1999) described exactly how an error could occur if the wrong statistical analysis was performed. Packard & Boardman (1999) produced a hypothetical data set (Table 3.2) to show the how the results of analysis of physiological data can be confounded when ratios or percentages are used to remove the effect of BM instead of ANCOVA. The BM and a value of a physiological variable were produced for two groups of nine animals (Table 3.2). The data for the two groups were plotted against the measure of BM, with the group 2 animals being larger than the group 1 animals overall (Figure 3.1). The data were then analysed by

ANOVA without being adjusted for the effect of BM ($F_{1,16}$ = 45.03, P ≤ 0.001, Figure 3.2A). However, this difference may not be a true difference but actually due to the difference in body size.

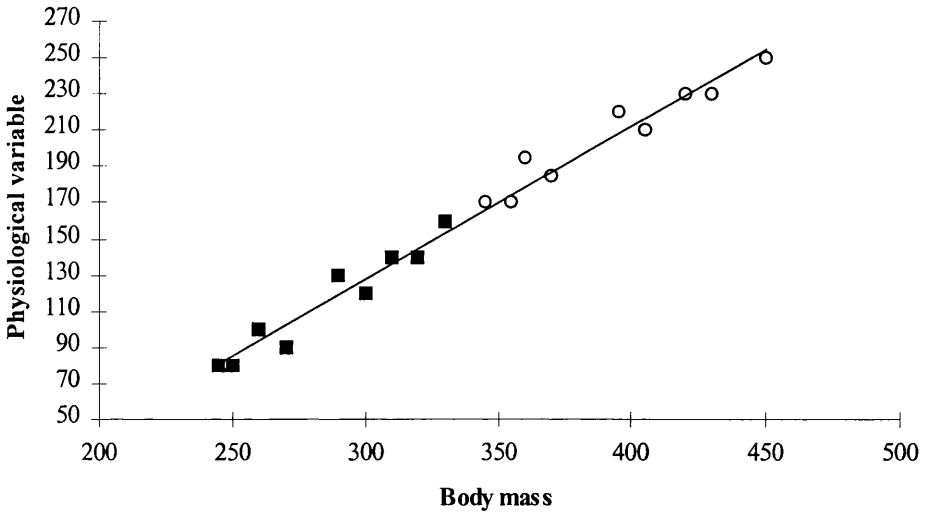


Figure 3.1 Hypothetical values for a physiological variable plotted against body mass in two groups of animals, group 1 (■) and group 2 (o).

When the physiological variable was made size-specific i.e. producing a ratio by dividing the physiological variable by BM, for each animal, these ratios were then analysed using ANOVA ($F_{1,16}$ =29.14, P ≤ 0.001, Figure 3.2B). Again a significant difference can be seen between the two groups. Therefore, the conclusion could be that there was a difference between these two groups even when BM was taken into account.

However, when an ANCOVA, with BM as the covariate, was performed on the data a different result was produced. In the ANCOVA the response was the physiological variable, the model was the group and the covariate was BM. The result ($F_{1,15}$ =0.37, P > 0.554, Figure 3.2C), indicated that when using the ANCOVA there no longer was a significant difference between the two groups. The reason for this is that the ratios did not correct fully for the variation in BM and this introduced a bias into the

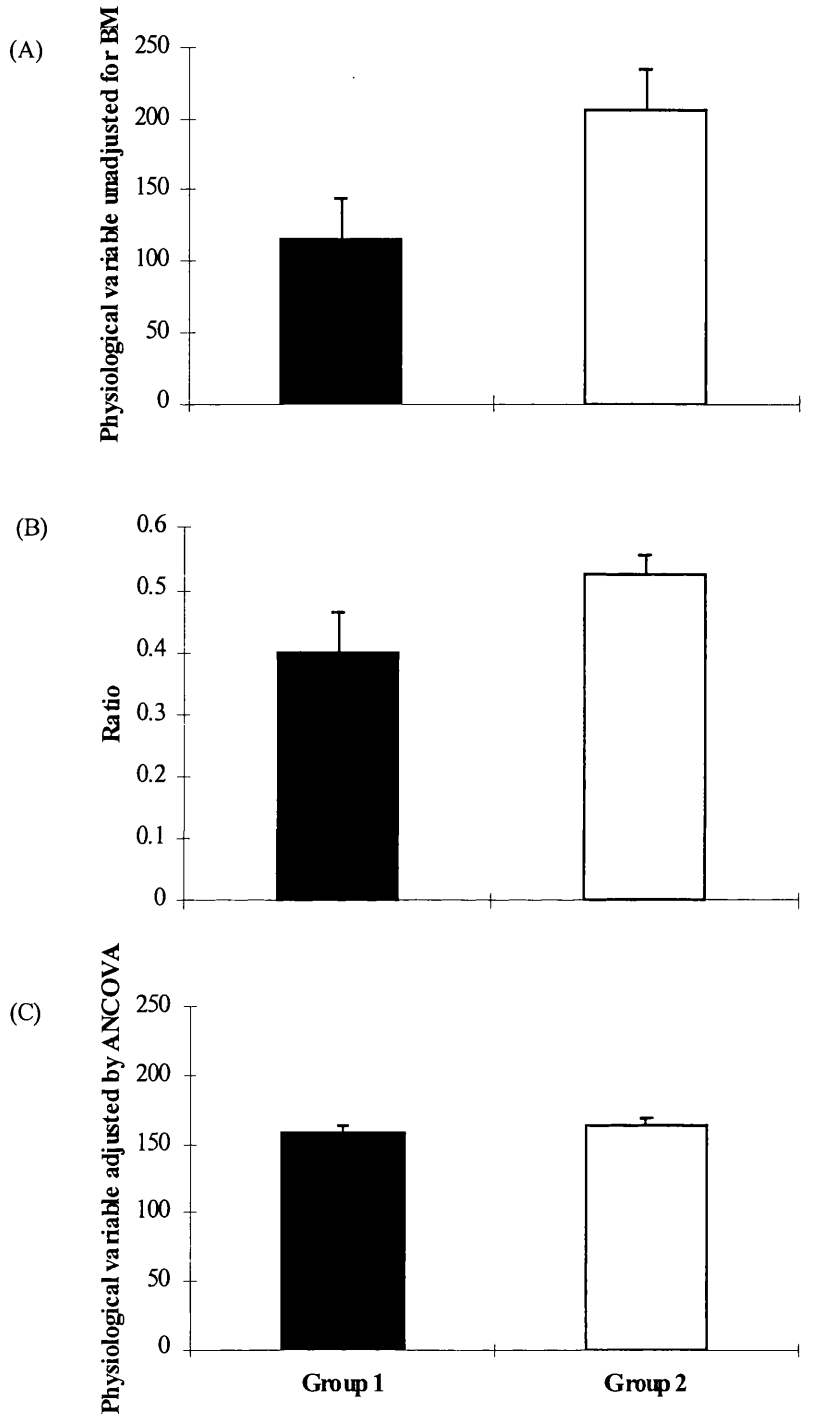


Figure 3.2 The (A) mean physiological variable (± SD) after analysis of variance, (B) mean ratio of the physiological variable to body mass after analysis of varaince and (C) mean measure of the physiological variable which has been adjusted for body mass using analysis of covariance, for group 1 (black) and group 2 (white).

data. The bias resulted in a statistical test which lead to an incorrect conclusion being reached (Packard & Boardman, 1999). Therefore, the correct way to analyse comparative data is to use ANCOVA, with corrected BM as the covariate.

3.3.3 The Analysis of Morphological Data in Relative Terms

The analysis of the morphological data from the present study presented a number of problematic issues that required careful consideration. Firstly, there was the fact that BM and age tended to be highly correlated and that the genotypes were grown to a similar end weight but different ages. Age and BM were strongly, positively correlated within each genotype, the r^2 values from regression analysis being 93.5%, 94.4% and 91.7% for FB, SB and L respectively. When the BM and growth rate data were plotted they were curvilinear or sigmoidal in appearance, so the data were then transformed using $\log_e$, so that all of the data became linear. Therefore, linear regressions were performed on the log BM and log growth rates with log age as the predictor variable for the full data set (Genstat 5, release 4.1 for windows (Lawes Agricultural Trust, Rothamsted Experimental Station)). The slopes created from these regressions were then analysed by a general linear model (GLM, Minitab 10.5 Xtra, Minitab Inc., Birmingham, UK) to test for the effect of genotype, sex and diet on BM and growth rates.

Secondly, since organ sizes in animals of different BM were being compared, BM had to be taken into account, and this traditionally has included generating relative organ masses. As stated previously expressing the organ size as a percentage of BM or as a ratio is inherently flawed as the mass of each individual organ contributes to the overall BM, so there is a very high inherent degree of correlation between organ mass and BM. Body mass was therefore corrected for the contribution of individual organ masses by subtracting the mass of each organ from BM in turn. Thus, the mass of an individual organ was always compared to BM adjusted for the mass of that organ. However, due to some of the relationships between organ mass and BM being curvilinear, the organ mass and BM minus organ mass (BM-OM) were then

transformed using a natural logarithmic scale for ANCOVA analyses (Genstat 5). Due to the possibility of fat or water content confounding the results lean body mass (LBM-OM) was used as a covariate for wet organ mass data, LBM was generated when BM was corrected for fat content. The dry mass of the organ was subtracted from the total dry weight of the bird (DM-OM) for dry organ mass data before each computation (Christians, 1999). The statistical model used to test the effect of BM-OM, age, genotype, sex and their interactions on organ mass was:

$$Y = g_i + s_j + gs_{ij} + b_iBM + c_jBM + bc_{ij}BM + d_iAge + f_jAge + df_{ij}Age + e$$

Where Y = the response variable, g_i = the intercept for genotype i, s_j = the intercept for sex j, gs_{ij} = the intercept for genotype i by sex j interaction, b_i = slope for genotype i with BM, c_j = slope for sex j with BM, bc_{ij} = slope for genotype i by sex j interaction with BM, d_i = slope for genotype i with age, f_j = slope for sex j with age, df_{ij} = slope for genotype i by sex j interaction with age, e = residual error. For full Genstat programme see Appendix 2.

The same ANCOVA model was also used to test for the effect of; genotype, sex, BM and their interactions on the total muscle mass, total organ mass and M:O with BM corrected for total muscle mass, total organ mass and carcass mass respectively. Again due to the relationship being curvilinear the values were log-transformed to the natural log before analyses were performed. The model was also used to examine the level of hydration of the total muscle using BM corrected for total muscle. The hydration values were also logged transformed prior to any analyses, due to the relationship being curvilinear.

Birds from the L genotype had been fed one of two different diets, either a layer or broiler type diet. Therefore, the effect of diet was tested on these data. The same ANCOVA model was therefore used to analyse for the effect of diet in the L genotype by replacing genotype with diet in the model. There was no significant difference in growth rate between the two groups of L birds, but there were some differences in the individual organ masses between the groups of L chickens fed the two dietary regimens. Therefore, the L chickens that had been fed a layer diet were

removed from the subsequent analysis with the FB and SB data. The data presented are means ± SEM. Unless stated otherwise means that differed at the 5% level were considered statistical significant.

Where significant interactions were revealed in the ANCOVA model, which occurred in all the ANCOVA analyses, another method of predicting the mass of the organ was used. A stepwise regression model was used to test for the effect of BM-OM, age, genotype, sex and their interactions on the mass of each organ.

The significant terms generated from the stepwise regression were then entered into a prediction model in Genstat 5. The model allows the respective organ mass to be predicted for each genotype at different BM, whilst any significant variates, factors or interactions from the stepwise model are taken into account.

When sex was a significant factor in the ANCOVA model, the predictive model was manipulated to predict for both genotype and sex.

The predicted organ masses that were then generated by this model were the logged values, these values were then back transformed using the exponential of the log and were described graphically to indicate how the genotypes and sexes differed from one another.

3.3.4 Statistical Problem

Since most of the data had a curvilinear relationship all the data was transformed, using the natural log, before any analysis was undertaken. The log transformation made the data more linear and therefore easier to analyse and apply the statistical models. An exception was the pectoral muscle mass, for which, even after the log transformation, there was not a linear relationship (Figure 3.3A).

When the full set of data was analysed using the statistical models as previously described, a significant effect of genotype was produced with the L genotype having

larger pectoral muscle mass compared to either of the broiler genotypes. This result was unlikely to be correct due to the current selection procedures used by geneticists for increased pectoral muscle mass in the broiler chicken.

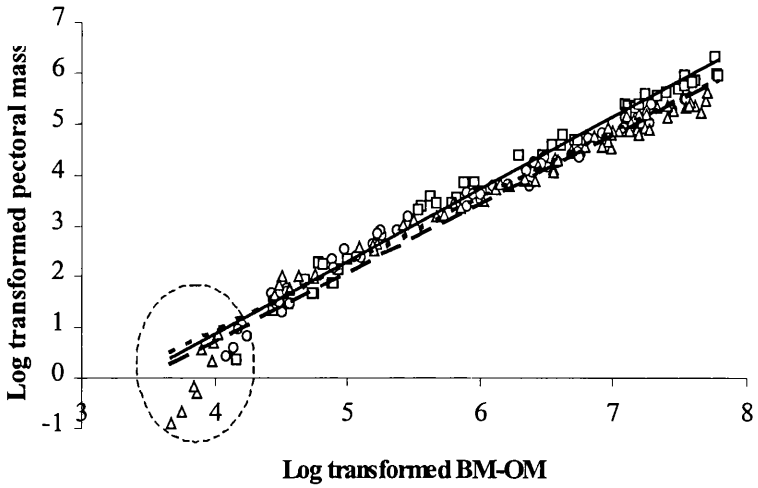
An attempt was made to clarify why the pectoral muscle mass data gave this unexpected result. The relationship between log pectoral mass and log BM-OM indicated a small cluster of data points at the lower BM's which could have a strong weighting on the statistical results (Figure 3.3A).

To account for this cluster of data points the total data set was analysed using a curvilinear model rather than the linear ANCOVA model. Unfortunately there were many limitations within the expontential curve model. Primarily only one explanatory variable and one factor could be used in the analysis, and as shown in the previously described ANCOVA model there are two variables (BM-OM and age), two factors (genotype and sex) and the interactions of all of these variables and factors. Therefore it was concluded that using this analysis was not appropriate.

Another potential way of reducing the curvature of the data caused by this cluster of data points at low BM was to use the square of the log transformed BM-OM as another variable in the ANCOVA model. There were three problems with this analysis, firstly, the data which is being transformed using the square has already been log transformed. By transforming the data then transforming again one appears to be drifting away from the real data. Secondly, the graphical output from the squared data compared to the unsquared data were not disimilar and the curvature had not been significantly improved. Thirdly, the output produced from the ANCOVA model with added square variable was extremely difficult to interpret.

The final analysis of this data set involved the removal of the cluster of data (values below 4.4 log transformed BM-OM (Figure 3.3B)) and to repeat the ANCOVA and predictive models that were used on all the other data sets. This analysis although was not ideal, i.e. removing real, valid data from the data set, appeared to be the most realistic approach and comparable way of dealing with the problem within this specific data set.

(A)



(B)

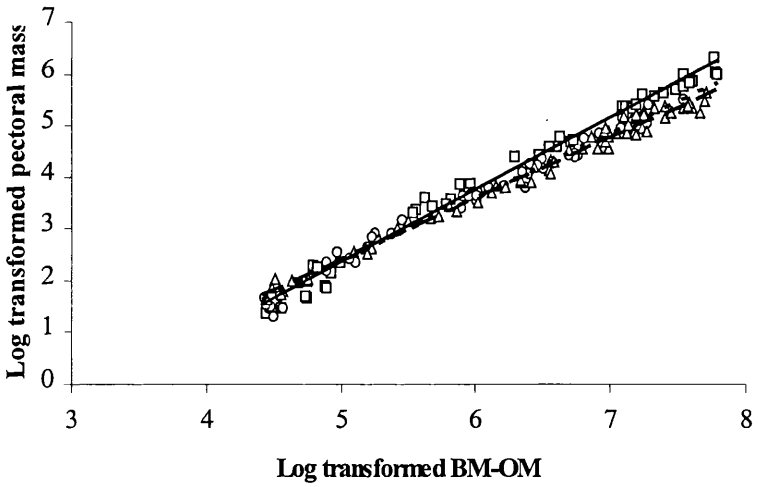


Figure 3.3 Log transformed pectoral muscle mass with (A) the full data set and (B) with data removed in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ). The data points, which were removed due to their weighting effect, are indicated on figure 3.3A.

3.4 Results

3.4.1 Body Mass and Growth Rate

All three genotypes of chicken were grown until growth rate (g/week) reached a peak. As expected, this varied with genotype and was approximately; 700 g/week by day 35 for FB, 275 g/week by day 49 for SB and was around 175 g/week by day 63 for the L genotype (Figure 3.4B). The mean BM at the end point of the studies was highest in the FB compared to SB or L genotypes (Figure 3.4A and Table 3.3). The FB genotype also had the largest BM when the genotypes were compared at 42 days of age and the SB had a larger BM at 56 days of age compared to the L genotype (Table 3.3). There was a significant effect of genotype ($F_{2,590} = 278.85$, $P \leq 0.001$) and sex ($F_{1,590} = 30.51$, $P \leq 0.001$) on the slopes of the regressions of BM on age, but no genotype X sex interaction. Genotype ($F_{2,587} = 7.35$, $P \leq 0.001$) also had a significant effect on the slopes of the regressions of growth rate on age. There was no significant effect of sex on growth rate, nor was there a significant genotype X sex interaction (Figure 3.4B).

Table 3.3 Mean body mass (g) (± SEM) of three genotypes of chicken and both sexes within each genotype, at comparative ages and at the end point in each genotype.

	[1]FB		[2]SB		[3]L	
Age (days)	[4]F	[5]M	F	M	F	M
42	2346 (30.61)	2774 (65.45)	1078 (15.89)	1344 (20.95)	494.8 (3.740	614.8 (6.19)
56			1550 (19.06)	2034 (39.33)	780.6 (7.98)	1029 (9.65)
112					1544 (13.56)	2443 (23.16)

Where;[1]FB-fast broiler, [2]SB-slow broiler, [3]L-layer, [4]F-female and [5]M-male.

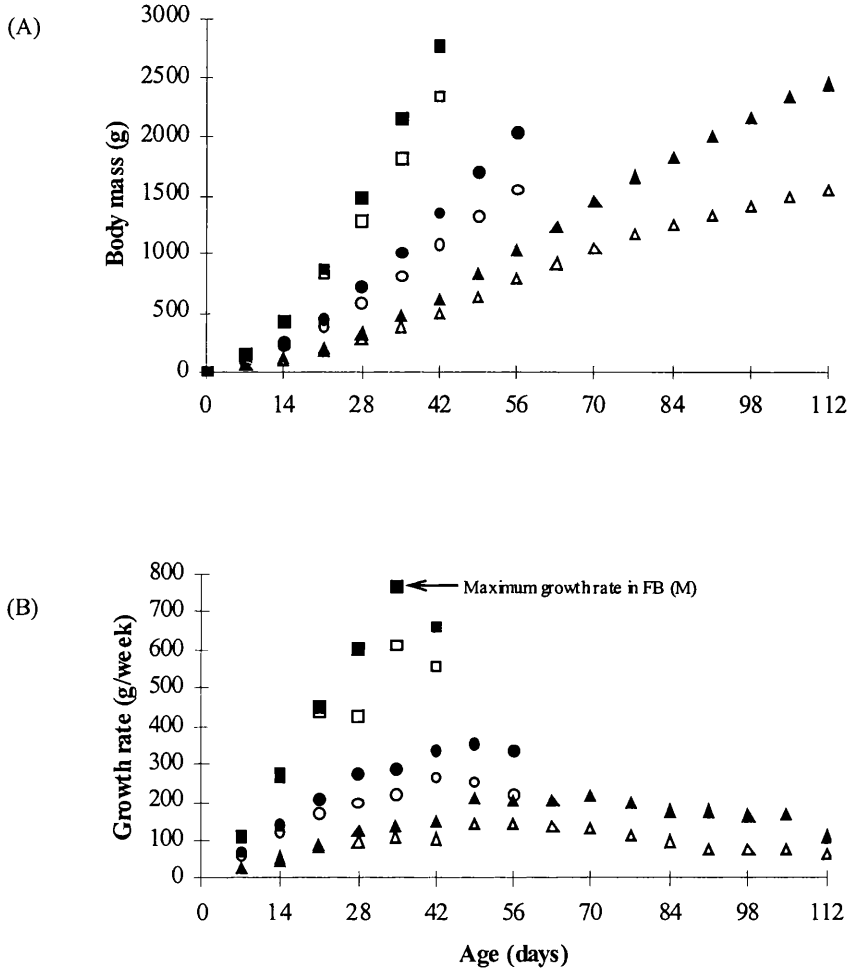


Figure 3.4 The relationship between age and (A) body mass and (B) growth rate in three genotypes of chicken; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ). The data points are mean (±SEM). The maximum growth rate for the male fast broilers is indicated on figure 3.4B.

3.4.2 Absolute Organ Masses

The absolute masses for all the different organs, the combined muscle and combined organs and M:O ratio were all larger in the male compared to the female within each genotype (Figure 3.5 and 3.6). For the graphs of all other muscle and individual organs refer to Appendix 4. These graphs also clearly demonstrate the difference in the growth rate of each organ between the three genotypes, with the FB genotype producing larger tissues much more rapidly than the other two genotypes, this is expected due to the genetic improvements in growth rate of the FB genotype.

The absolute pectoral and leg muscle masses correlated significantly with both the wet and dry masses of all the other organ masses, for all three genotypes combined (Table 3.4). All of the tisues had highly significant and positive correlations with the muscle masses, this is most likely due the tissue masses all increasing with age and BM, as one would expect.

Correlations between absolute pectoral and leg muscle masses and the other tissues were also examined within two BM intervals, 0-500g and 2000-2500g (Table 3.5). Within the first BM interval, there were significant, positive correlations between the wet and dry muscle masses and all of the other organs ($P \leq 0.001$). In contrast, in the wet masses at the larger BM interval there were positive, significant correlations between pectoral muscle mass and intestine ($R^2=0.633$, $P \leq 0.05$) and liver mass ($R^2=0.753$, $P \leq 0.001$), and between leg muscle mass and carcass mass ($R^2=0.692$, $P \leq 0.01$). There were also significant, negative correlations between pectoral muscle mass and brain mass ($R^2=-0.589$, $P \leq 0.05$) and a correlation between pectoral muscle mass and gizzard mass which tended towards significance ($R^2=-0.482$, $P = 0.069$) (Table 3.5).

The dry absolute data at the larger BM interval had significant, positive correlations between pectoral muscle mass and intestine ($R^2=0.805$, $P \leq 0.001$) and liver mass ($R^2=0.844$, $P \leq 0.001$), and between leg muscle mass and liver ($R^2=0.533$, $P \leq 0.05$) and lung mass ($R^2=0.640$, $P \leq 0.01$). There was a negative correlation between

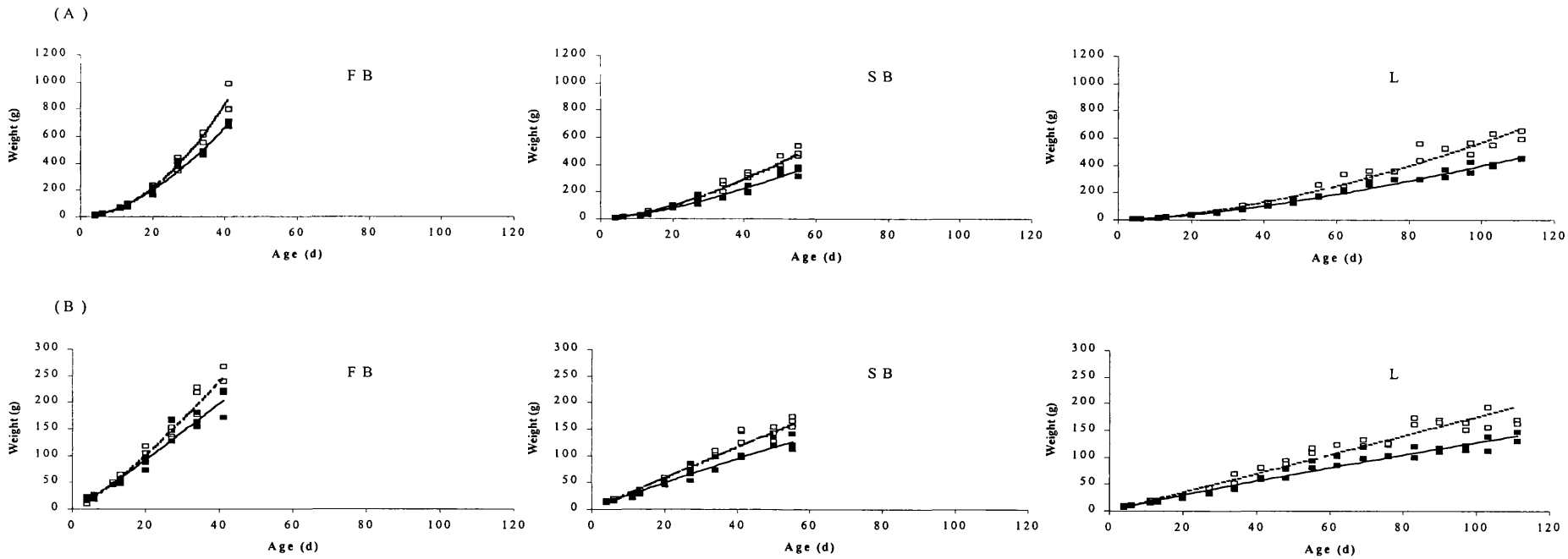


Figure 3.5 The absolute mean (A) total muscle mass and (B) total organ mass for fast broiler, slow broiler and layer genotypes and male (□) and female (■) chickens. The data presented are shown for each individual bird. For comparative purposes, the x-axis is from 0-120 d, which is the final age that the layer genotype was grown to.

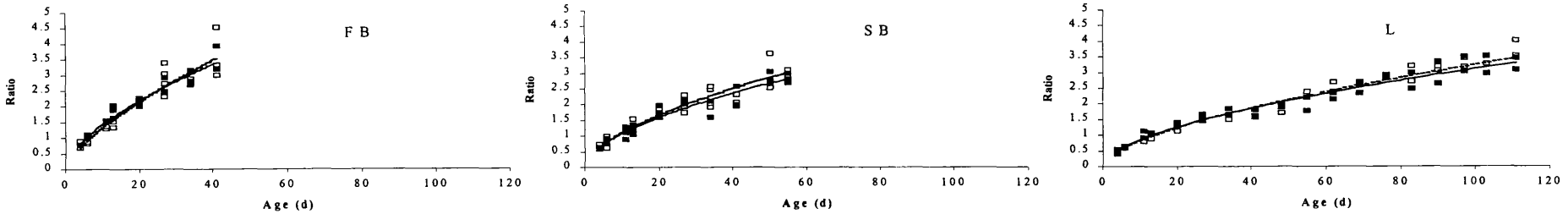


Figure 3.6 The absolute Muscle:Organ ratio for fast broiler, slow broiler and layer genotypes and male (□) and female (■) chickens. The data presented are shown for each individual bird. For comparative purposes, the x-axis is from 0-120 d, which is the final age that the layer genotype was grown to.

Table 3.4 The correlation of the absolute pectoral and leg muscle masses with all organ masses over the whole growth phase of three genotypes of chicken, for both (A) wet and (B) dry masses (g). The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

(A)

	Genotype					
	[1]FB		[2]SB		[3]L	
Organ	Pectorals	Legs	Pectorals	Legs	Pectorals	Legs
Intestine	0.923	0.955	0.925	0.950	0.934	0.943
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
Caeca	0.948	0.953	0.949	0.961	0.949	0.930
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
Gizzard	0.857	0.903	0.914	0.929	0.888	0.876
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
Liver	0.948	0.956	0.960	0.972	0.938	0.951
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
Heart	0.955	0.961	0.969	0.983	0.941	0.974
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
Lungs	0.974	0.987	0.971	0.971	0.948	0.976
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
Brain	0.947	0.960	0.928	0.940	0.895	0.882
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
Carcass	0.984	0.996	0.987	0.993	0.984	0.994
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)

(B)

	Genotype					
	FB		SB		L	
	Pectorals	Legs	Pectorals	Legs	Pectorals	Legs
	0.951	0.963	0.929	0.933	0.948	0.937
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
	0.866	0.874	0.951	0.945	0.955	0.920
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
	0.853	0.888	0.928	0.936	0.900	0.891
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
	0.942	0.958	0.967	0.972	0.941	0.946
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
	0.887	0.912	0.969	0.960	0.942	0.965
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
	0.975	0.975	0.967	0.967	0.958	0.983
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
	0.927	0.945	0.926	0.914	0.903	0.890
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
	0.986	0.981	0.986	0.995	0.992	0.992
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)

Where; [1]FB-fast broiler, [2]SB-slow broiler, [3]L-layer

Table 3.5 The correlation of the absolute pectoral and leg muscle masses and with all organ masses at 0-500 g and 2000-2500 g body mass intervals, for both (A) wet and (B) dry masses (g). The data within these body mass intervals are combined from all three genotypes of chicken. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

(A)

	Body mass interval			
	0-500g		2000-2500g	
Organ	Pectorals	Legs	Pectorals	Legs
Intestine	0.939	0.918	0.633	-0.183
	(0.000)	(0.000)	(0.011)	(0.515)
Caeca	0.929	0.944	0.020	-0.349
	(0.000)	(0.000)	(0.943)	(0.202)
Gizzard	0.883	0.921	-0.482	0.128
	(0.000)	(0.000)	(0.069)	(0.650)
Liver	0.958	0.952	0.753	-0.018
	(0.000)	(0.000)	(0.001)	(0.948)
Heart	0.965	0.978	0.189	0.387
	(0.000)	(0.000)	(0.499)	(0.155)
Lungs	0.931	0.926	0.317	0.374
	(0.000)	(0.000)	(0.250)	(0.169)
Brain	0.860	0.910	-0.589	0.325
	(0.000)	(0.000)	(0.021)	(0.237)
Carcass	0.967	0.992	-0.372	0.692
	(0.000)	(0.000)	(0.173)	(0.004)

(B)

	Body mass interval			
	0-500g		2000-2500g	
Pectorals	Legs	Pectorals	Legs
0.946	0.944	0.805	0.408
(0.000)	(0.000)	(0.000)	(0.131)
0.942	0.946	0.063	-0.010
(0.000)	(0.000)	(0.823)	(0.971)
0.921	0.925	-0.312	0.025
(0.000)	(0.000)	(0.258)	(0.928)
0.960	0.963	0.844	0.533
(0.000)	(0.000)	(0.000)	(0.041)
0.961	0.974	0.112	0.279
(0.000)	(0.000)	(0.691)	(0.314)
0.938	0.936	0.286	0.640
(0.000)	(0.000)	(0.302)	(0.010)
0.821	0.828	-0.492	-0.109
(0.000)	(0.000)	(0.063)	(0.700)
0.965	0.980	-0.169	0.340
(0.000)	(0.000)	(0.548)	(0.214)

pectoral muscle and brain mass which tended towards significance (R^2=-0.492, P = 0.063) (Table 3.5).

Due to the low sample size (n=15) of the combined genotypes within the 2000-2500 g BM interval, the correlation statistics for this BM interval are less robust and therefore should only be taken as an indication of what may be happening biologically.

The absolute values of the individual organ masses at different BM's and ages gave an indication of what effect genotype has had on these organs (Table 3.6-3.9). Due to the FB genotype reaching their maximum growth rate at a heavier BM, the mean organ masses were calculated for the BM interval 2500-3000 g only for this genotype. For full weekly values refer to Appendix 6.

The absolute values for the demand organs indicated that the pectoral muscle mass was larger in the FB genotype in both the first BM interval ($P \leq 0.05$) and the final interval ($P \leq 0.001$). Also at the higher BM interval the females had heavier pectoral muscle than the male birds ($P \leq 0.001$). In the lower BM interval there was no difference between the genotypes for the leg muscle mass and carcass mass, but at the larger BM interval the leg muscle ($P \leq 0.05$) and carcass mass ($P \leq 0.05$) were both larger in the L genotype (Table 3.6).

The GIT organs also showed differences between the genotypes, the intestine mass was larger in the FB genotype for both the first ($P \leq 0.001$) and final BM intervals ($P \leq 0.001$). There were no significant differences between the genotypes in caeca or gizzard mass for either of the BM intervals (Table 3.7).

The other support organs, such as the liver, were larger in the FB genotype compared to the other two genotypes at both the lower BM interval ($P \leq 0.01$) and the heavier BM interval ($P \leq 0.05$) (Table 3.7). Also, the females had larger liver mass than the male chickens at the heavier BM interval ($P \leq 0.05$). There was no difference evident between the heart, lung and brain mass of the three genotypes at the lower

Table 3.6 The mean (±SEM) absolute wet mass (g) of the demand organs, for each of the three genotypes of chicken at 500 g body mass intervals.

Organ	Genotype	Body Mass (g)					
		0-500*	500-1000	1000-1500	1500-2000	2000-2500*	2500-3000
Pectoral	[1]FB	22.22[a]	99.14	199.77	244.14	340.34[a]	452.87
		(3.43)	(4.79)	(9.44)	(13.88)	(16.20)	(53.95)
	[2]SB	16.45[ab]	68.79	140.87	176.22	242.0[b]	-
		(2.42)	(3.49)	(5.80)	(15.12)	(1.8)	
	[3]L	12.21[b]	62.88	126.04	175.04	221.37[b]	-
		(2.17)	(4.99)	(5.64)	(8.71)	(9.21)	
Leg	FB	28.31[a]	107.34	198.35	237.96	324.62[ab]	409.87
		(3.59)	(5.47)	(7.62)	(11.32)	(7.97)	(10.21)
	SB	23.95[a]	92.70	175.86	237.92	267.75[b]	-
		(3.09)	(4.80)	(6.86)	(10.13)	(27.55)	
	L	18.91[a]	88.71	170.81	237.4	354.81[a]	-
		(3.10)	(5.93)	(5.31)	(8.07)	(13.38)	
Carcass	FB	135.42[a]	460.5	797.53	943.4	1264.8[b]	1595.4
		(15.38)	(22.20)	(6.19)	(53.43)	(44.54)	(19.59)
	SB	124.61[a]	466.11	804.45	1014.4	1298.1[ab]	-
		(15.34)	(20.32)	(28.90)	(39.76)	(2.9)	
	L	101.89[a]	435.55	779.33	1040.3	1426.2[a]	-
		(15.06)	(25.25)	(22.84)	(32.25)	(32.65)	

*Values within a column not sharing a common superscript are significantly ($P<0.05$) different.

Where; [1]FB-fast broiler, [2]SB-slow broiler, [3]L-layer.

Table 3.7 The mean (±SEM) absolute wet mass (g) of organs of the gastro-intestinal tract and liver, for each of the three genotypes of chicken at 500 g body mass intervals.

Organ	Genotype	Body Mass (g)					
		0-500*	500-1000	1000-1500	1500-2000	2000-2500*	2500-3000
Intestine	[1]FB	10.99[a]	30.88	49.72	57.95	63.79[a]	77.09
		(1.03)	(1.84)	(3.19)	(3.17)	(5.97)	(3.72)
	[2]SB	7.91[b]	24.56	34.48	37.32	46.29[ab]	-
		(0.72)	(1.42)	(1.57)	(2.26)	(6.89)	
	[3]L	5.63[b]	18.99	24.31	28.84	38.60[b]	-
		(0.61)	(1.03)	(0.83)	(0.91)	(1.75)	
Caeca	FB	1.23[a]	3.43	5.18	6.07	6.67[a]	9.60
		(0.12)	(0.23)	(0.12)	(0.49)	(0.59)	(0.73)
	SB	0.95[a]	3.40	5.92	6.55	8.94[a]	-
		(0.09)	(0.35)	(0.31)	(0.25)	(1.73)	
	L	0.87[a]	3.52	5.00	6.05	6.63[a]	-
		(0.12)	(0.20)	(0.15)	(0.27)	(0.19)	
Gizzard	FB	7.94[a]	17.60	18.40	24.70	31.89[a]	37.69
		(0.69)	(0.98)	(2.41)	(2.06)	(3.01)	(5.03)
	SB	7.63[a]	18.40	25.06	29.12	33.24[a]	-
		(0.56)	(0.98)	(1.46)	(2.27)	(3.99)	
	L	6.97[a]	21.70	30.24	34.18	34.85[a]	-
		(0.82)	(1.14)	(1.29)	(1.88)	(2.19)	
Liver	FB	10.68[a]	29.23	46.02	50.99	72.42[a]	74.78
		(1.01)	(2.19)	(1.97)	(2.69)	(7.48)	(6.22)
	SB	8.20[ab]	21.38	33.53	42.37	46.90[b]	-
		(0.77)	(1.29)	(1.65)	(2.01)	(1.33)	
	L	6.23[b]	24.20	31.93	39.59	52.01[b]	-
		(0.81)	(1.60)	(1.10)	(2.18)	(2.42)	

*Values within a column not sharing a common superscript are significantly ($P<0.05$) different.

Where; [1]FB-fast broiler, [2]SB-slow broiler, [3]L-layer.

Table 3.8 The mean (±SEM) absolute wet mass (g) of cardio-pulmonary organs and brain, for each of the three genotypes of chicken at 500 g body mass intervals.

Organ	Genotype	Body Mass (g)					
		0-500*	500-1000	1000-1500	1500-2000	2000-2500*	2500-3000
Heart	[1]FB	1.99[a]	5.99	10.14	11.52	14.34[a]	16.05
		(0.20)	(0.22)	(0.51)	(0.54)	(1.15)	(1.54)
	[2]SB	1.68[a]	5.41	8.58	11.46	13.24[a]	-
		(0.17)	(0.26)	(0.38)	(0.35)	(2.16)	
	[3]L	1.34[a]	5.22	7.73	10.12	14.15[a]	-
		(0.19)	(0.34)	(0.32)	(0.65)	(0.69)	
Lungs	FB	3.52[a]	6.49	11.22	14.69	20.28[a]	23.41
		(0.15)	(0.43)	(0.37)	(1.05)	(0.67)	(0.58)
	SB	1.98[a]	5.75	10.89	14.62	17.82[a]	-
		(0.20)	(0.46)	(0.51)	(1.48)	(1.43)	
	L	1.34[a]	5.02	9.10	12.44	19.82[a]	-
		(0.19)	(0.32)	(0.33)	(0.97)	(1.09)	
Brain	FB	1.58[a]	2.18	2.55	2.72	3.04[b]	3.44
		(0.05)	(0.04)	(0.05)	(0.09)	(0.06)	(0.18)
	SB	1.55[a]	2.44	2.95	3.18	3.20[ab]	-
		(0.06)	(0.04)	(0.07)	(0.12)	(0.08)	
	L	1.57[a]	2.61	3.05	3.31	3.49[a]	-
		(0.09)	(0.05)	(0.03)	(0.06)	(0.06)	

*Values within a column not sharing a common superscript are significantly ($P<0.05$) different.

Where; [1]FB-fst broiler, [2]SB-slow broiler, [3]L-layer.

Table 3.9 The mean (±SEM) absolute wet mass (g) of total muscle and organ, muscle:organ ratio (M:O) and muscle water content, for the three genotypes of chicken at 500 g body mass intervals.

Body Component	Genotype	Body Mass (g)					
		0-500*	500-1000	1000-1500	1500-2000	2000-2500*	2500-3000
Total Muscle	[1]FB	50.53[a] (6.99)	206.5 (9.49)	398.1 (14.52)	482.1 (22.04)	665.0[a] (19.44)	862.7 (63.49)
	[2]SB	40.40[a] (5.50)	161.5 (8.08)	316.7 (12.38)	414.1 (21.49)	509.8[b] (29.35)	-
	[3]L	31.12[a] (5.26)	151.6 (10.73)	296.9 (9.83)	412.4 (14.29)	576.2[b] (17.39)	-
Total Organ	FB	36.17[a] (3.36)	95.81 (4.70)	143.2 (5.81)	168.6 (4.63)	212.4[a] (10.50)	242.1 (14.21)
	SB	29.90[ab] (2.53)	81.33 (4.32)	121.4 (4.92)	144.6 (4.78)	169.6[b] (3.95)	-
	L	23.95[b] (2.78)	81.25 (3.77)	111.4 (2.37)	134.5 (4.70)	169.5[b] (4.01)	-
M:O	FB	1.24[a] (0.08)	2.16 (0.03)	2.80 (0.16)	2.86 (0.13)	3.17[a] (0.21)	3.62 (0.47)
	SB	1.17[a] (0.07)	2.00 (0.05)	2.62 (0.09)	2.88 (0.20)	3.00[a] (0.10)	-
	L	1.07[a] (0.08)	1.86 (0.08)	2.67 (0.08)	3.08 (0.10)	3.41[a] (0.11)	-
Water Content	FB	37.05[a] (5.01)	146.0 (6.08)	277.1 (8.01)	348.8 (14.71)	475.5[a] (11.15)	609.8 (39.99)
	SB	29.99[a] (4.05)	119.3 (5.95)	231.7 (8.98)	303.0 (16.37)	335.9[b] (34.37)	-
	L	23.05[a] (3.91)	114.1 (7.91)	220.8 (7.18)	305.5 (10.31)	428.6[ab] (13.01)	-

*Values within a column not sharing a common superscript are significantly ($P<0.05$) different.

Where; [1]FB-fast broiler, [2]SB-slow broiler, [3]L-layer.

BM interval, and heart and lung mass at the heavier BM interval. However, the brain mass was larger in the L genotype compared to the other two genotypes at the heavier BM interval ($P \leq 0.001$) (Table 3.8).

The FB genotype had larger total organ mass compared to the SB and L genotypes at the lower BM interval ($P \leq 0.05$), but there was no difference between the genotypes for total muscle mass, muscle water content and M:O ratio at the same interval. At the heavier BM interval, total muscle mass ($P \leq 0.01$), total organ mass ($P \leq 0.01$) and muscle water content ($P \leq 0.01$) were all larger in the FB genotype, whereas no difference between the genotypes was evident for the M:O ratio (Table 3.9). At the heavier BM interval females had larger total muscle mass ($P \leq 0.01$) and muscle water content ($P \leq 0.05$) than the male chickens. Some of the genotype and sex effects that were presented here as absolute data did not change when the organs were analysed relative to BM and age.

3.4.3 Relative Organ Masses

In the following comparisons, the way in which the masses of key organs and tissues changed relative to BM and age was examined. In particular, the effect of BM, age, genotype, sex and all their interactions were tested on the different organ masses. Body mass, corrected for individual organ masses, consistently had a significant effect both on the wet and dry masses of each organ ($P \leq 0.01$) and in the analyses for M:O and muscle hydration. All of the ANCOVA analyses produced positive linear relationships.

The organ mass results in this study were examined in three ways, firstly corrected BM was used as the covariate in the model, secondly due to the possibility of fat content confounding the results corrected LBM was used as a covariate. Thirdly the results of the dry organ weights were examined using corrected DM as the covariate.

Within the following organ morphology and carcass composition results there were many significant interactions. Explanations for each of these interaction terms are

described below. To simplify the explanations, the term BM was used, but BM-OM, LBM-OM or DM-OM depending on the covariate being used in the model for that particular organ could replace this.

The BM X age interaction indicates that the organ mass at the initial ages of the birds was similar but as age and BM increased the organ mass differed. The BM X genotype interaction indicates that each genotype had a similar initial organ mass but that it subsequently increased at a different rate with BM for each genotype. The age X genotype interaction indicates that each genotype had a similar initial organ mass but that it subsequently increased at a different rate with age for each genotype. The BM X sex interaction indicates that each sex had a similar initial organ mass but that it subsequently increased at a different rate with BM for each sex. The age X sex interaction indicates that each sex had a similar initial organ mass but that it subsequently increased at a different rate with age for each sex. The genotype X sex interaction inidicates that there was a significant sex effect within each genotype for organ mass.

3.4.3.1 Relative Masses of the Muscle and Carcass

There was a significant effect of genotype on wet pectoral muscle mass ($F_{2,173}$ = 15.82, $P \leq 0.001$; BM-OM and age were significant covariates), there was also a significant effect of sex on wet pectoral mass ($F_{1,173}$ = 53.87, $P \leq 0.001$), and BM-OM X genotype and age X genotype interactions. The predicted wet pectoral muscle mass was greater in the FB chickens compared to the SB or L chickens and greater in the females compared to the males within all genotypes (Figure 3.7A).

The wet pectoral muscle mass of the FB chickens corrected for LBM was significantly larger than those of the SB or L genotypes ($F_{2,172}$ = 72.94, $P \leq 0.001$; LBM-OM and age were significant covariates, Figure 3.7B), and there were also significant interactions between LBM-OM X age, age X genotype, LBM-OM X genotype and age X sex and a significant effect of sex ($F_{1,172}$ = 86.86, $P \leq 0.001$). The female chickens had a larger predicted pectoral mass than the male chickens.

Dry pectoral muscle mass was also significantly affected by genotype ($F_{2,173} = 21.70$, $P \leq 0.001$; DM-OM and age were significant covariates). There were also significant age X genotype and DM-OM X genotype interactions and a significant effect of sex ($F_{1,173} = 10.09$, $P \leq 0.01$). Predicted dry pectoral muscle mass was greater in the FB genotype compared to the SB with the L genotype being intermediate and greater in the females compared to the male birds (Figure 3.7C). Overall the FB genotype had a larger pectoral muscle mass compared to other genotypes.

There was a significant effect of genotype on wet leg muscle mass ($F_{2,186} = 3.46$, $P \leq 0.05$; BM-OM was a significant covariate), there were also significant BM-OM X age, age X genotype and age X sex interactions. The predicted wet leg muscle mass was larger in the L and FB chickens compared to the SB chickens and the male birds had slightly more predicted leg muscle than the female birds (Figure 3.8A).

The wet leg muscle mass of the FB chickens corrected for LBM were significantly larger than those of the SB or L chickens ($F_{2,185} = 94.63$, $P \leq 0.001$; LBM-OM and age were significant covariates, Figure 3.8B), there were also significant LBM-OM X age, age X genotype, LBM-OM X genotype and age X sex interactions and a significant effect of sex ($F_{1,185} = 4.68$, $P \leq 0.05$). The predicted wet leg muscle mass corrected for LBM was greater in the female chickens compared to the male chickens (Figure 3.8B).

The dry leg muscle mass of the FB chickens were significantly larger than those of the SB or L chickens ($F_{2,186} = 19.67$, $P \leq 0.001$; DM-OM and age were significant covariates, Figure 3.8C), there were also significant age X genotype, DM-OM X genotype and age X sex interactions and a significant effect of sex ($F_{1,186} = 36.35$, $P \leq 0.001$). The male birds also had significantly more dry leg muscle than female birds. Therefore, overall the FB chickens had a larger lean wet and dry leg muscle mass when compared to the other genotypes.

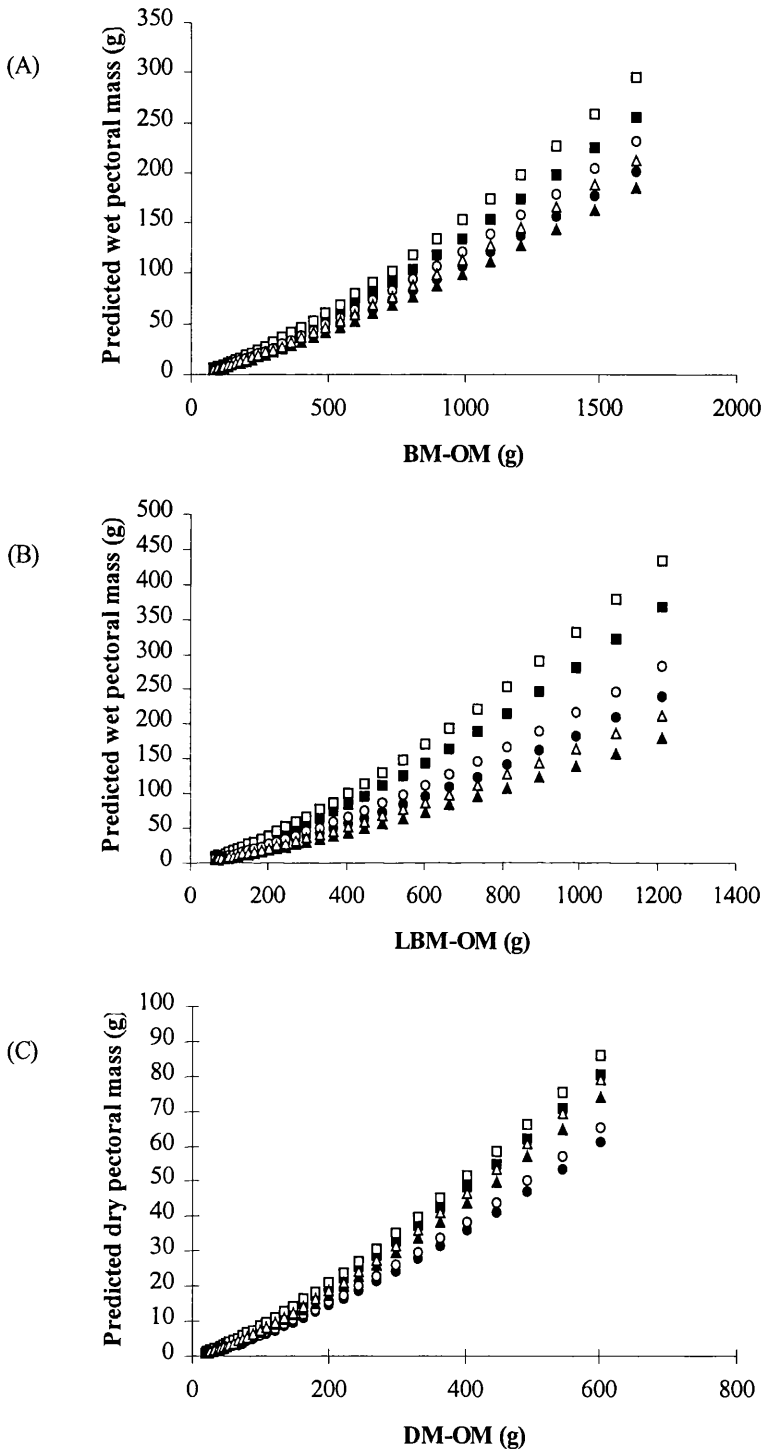


Figure 3.7 The relationship between corrected body mass and pectoral muscle mass in three genotypes of chicken; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ). The predicted pectoral mass was adjusted for age, sex and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM).

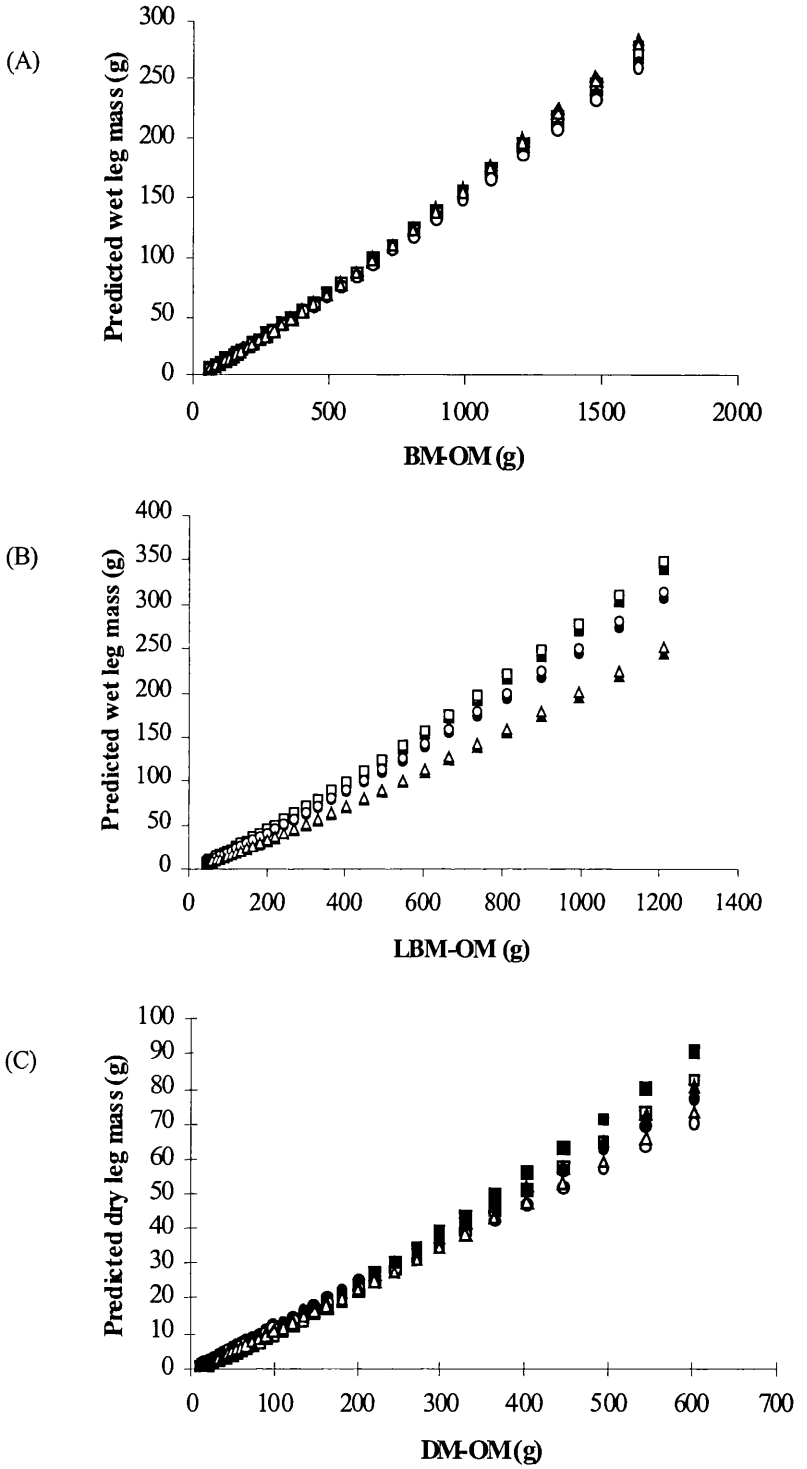


Figure 3.8 The relationship between corrected body mass and leg muscle mass in three genotypes of chicken; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ). The predicted leg mass was adjusted for age, sex and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM).

The carcass mass was the residual tissues of the bird after the removal of the muscle and key organs. Wet carcass mass was significantly affected by genotype ($F_{2,186}$ = 99.11, $P \leq 0.001$; BM-OM and age were significant covariates), there were also significant BM-OM X age, age X genotype, BM-OM X genotype, genotype X sex, age X sex and BM-OM X sex interactions. Predicted wet carcass mass was greater in the L and SB genotypes compared to the FB genotype and was greater in the female compared to the male chickens (Figure 3.9A).

There was a significant effect of genotype on wet carcass mass corrected for LBM ($F_{2,185}$ = 388.26, $P \leq 0.001$; LBM-OM and age were significant covariates), there were also significant LBM-OM X age, age X genotype, LBM-OM X genotype and age X sex interactions and a significant effect of sex ($F_{1,185}$ = 6.40, $P \leq 0.05$). The predicted wet carcass mass was greater in the FB chickens than in the SB or L chickens and was greater in the female compared to the male chickens (Figure 3.9B).

Dry carcass mass was also significantly affected by genotype ($F_{2,186}$ = 56.62, $P \leq 0.001$; DM-OM and age were significant covariates), there were also significant DM-OM X age, age X genotype, DM-OM X genotype, genotype X sex and DM-OM X sex interactions and a significant effect of sex ($F_{1,186}$ = 18.69, $P \leq 0.001$). The predicted dry carcass mass was greater in the L chickens compared to the SB and FB chickens and was greater in the female compared to the male chickens (Figure 3.9C). Overall the L genotype had more residual tissue mass than the other groups except when LBM was used as the covariate.

3.4.3.2 Relative Masses of the GIT and Liver

Wet intestine mass was significantly affected by genotype ($F_{2,186}$ = 25.67, $P \leq 0.001$; BM-OM and age were significant covariates, Figure 3.10A) with FB birds having larger intestines than the L birds with the SB birds being intermediate. There was also a significant effect of sex on wet intestine mass ($F_{1,186}$ = 6.72, $P \leq 0.01$). The male chickens had larger predicted intestine mass than the female chickens.

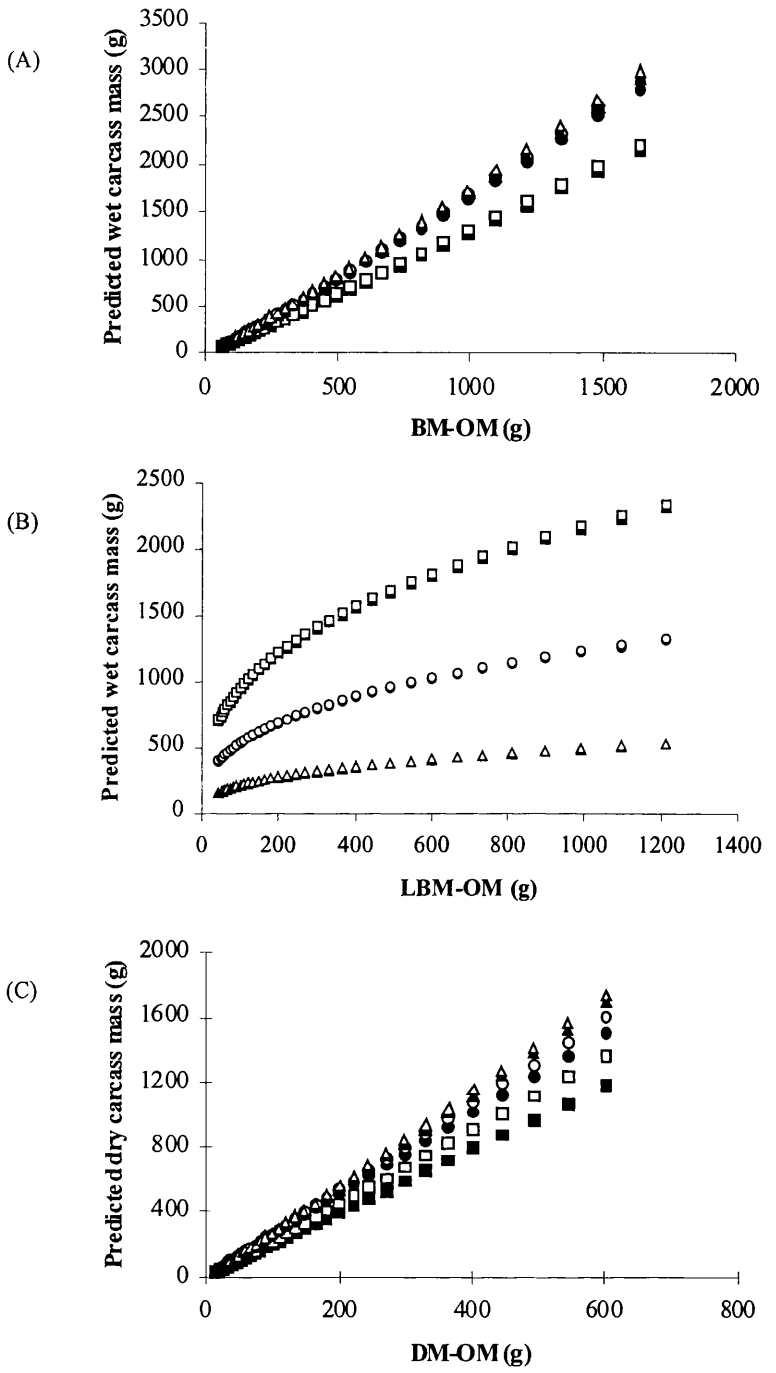


Figure 3.9 The relationship between corrected body mass and carcass mass in three genotypes of chicken; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ). The predicted carcass mass was adjusted for age, sex and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM).

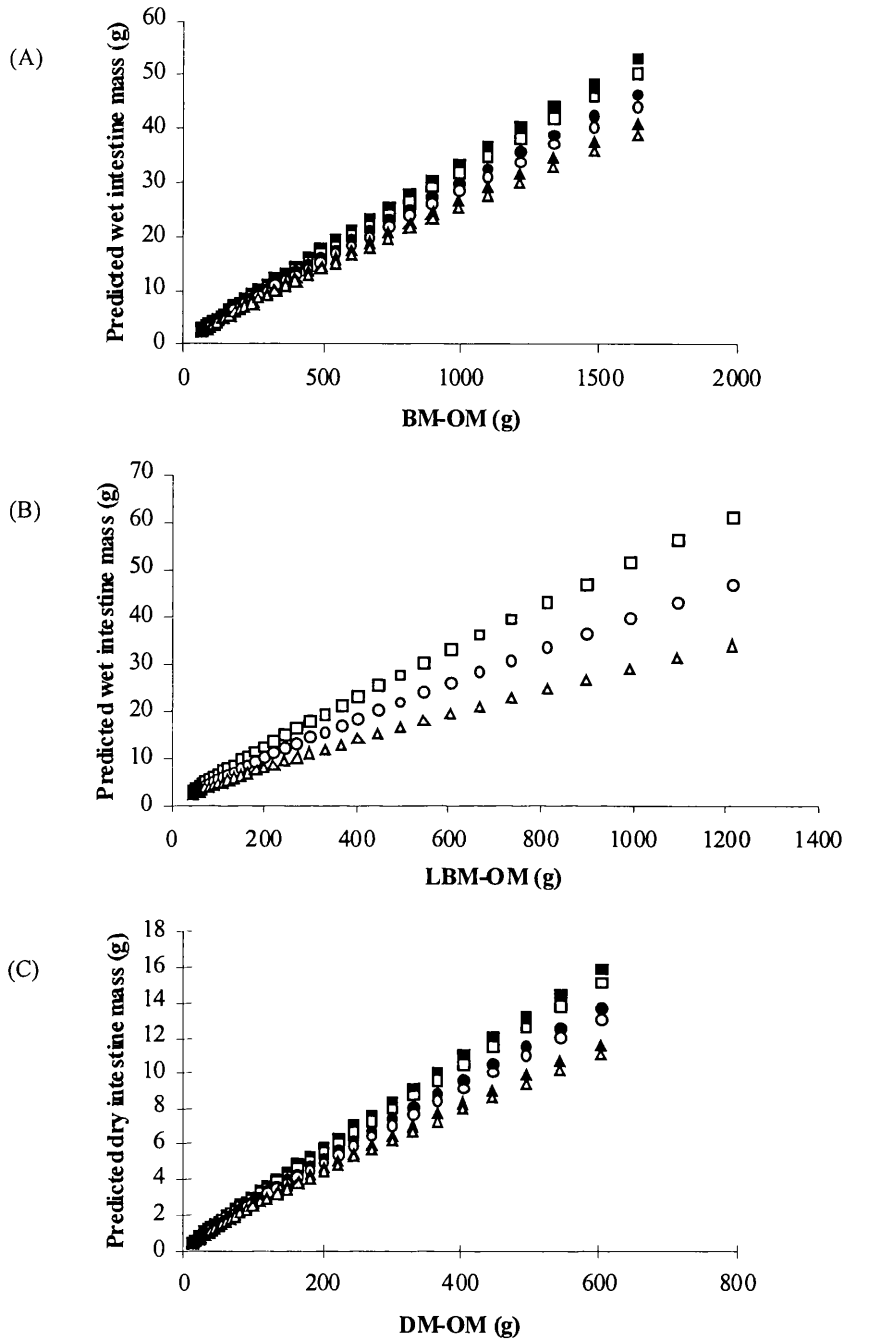


Figure 3.10 The relationship between corrected body mass and intestine mass in three genotypes of chicken; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ). The predicted intestine mass was adjusted for age, sex and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM). For lean body mass the genotypes were; fast broiler (□), slow broiler (O) and layer (Δ).

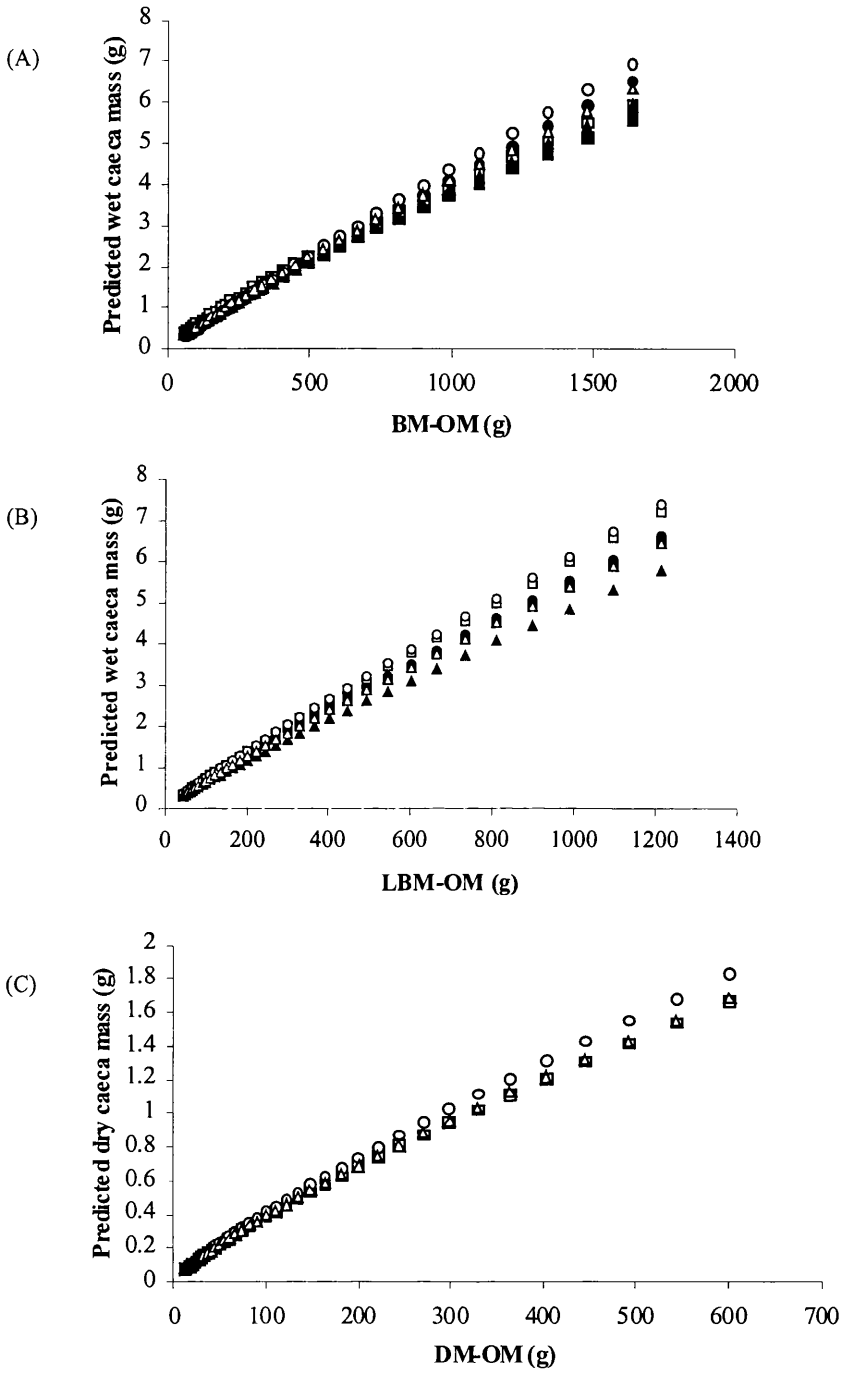


Figure 3.11 The relationship between corrected body mass and caeca mass in three genotypes of chicken; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ). The predicted caeca mass was adjusted for age, sex and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM). For dry body mass the genotypes were; fast broiler (□), slow broiler (O) and layer (Δ).

There was a similar pattern in intestine mass between the genotypes when LBM and DM replaced BM as the covariate in the analyses. The wet intestine mass corrected for LBM of the FB chickens was significantly larger than those of the SB or L genotypes ($F_{2,185} = 66.42$, $P \leq 0.001$; LBM-OM and age were significant covariates, Figure 3.10B), and there were significant interactions between LBM-OM X age and LBM-OM X genotype.

When DM-OM was placed in the model, genotype had a significant effect on dry intestine mass ($F_{2,186} = 27.21$, $P \leq 0.001$; DM-OM and age were significant covariates, Figure 3.10C). Sex ($F_{1,186} = 5.46$, $P \leq 0.05$), age X sex and DM-OM X sex were also significant interactions. The FB chickens had larger dry intestine mass compared to the SB or L chickens and the male chickens had larger dry intestine mass than the female chickens (Figure 3.10C). Therefore the FB chickens consistently had larger intestine mass whichever covariate was used in the analyses.

The wet caeca mass was not affected by genotype but there were significant age X genotype and BM-OM X genotype interactions and BM-OM was a significant covariate. The SB chickens had larger wet caeca mass compared to either the L or FB chickens. There was also a significant effect of sex ($F_{1,186} = 5.21$, $P \leq 0.05$) and a BM-OM X age interaction. The female birds had larger wet caeca mass compared to the male birds (Figure 3.11A).

When LBM-OM was the covariate in the analysis, there was a significant effect of genotype on wet caeca mass ($F_{2,185} = 7.73$, $P \leq 0.001$; LBM-OM and age were significant covariates). There was also a significant effect of sex ($F_{1,185} = 13.11$, $P \leq 0.001$), LBM-OM X age, age X genotype and LBM-OM X genotype interactions. The predicted wet caeca mass corrected for LBM was greater in the SB chickens compared to the FB and L chickens and greater in the females compared to the male chickens (Figure 3.11B).

No significant affect of genotype on dry caeca mass was observed ($P > 0.271$), but there were significant age X genotype and DM-OM X genotype interactions and

DM-OM was a significant covariate. The predicted dry caeca mass was greater in the SB chickens compared to the L or the FB chickens (Figure 3.11C). Overall the SB chickens had a larger caeca mass, when all covariates were used in the analyses.

The same significant results emerged for gizzard mass when either BM-OM, LBM-OM or DM-OM were used in the statistical model. In all cases the gizzard mass of the L chickens was significantly larger than those of the SB or L chickens (Figure 3.12A-C), BM-OM and age were significant covariates, and there was a significant BM-OM X age interaction. The effect of genotype was $F_{2,186} = 9.74$, $P \leq 0.001$, $F_{2,185} = 4.88$, $P \leq 0.01$ and $F_{2,186} = 3.28$, $P \leq 0.05$, for BM, LBM and DM respectively.

Wet liver mass was significantly affected by genotype ($F_{2,186} = 22.91$, $P \leq 0.001$; BM-OM and age were significant covariates). There were also significant age X genotype and BM-OM X genotype interactions. Predicted wet liver mass was greater in the L and FB chickens compared to the SB chickens (Figure 3.13A).

The replacement of BM-OM with LBM-OM as a covariate resulted in a significant effect of genotype wet liver mass corrected for LBM ($F_{2,185} = 45.32$, $P \leq 0.001$; LBM-OM and age were significant covariates). There was also a significant LBM-OM X genotype interaction. Predicted wet liver mass was greater in the FB chickens compared to the SB and L chickens (Figure 3.13B).

Dry liver mass was also significantly affected by genotype ($F_{2,186} = 22.75$, $P \leq 0.001$; DM-OM and age were significant covariates). There were also significant age X genotype, DM-OM X genotype, age X sex and DM-OM X sex interactions. Predicted dry liver mass was greater in the FB birds compared to the L or SB birds and male birds had a greater dry liver mass than the female birds (Figure 3.13C). In general the FB chickens had a larger liver mass compared to the other genotypes.

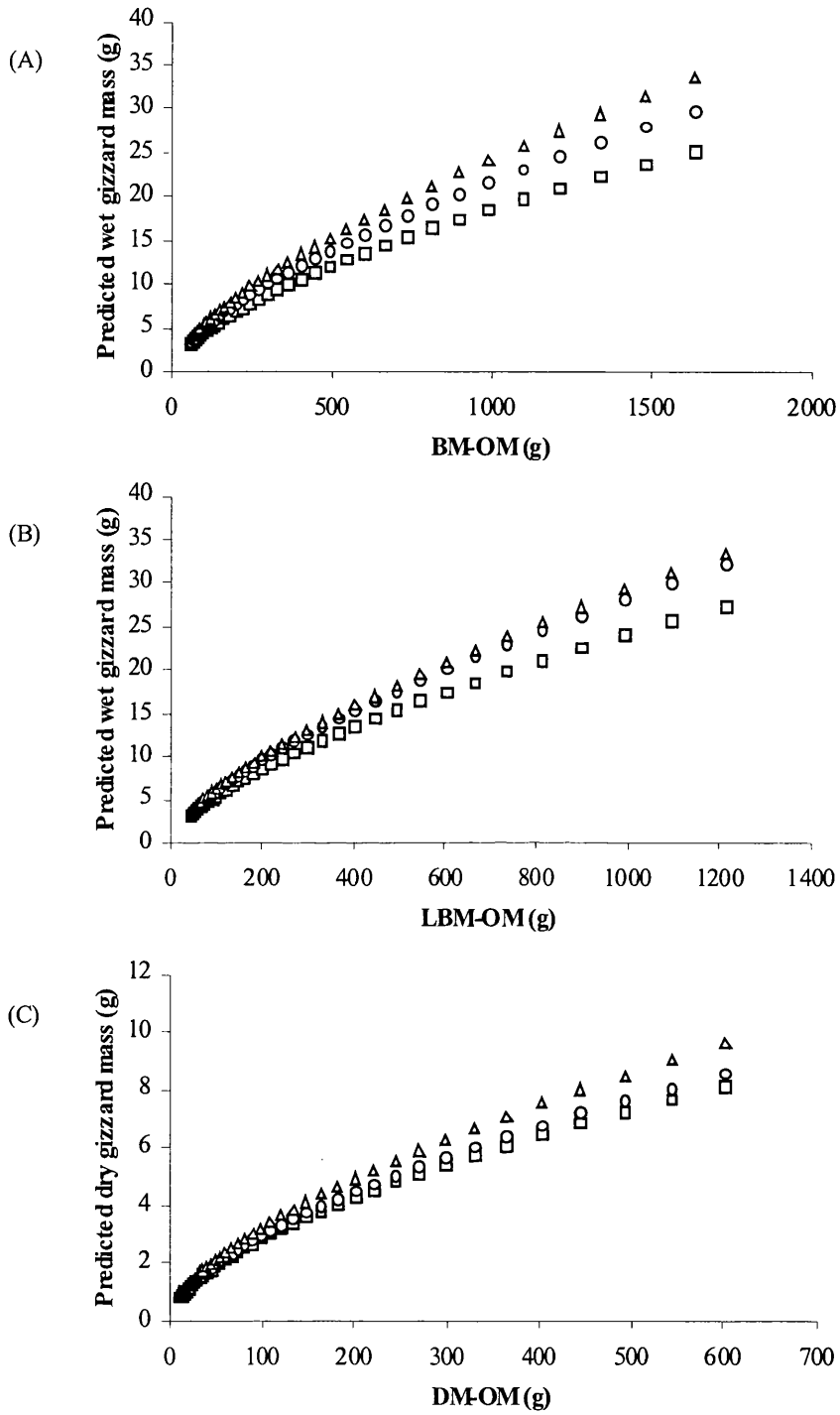


Figure 3.12 The relationship between corrected body mass and gizzard mass in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ). The predicted gizzard mass was adjusted for age, sex and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM).

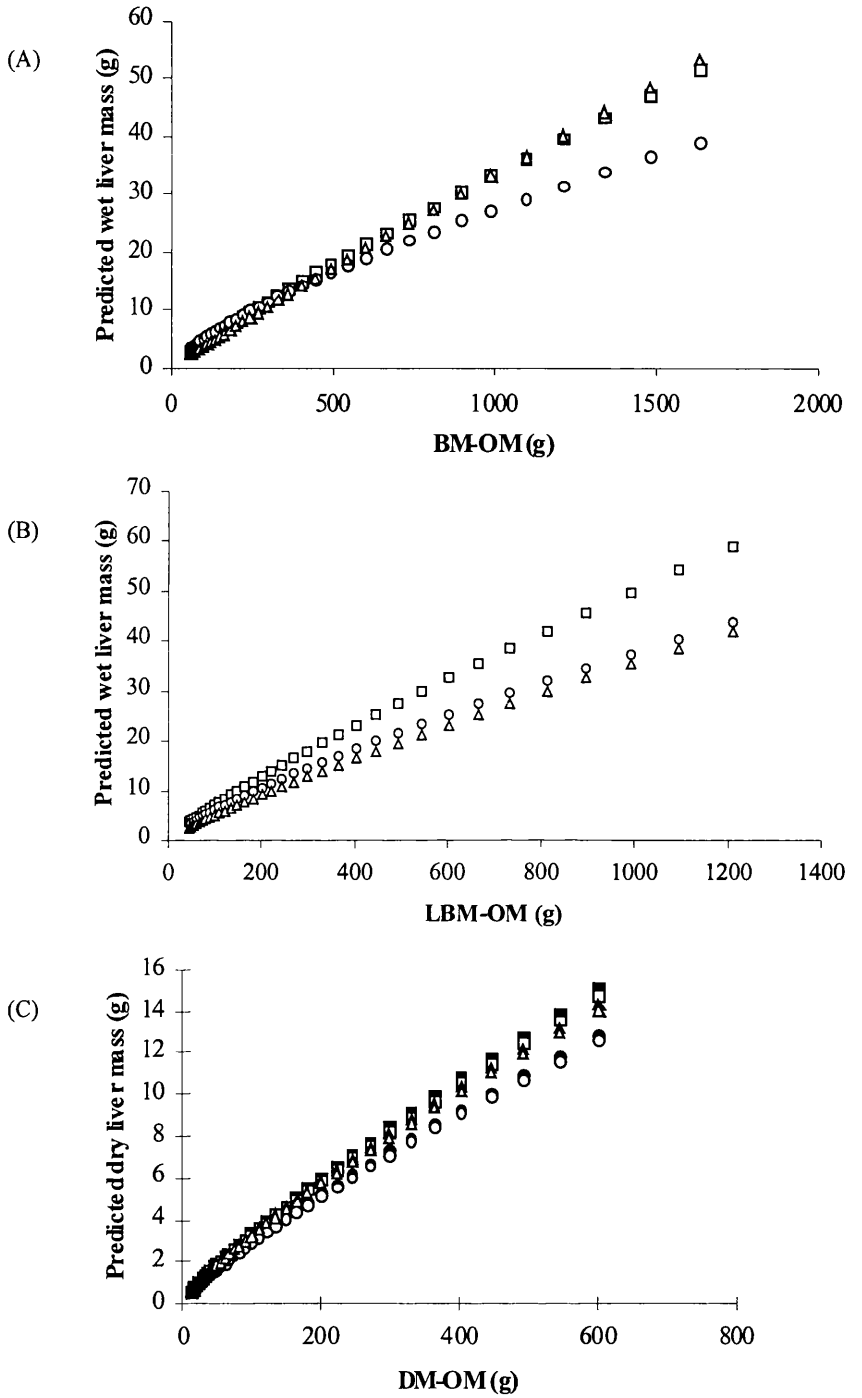


Figure 3.13 The relationship between corrected body mass and liver mass in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ). The predicted liver mass was adjusted for age, sex and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM). For dry body mass the genotypes were; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ).

3.4.3.3 Relative Masses of the Cardio-pulmonary System and Brain

The wet heart mass was not affected by genotype but there was a significant age X genotype interaction ($F_{2,186}$ = 3.68, $P \leq 0.05$; BM-OM and age were significant covariates). There was also a significant effect of sex on wet heart mass ($F_{1,186}$ = 24.98, $P \leq 0.001$). The predictive model indicates that the male chickens had a greater heart mass than the female chickens. The predicted wet heart mass was indistinguishable between genotypes (Figure 3.14A), probably due to no significant genotype or BM-OM X genotype interaction since the prediction model is predicting for BM-OM values.

However, with LBM-OM as a covariate the wet heart mass of the FB chickens was significantly larger than those of the SB or L chickens ($F_{2,185}$ = 17.87, $P \leq 0.001$; LBM-OM and age were significant covariates, Figure 3.14B). There was also significant LBM-OM X genotype interaction and a significant effect of sex on wet heart mass ($F_{1,185}$ = 8.01, $P \leq 0.01$). The wet heart mass corrected for LBM was greater in the male chickens compared to the female chickens.

The dry heart mass was not affected by genotype but there was a significant DM-OM X genotype interaction ($F_{2,186}$ = 5.08, $P \leq 0.01$; DM-OM and age were significant covariates). There was also a significant effect of sex on dry heart mass ($F_{1,186}$ = 8.19, $P \leq 0.01$). Predicted dry heart mass was greater in the FB chickens compared to the SB and L chickens and greater in the males compared to the female chickens (Figure 3.14C). Therefore generally the FB chickens had a larger heart mass than the other groups of chickens.

Wet lung mass was significantly affected by genotype ($F_{2,186}$ = 36.69, $P \leq 0.001$; BM-OM and age were significant covariates), there were also significant BM-OM X age and BM-OM X genotype interactions and a significant effect of sex on wet lung mass ($F_{1,186}$ = 14.04, $P \leq 0.001$). The predicted wet lung mass was larger in the FB

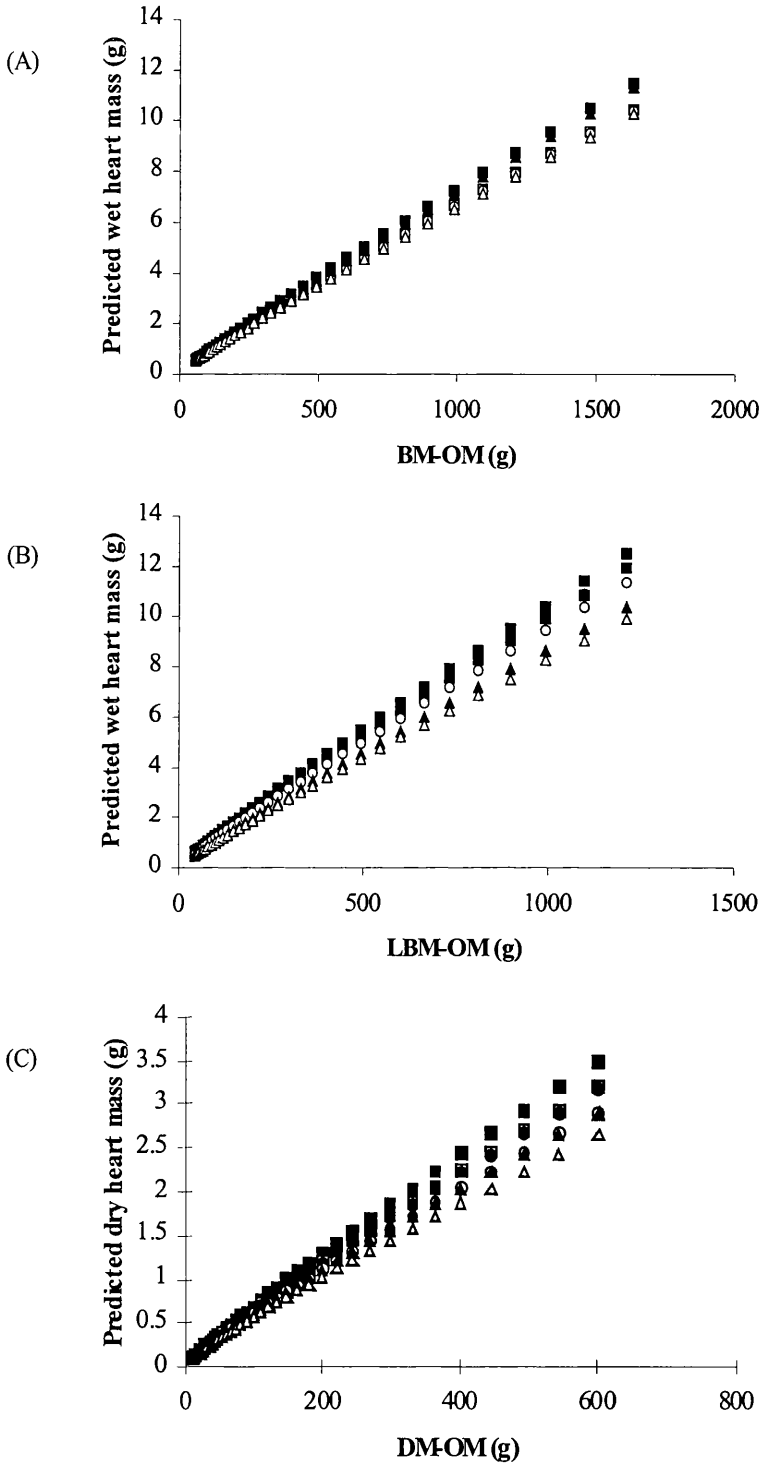
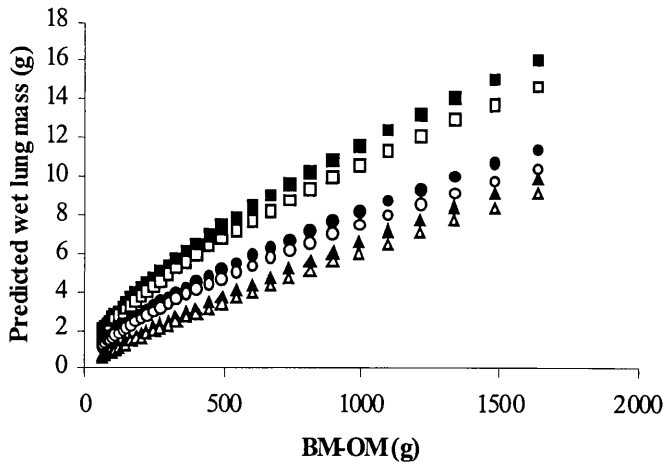
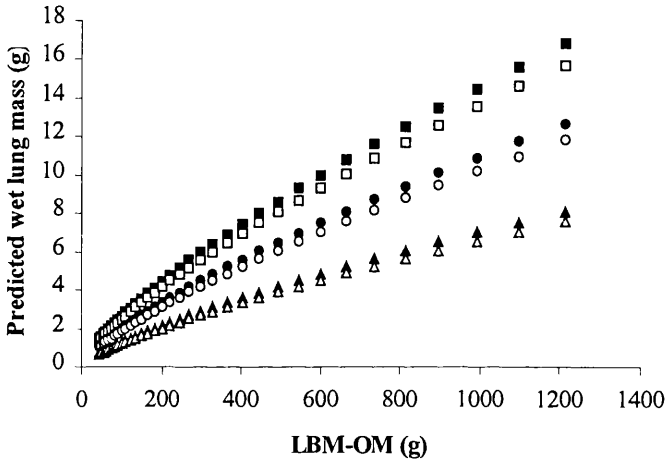


Figure 3.14 The relationship between corrected body mass and heart mass in three genotypes of chicken; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ). The predicted heart mass was adjusted for age, sex and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM).

(A)



(B)



(C)

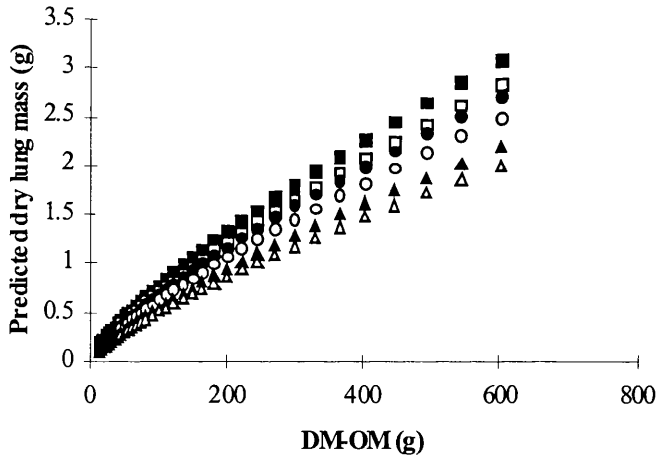


Figure 3.15 The relationship between corrected body mass and lung mass in three genotypes of chicken; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ). The predicted lung mass was adjusted for age, sex and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM).

birds compared to the SB or L birds and larger in the males compared to the female birds (Figure 3.15A).

A similar pattern between the genotypes was shown when BM was replaced by LBM and DM in the analyses. The wet lung mass corrected for LBM of the FB chickens was significantly larger than those of the L or SB genotypes ($F_{2,185} = 93.05$, $P \leq 0.001$; LBM-OM and age were significant covariates, Figure 3.15B). There were also significant LBM-OM X age and LBM-OM X genotype interactions and a significant effect of sex on wet lung mass ($F_{1,185} = 4.19$, $P \leq 0.05$). The male chickens had greater wet lung mass compared to the female chickens.

The dry lung mass was also significantly affected by genotype ($F_{2,186} = 23.34$, $P \leq 0.001$; DM-OM and age were significant covariates). There were also significant DM-OM X age, DM-OM X genotype, genotype X sex and age X sex interactions and a significant effect of sex on dry lung mass ($F_{1,186} = 13.87$, $P \leq 0.001$). Predicted dry lung mass was greater in the FB chickens compared to the SB or L chickens and greater in the male chickens compared to the female chickens (Figure 3.15C). Overall the FB chickens had a greater lung mass compared to the other genotypes.

There was a significant effect of genotype on wet brain mass ($F_{1,186} = 68.47$, $P \leq 0.001$; BM-OM and age were significant covariates). There were also significant BM-OM X age, age X genotype and BM-OM X genotype interactions. Predicted wet brain mass was larger in the L genotype than in the SB or FB genotypes (Figure 3.16A).

A similar pattern between the genotypes was shown when LBM and DM replaced BM in the analyses. The wet brain mass of the L chickens corrected for LBM was significantly larger than those of the FB or SB chickens ($F_{1,185} = 33.77$, $P \leq 0.001$; LBM-OM and age were significant covariates, Figure 3.16B), there were also significant LBM-OM X age, age X genotype and LBM-OM X genotype interactions.

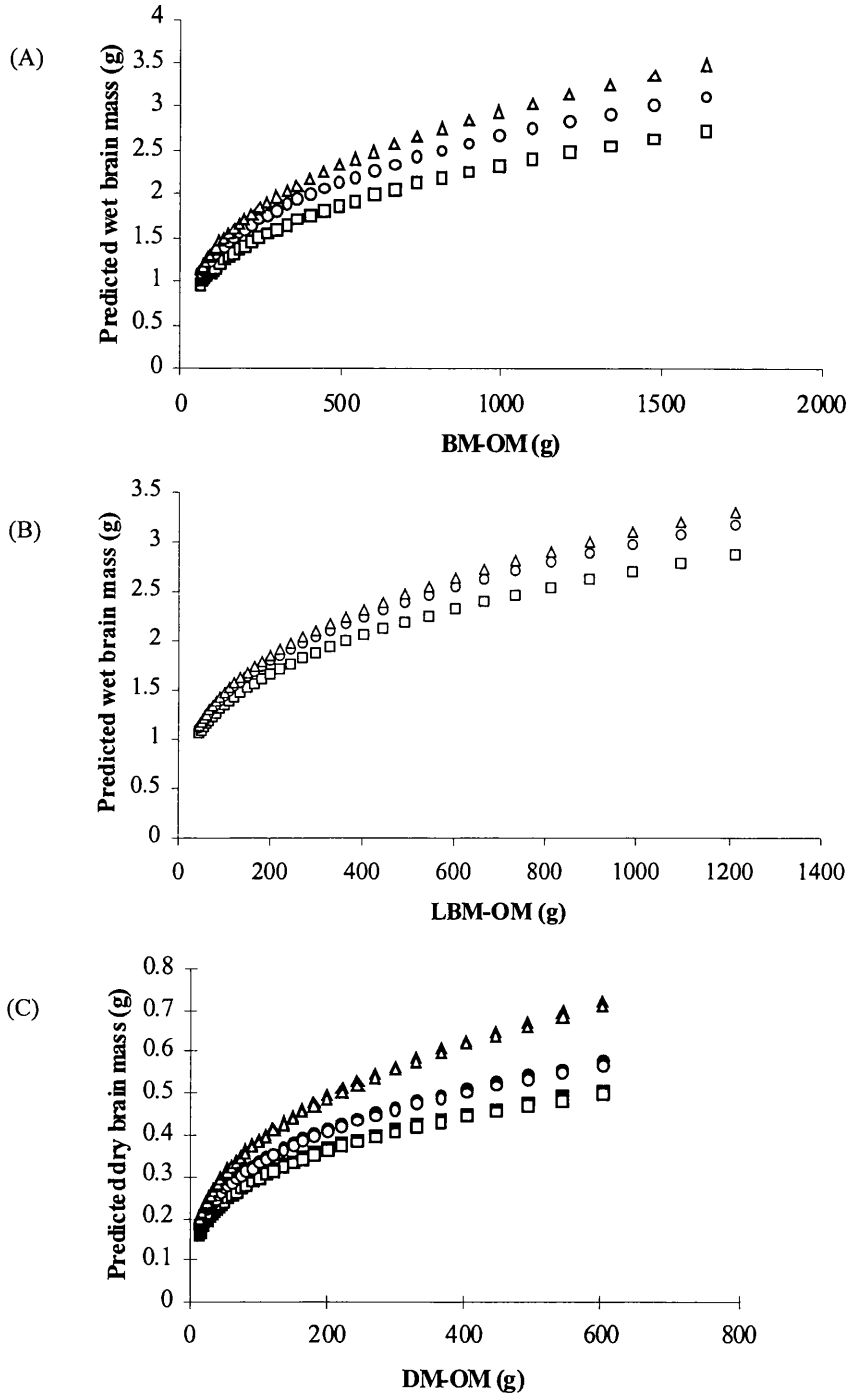


Figure 3.16 The relationship between corrected body mass and brain mass in three genotypes of chicken; fast broiler (□), slow broiler (O) and layer (Δ). The predicted brain mass was adjusted for age, sex and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM). For dry body mass the genotypes were; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ).

The dry brain mass was significantly affected by genotype ($F_{2,186} = 69.51$, $P \leq 0.001$; DM-OM and age were significant covariates). There were also significant DM-OM X age, age X genotype and DM-OM X genotype interactions and a significant effect of sex on dry brain mass ($F_{1,186} = 6.37$, $P \leq 0.05$). Predicted dry brain mass was greater in the L chickens compared to the SB and FB chickens and males had a larger dry brain mass compared to the female chickens (Figure 3.16C). Overall the L chickens had a larger brain mass compared to the other groups of chickens.

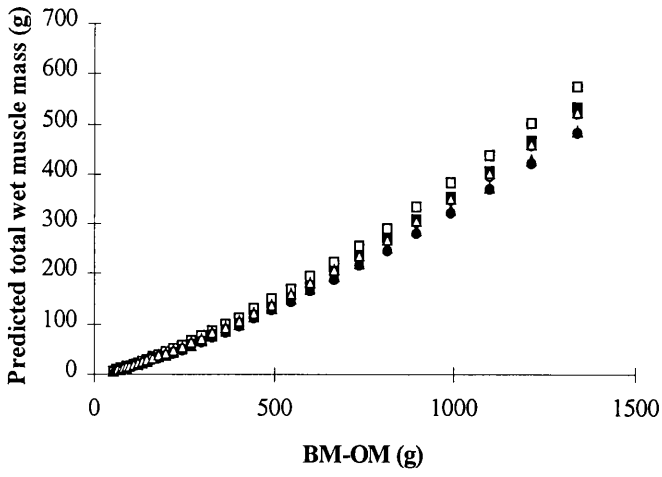
3.4.4 The Ratio of Muscle Mass to Organ Mass (M:O)

The analysis of the M:O ratio was divided into three parts, firstly the analysis of the total muscle mass, secondly the analysis of the total organ mass and thirdly the analysis of the M:O ratio. These analyses were performed both on wet and dry data.

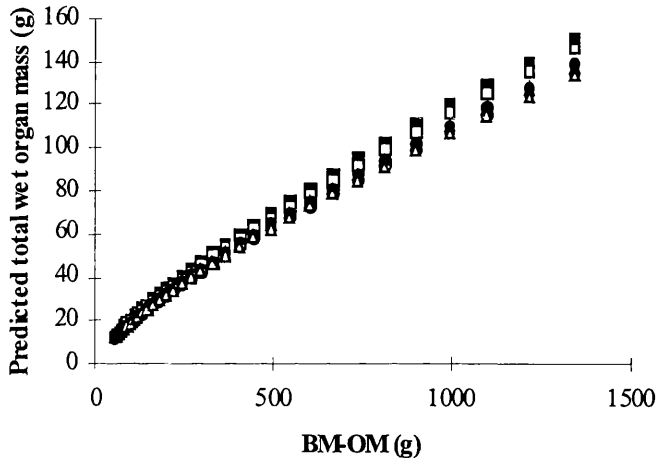
There was a significant effect of genotype on wet total muscle mass ($F_{2,186} = 12.76$, $P \leq 0.001$; BM-OM and age were significant covariates), there were also BM-OM X age, age X genotype, BM-OM X genotype and age X sex interactions and a significant effect of sex ($F_{1,186} = 15.56$, $P \leq 0.001$). Predicted wet total muscle mass was greater in the FB chickens compared to the SB and L chickens and greater in the female chickens compared to the male chickens (Figure 3.17A).

The total wet organ mass was also significantly affected by genotype ($F_{2,186} = 7.52$, $P \leq 0.001$; BM-OM and age were significant covariates), there were also significant age X genotype and BM-OM X genotype interactions and a significant effect of sex ($F_{1,186} = 4.96$, $P \leq 0.05$). Predicted total organ mass was greater in the FB genotype than in the SB or L genotypes and greater in the male compared to the female chickens (Figure 3.17B).

(A)



(B)



(C)

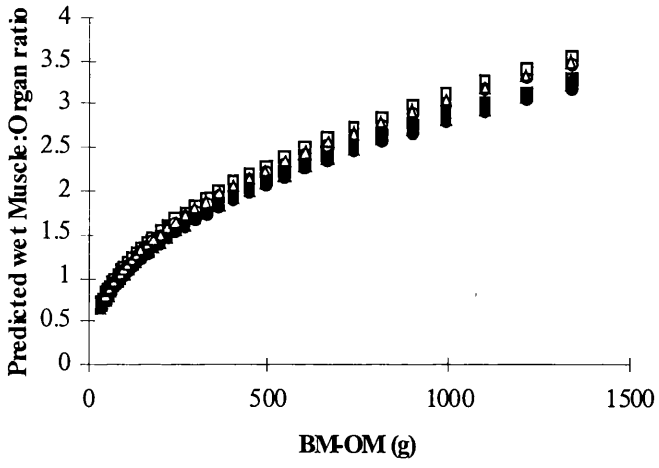


Figure 3.17 The relationship between corrected body mass and muscle and organ mass in three genotypes of chicken; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ). The predicted (A) total wet muscle mass, (B) total wet organ mass and (C) wet muscle to organ ratio adjusted for age, sex and corrected body mass (BM-OM).

There was no significant effect of genotype on wet M:O ratio ($P > 0.266$, Figure 3.17C), but BM-OM was a significant covariate. There was a significant BM-OM X age interaction and there was a significant effect of sex ($F_{1,186} = 14.16$, $P \leq 0.001$). The female chickens had a larger M:O ratio than the male chickens.

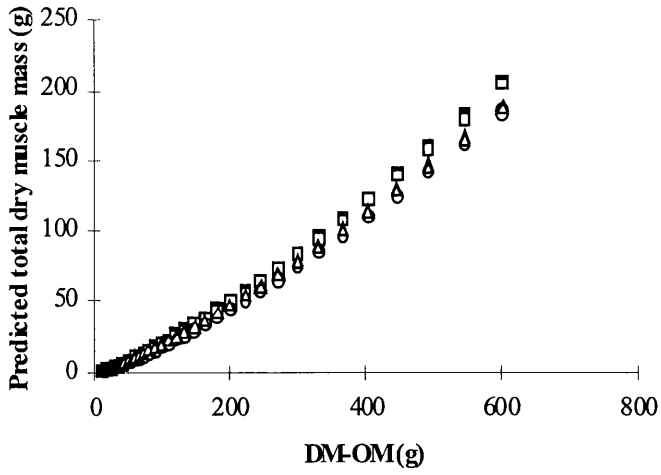
A similar pattern between the genotypes was evident when DM replaced BM as the covariate in the analyses. Total dry muscle mass was significantly affected by genotype ($F_{2,186} = 17.20$, $P \leq 0.001$; DM-OM and age were significant covariates) there were also significant DM-OM X age, age X genotype, DM-OM X genotype and age X sex interactions. Predicted total dry muscle mass was larger in the FB chickens compared to the SB or L chickens (Figure 3.18A). The predicted total dry muscle mass was indistinguishable between sexes.

There was a significant effect of genotype on total dry organ mass ($F_{2,186} = 14.69$, $P \leq 0.001$; DM-OM and age were significant covariates), there were also significant age X genotype, DM-OM X genotype and DM-OM X sex interactions and a significant effect of sex ($F_{1,186} = 11.06$, $P \leq 0.001$). The predicted total dry organ mass was greater in the FB chickens than in the SB or L chickens and greater in the males compared to the female chickens (Figure 3.18B).

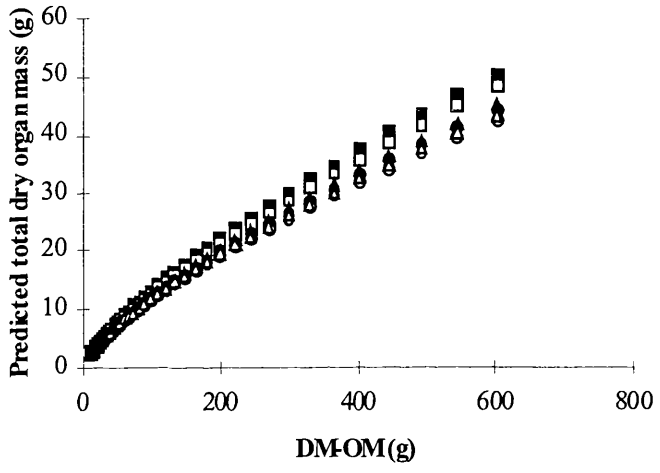
There was no significant effect of genotype on dry M:O ratio, but there was a significant age X genotype and DM-OM X genotype interactions and DM-OM and age were significant covariates. There was also a significant age X sex interaction. The predicted values show that the L chickens had a larger dry M:O ratio at the lower range of DM-OM but that this result was reversed at the higher range of DM-OM with SB and FB chickens have greater dry M:O ratio than the L chickens (Figure 3.18C). The predicted dry M:O ratio was indistinguishable between sexes.

The absolute data for total dry muscle and total dry organ mass in the FB and L chickens shows a large difference between these two genotypes when compared at a similar dry BM. The FB chickens had a much larger difference between the muscle mass and organ mass when compared to the L chickens (Figure 3.19).

(A)



(B)



(C)

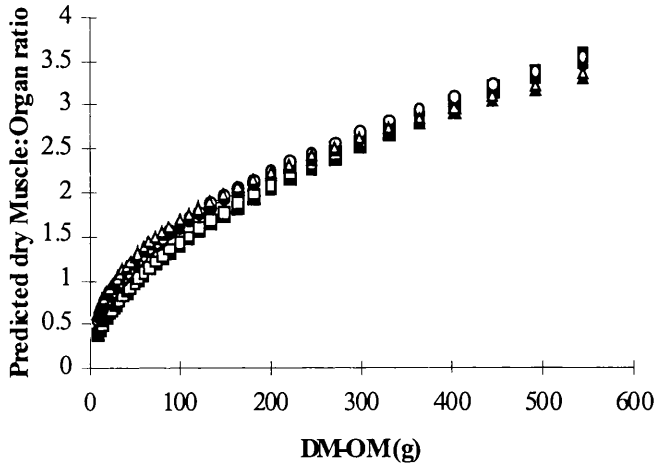


Figure 3.18 The relationship between corrected body mass and muscle and organ mass in three genotypes of chicken; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ). The predicted (A) total dry muscle mass, (B) total dry organ mass and (C) dry muscle to organ ratio corrected for age, sex and corrected dry body mass (DM-OM).

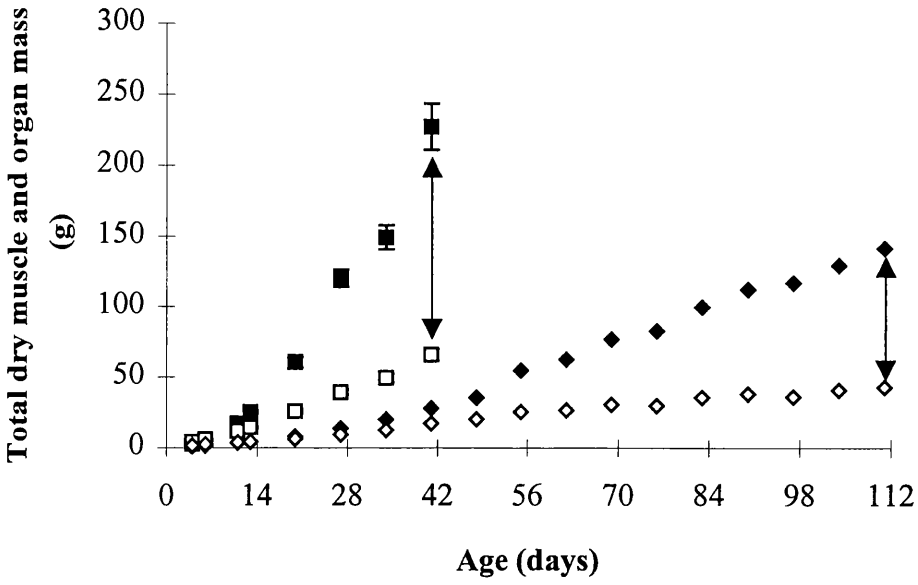


Figure 3.19 Total dry muscle mass and total dry organ mass relative to age in two genotypes of chicken; fast broiler muscle (■) and organ (□) and layer muscle (▲) and organ (Δ) (means ± SEM). The difference between total muscle and total organ mass is indicated on the graph for fast broiler at 42 days and for layer at 112 days, with the difference clearly greater in fast broiler than in layer. Slow broiler data is omitted for reasons of clarity.

3.4.5 Water Content of Muscle

The water content of total muscle mass was significantly affected by genotype ($F_{2,187}$ = 9.35, P ≤ 0.001; BM-OM and age were significant covariates). There were also significant BM-OM X age, age X genotype and age X sex interactions and a significant effect of sex ($F_{1,187}$ = 14.08, P ≤ 0.001). The predicted water content was greater in FB chickens compared to the SB and L chickens and there was a greater water content in the females than in the male chickens (Figure 3.20).

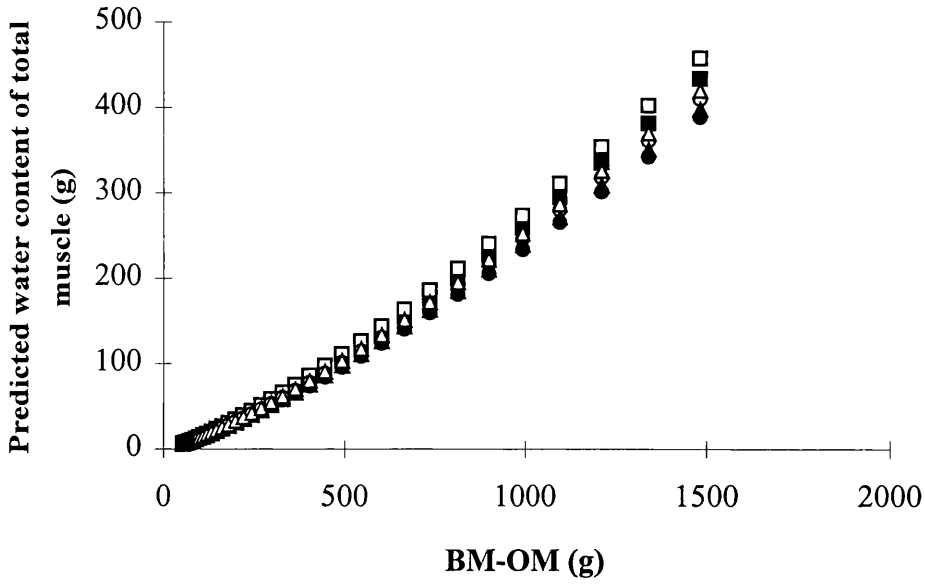


Figure 3.20 The relationship between corrected body mass and muscle water content in three genotypes of chicken; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ). The predicted water content of the total muscle mass adjusted for age, sex and corrected body mass (BM-OM).

3.4.6 Carcass Chemical Composition

The carcass composition results were determined from the analysis of a subsample from each whole chicken carcass that was dried and milled. The absolute values for the carcass composition (g/bird) indicated that there was no difference between the genotypes in CP content at the lower BM interval. However, at the larger BM interval the L genotype had greater CP content compared to either the SB or FB genotypes ($P \leq 0.001$). Also at the higher BM interval the male birds had greater CP content than the females ($P \leq 0.01$). The absolute fat content was greater in the FB and SB genotypes compared to the L genotype at the lower BM interval ($P \leq 0.001$), but at the higher BM interval there was no difference between the genotypes for fat content. There was no difference in absolute ash content at the lower BM interval, but at the larger BM interval the L genotype had a larger ash content than the SB

genotype ($P \leq 0.05$). There was also no difference between the sexes at either BM interval for fat or ash content (Table 3.10). Refer also to Appendix 7.18.

Table 3.10 The protein, fat and ash content of three genotypes of chicken at 500 g intervals of body mass. The data are absolute values (g/bird) and are mean (±SEM).

		Body Mass (g)					
Chemical Component	Genotype	0-500*	500-1000	1000-1500	1500-2000	2000-2500*	2500-3000
Crude protein (g)	[1]FB	134.4[a] (14.47)	425.8 (20.27)	718.4 (29.41)	867.7 (27.67)	1103[b] (39.51)	1461 (62.10)
	[2]SB	122.3[a] (14.43)	429.1 (20.49)	742.1 (26.45)	916.3 (48.50)	1154[b] (20.65)	-
	[3]L	112.1[a] (16.25)	466.7 (23.29)	790.3 (23.77)	1025 (50.63)	1424[a] (43.20)	-
Fat (g)	FB	76.26[a] (9.81)	280.6 (14.36)	496.5 (25.87)	556.3 (43.19)	707.3[a] (36.31)	999.6 (73.09)
	SB	56.12[a] (7.10)	229.8 (14.04)	420.5 (28.11)	547.8 (36.07)	762.1[a] (11.04)	-
	L	30.31[b] (4.29)	147.7 (13.79)	331.4 (22.76)	468.1 (44.70)	577.6[a] (34.86)	-
Ash (g)	FB	21.87[a] (2.45)	62.86 (4.16)	108.0 (6.45)	128.5 (4.82)	182.9[ab] (16.68)	228.2 (9.67)
	SB	20.61[a] (2.42)	64.44 (2.57)	113.7 (6.84)	133.4 (12.75)	128.2[b] (11.21)	-
	L	19.40[a] (2.89)	76.53 (3.60)	126.7 (5.43)	158.1 (8.69)	227.9[a] (14.53)	-

*Values within a column not sharing a common superscript are significantly ($P<0.05$) different.
Where; [1]FB-fast broiler, [2]SB-slow broiler, [3]L-layer.

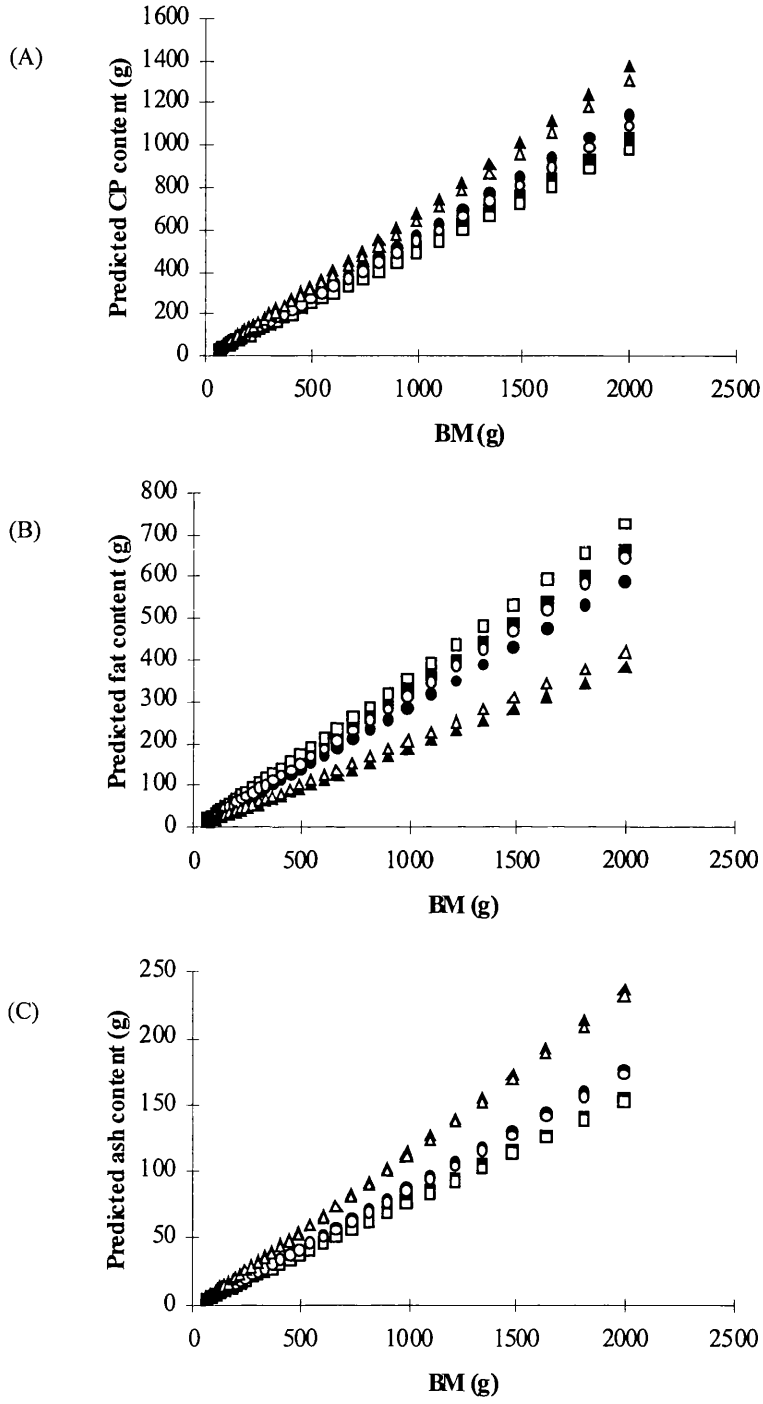


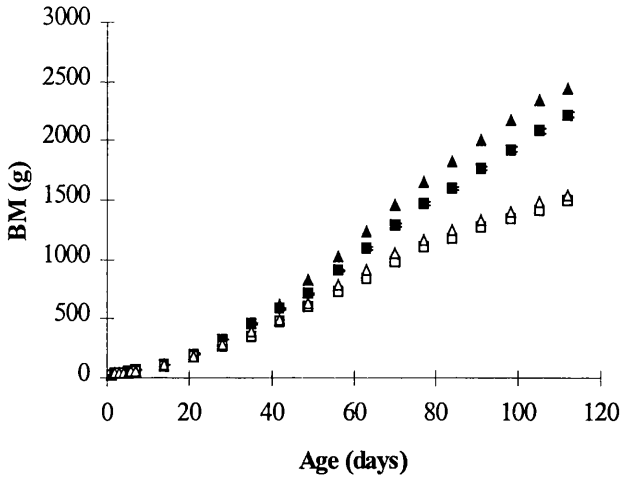
Figure 3.21 The relationship between body mass and carcass composition (g/bird) in three genotypes of chicken; fast broiler male (■) and female (□), slow broiler male (●) and female (O) and layer male (▲) and female (Δ). The predicted absolute mass (g/bird) of (A) crude protein, (B) fat and (C) ash mass of the total carcass adjusted for age, sex and body mass (BM).

When the carcass composition was analysed on a relative basis there were significant differences between genotypes with regard to total carcass CP ($F_{2,185}$ = 80.95, P ≤ 0.001), fat ($F_{2,185}$ = 136.24, P ≤ 0.001) and ash content ($F_{2,186}$ = 34.66, P ≤ 0.001). In all cases BM and age were significant covariates. There were also significant interactions for each of the chemical compositions. There were significant BM X age and age X sex interactions and a significant effect of sex on total CP content ($F_{1,185}$ = 28.19, P ≤ 0.001). The total fat content produced significant BM X genotype and age X sex interactions and a significant effect of sex ($F_{1,185}$ = 24.63, P ≤ 0.001), and in the total ash content there were significant BM X age, age X genotype, BM X genotype and age X sex interactions. The predicted CP and ash contents were larger in the L chickens compared to the FB and SB chickens and larger in the male chickens. This trend was reversed for fat content where the FB chickens had more fat than the SB or L chickens and the female chickens had more fat compared to the male chickens (Figure 3.21A-C).

3.4.7 The Effect of Diet on Organ Morphology in the Layer Genotype

All of the genotypes were fed a standard broiler ration but a second group of the L genotype was fed a typical layer diet. The L genotype was the only genotype fed two different diets, a broiler diet, which was high in CP and energy compared to the layer diet. The L birds were fed a layer diet to compare the organ morphology in the different genotypes when they had been reared under the commercial guidelines for that strain.

(A)



(B)

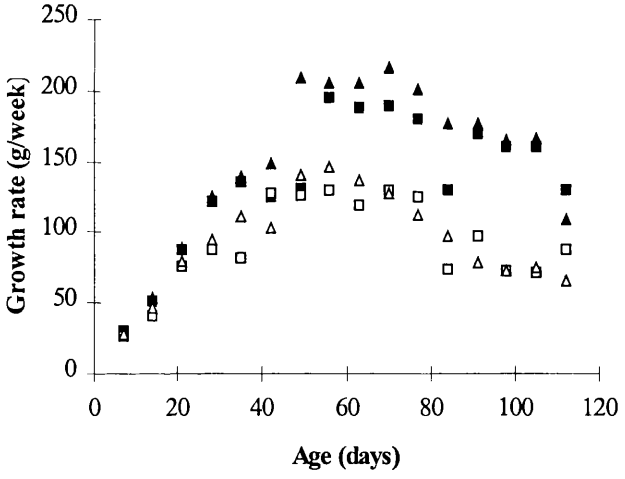


Figure 3.22 The relationship between age and (A) body mass and (B) growth rate in the layer genotype fed two diets; male fed broiler diet (▲), female fed broiler diet (Δ), male fed layer diet (■) and female fed layer diet (□). The data points are mean (±SEM)

Diet had no significant effect on the slopes of the regressions of BM on age. There was a significant effect of sex ($F_{1,392}$ = 92.33, P ≤ 0.001), but no diet X sex interaction on BM (Figure 3.22B).

When the relative organ mass results were analysed for the affect of diet using the same ANCOVA, stepwise regression and prediction models, that had been used previously, only some of the relative organ masses using certain covariates appeared significant.

Within the following results there were many significant interactions. Explanations for each of these interaction terms are described below. To simplify the explanations, the term BM was used, but BM-OM, LBM-OM or DM-OM depending on the covariate being used in the model for that particular organ could replace this.

The BM X age interaction indicates that the organ mass at the initial ages of the birds was similar but as age and BM increased the organ mass differed. The BM X diet interaction indicates that the birds fed each diet had a similar initial organ mass but that it subsequently increased at a different rate with BM for each diet. The age X diet interaction indicates that the birds fed each diet had a similar initial organ mass but that it subsequently increased at a different rate with age for each diet. The BM X sex interaction indicates that each sex had a similar initial organ mass but that it subsequently increased at a different rate with BM for each sex. The age X sex interaction indicates that each sex had a similar initial organ mass but that it subsequently increased at a different rate with age for each sex.

There was a significant effect of diet on wet leg muscle mass ($F_{1,143}$ = 4.18, P ≤ 0.05; BM-OM and age were significant covariates). There were also significant BM-OM X diet, age X sex and BM-OM X sex interactions. The predicted wet leg muscle mass was larger in the chickens fed a broiler diet compared to the layer fed chickens (Figure 3.23A).

There was a significant effect of diet on lean wet leg muscle mass ($F_{1,143}$ = 4.77, P ≤ 0.05; LBM-OM and age were significant covariates). There were also significant

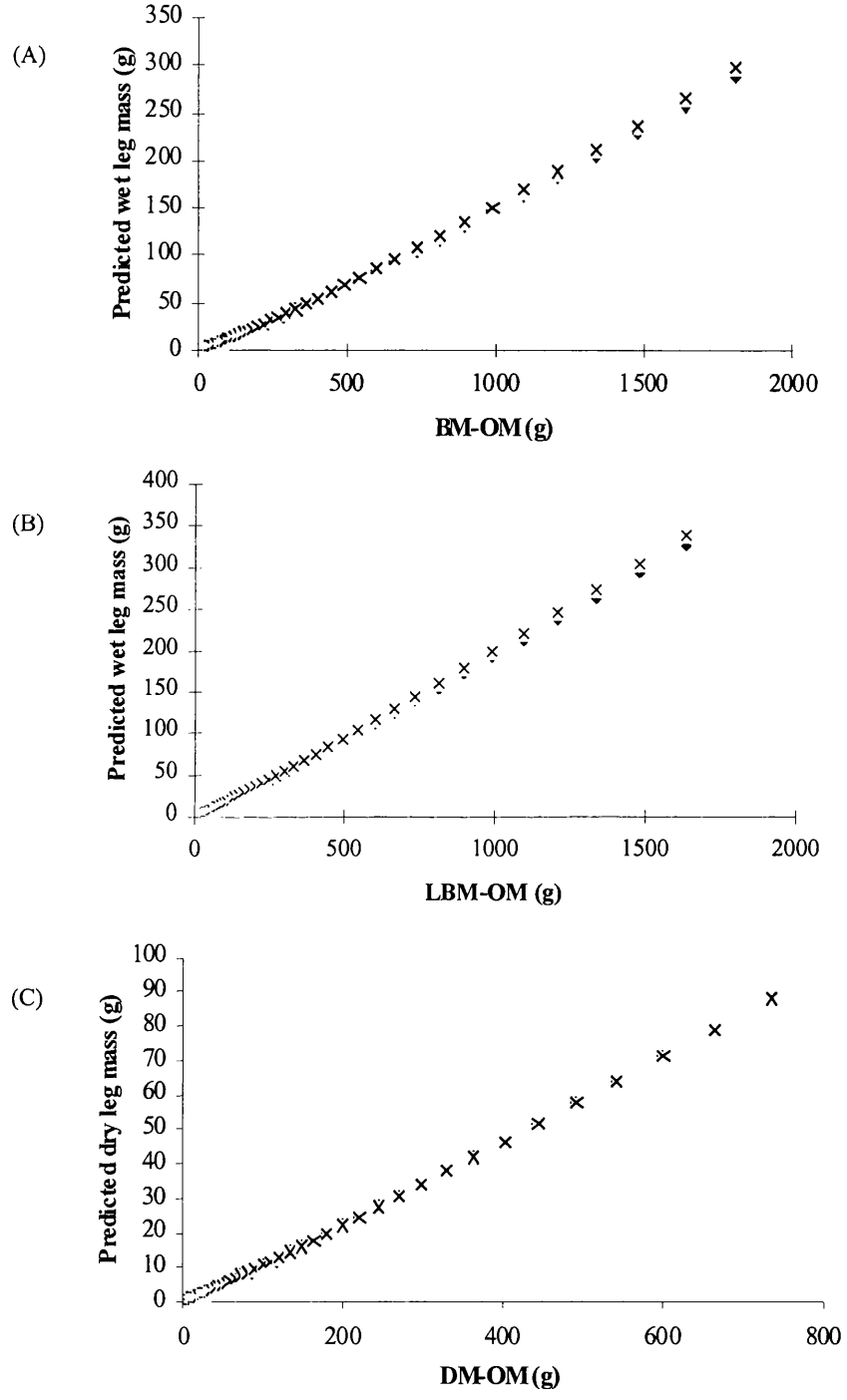
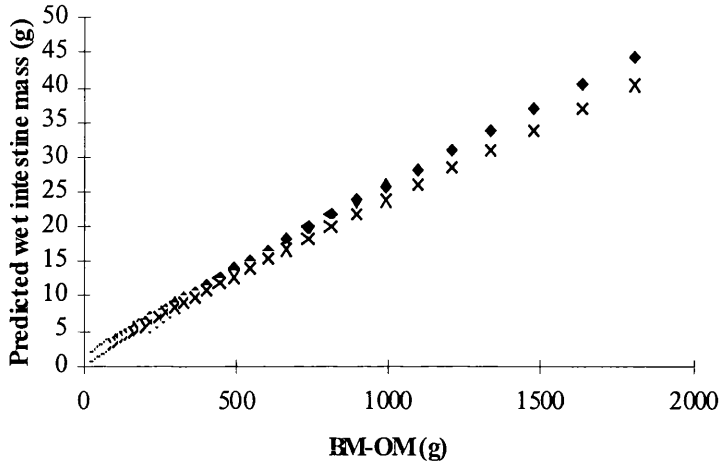


Figure 3.23 The relationship between corrected body mass and leg muscle mass in layer chickens fed a broiler (X) and a layer (◆) diet. The predicted leg mass was adjusted for age, sex and (A) corrected body mass (BM-OM), (B) corrected lean body mass (LBM-OM) and (C) corrected dry body mass (DM-OM).

(A)



(B)

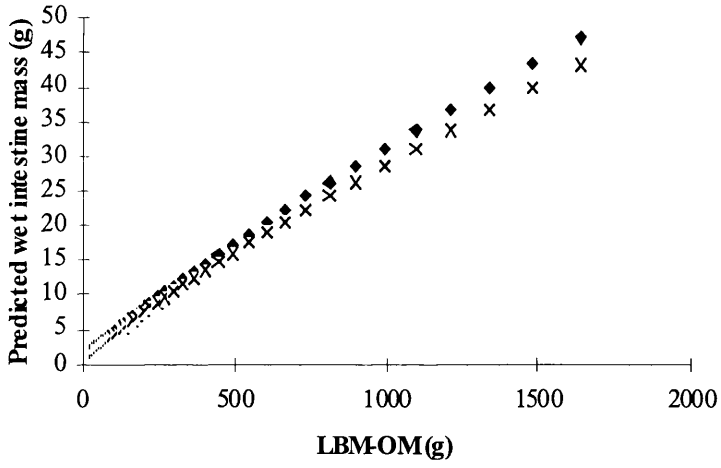


Figure 3.24 The relationship between corrected body mass and intestine mass in layer chickens fed a broiler (X) and a layer (◆) diet. The predicted intestine mass was adjusted for age, sex and (A) corrected body mass (BM-OM) and (B) corrected lean body mass (LBM-OM).

LBM-OM X age, age X diet and age X sex interactions. The predicted lean wet leg muscle mass was greater in the broiler fed chickens compared to the layer fed chickens (Figure 3.23B).

Diet had no significant effect on dry leg muscle mass, but DM-OM was a significant covariate, there were also significant DM-OM X diet and age X sex interactions and a significant effect of sex ($F_{1,143} = 38.69$, $P \leq 0.001$). The predicted dry leg mass was indistinguishable between diets (Figure 3.23C).

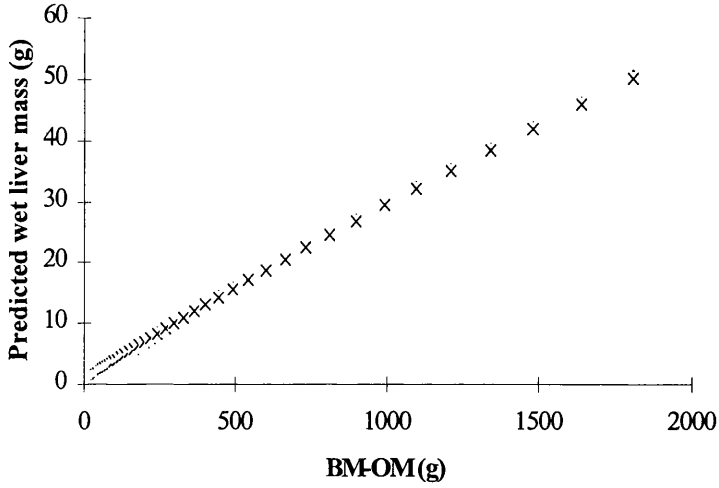
Wet intestine mass was significantly affected by diet ($F_{1,143} = 9.49$, $P \leq 0.01$; BM-OM and age were significant covariates, Figure 3.24A) with birds fed the layer diets having larger intestines than the birds fed the broiler diet. There was also a significant BM-OM X sex interaction.

The lean wet intestine mass of the chickens fed a layer diet were significantly larger than those fed a broiler diet ($F_{1,143} = 6.99$, $P \leq 0.01$; LBM-OM and age were significant covariates, Figure 3.24B), and there were significant interactions between LBM-OM X age and LBM-OM X sex.

There was no significant effect of diet on wet liver mass, but BM-OM and age were significant covariates. There were also significant BM-OM X diet and age X sex interactions. Predicted wet liver mass was indistinguishable between diets (Figure 3.25A).

Dry liver mass was not significantly affected by diet, but DM-OM was a significant covariate. There were also significant DM-OM X diet and age X sex interactions. Predicted dry liver mass was larger in birds fed a layer diet compared to birds fed a broiler diet (Figure 3.25B).

(A)



(B)

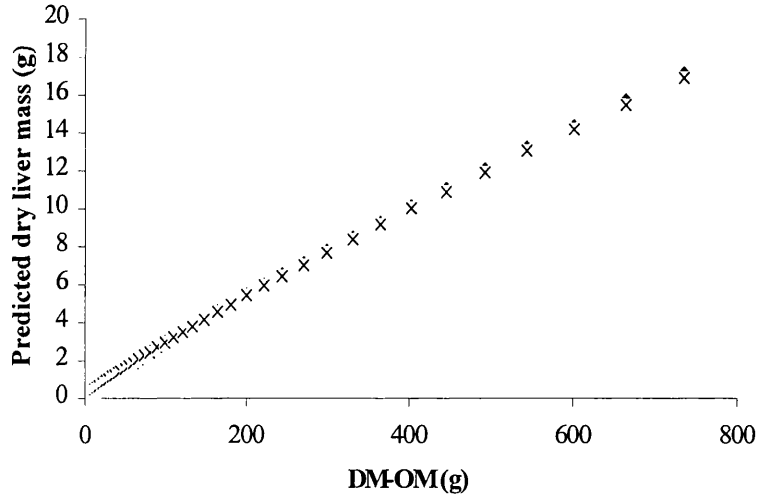
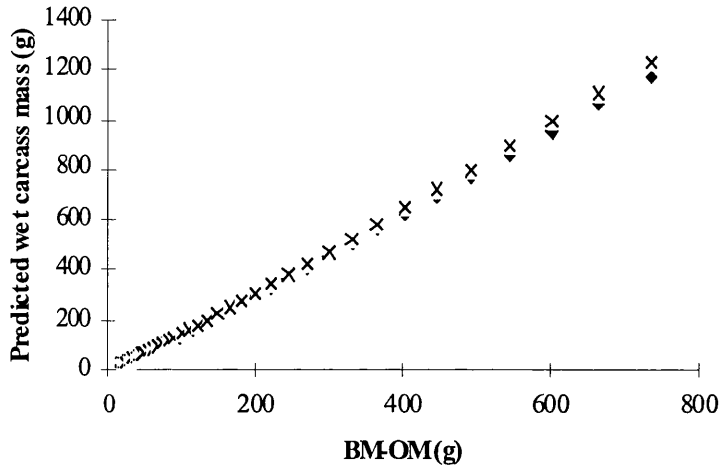


Figure 3.25 The relationship between corrected body mass and liver mass in layer chickens fed a broiler (X) and a layer (◆) diet. The predicted liver mass was adjusted for age, sex and (A) corrected body mass (BM-OM) and (B) corrected dry body mass (DM-OM).

(A)



(B)

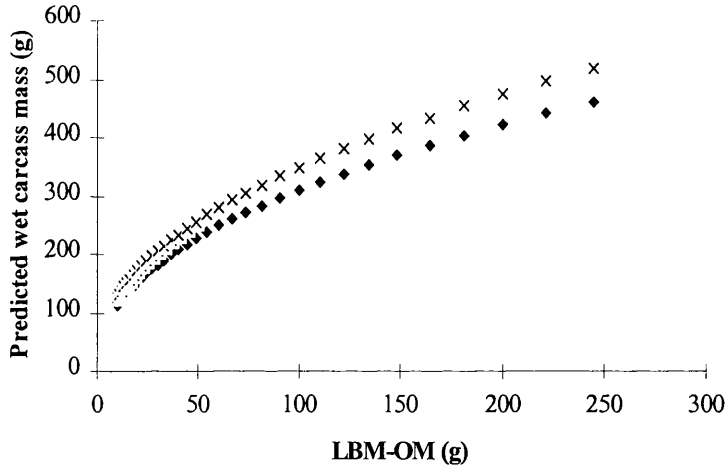


Figure 3.26 The relationship between corrected body mass and carcass mass in layer chickens fed a broiler (X) and a layer (◆) diet. The predicted carcass mass was adjusted for age, sex and (A) corrected body mass (BM-OM) and (B) corrected lean body mass (LBM-OM).

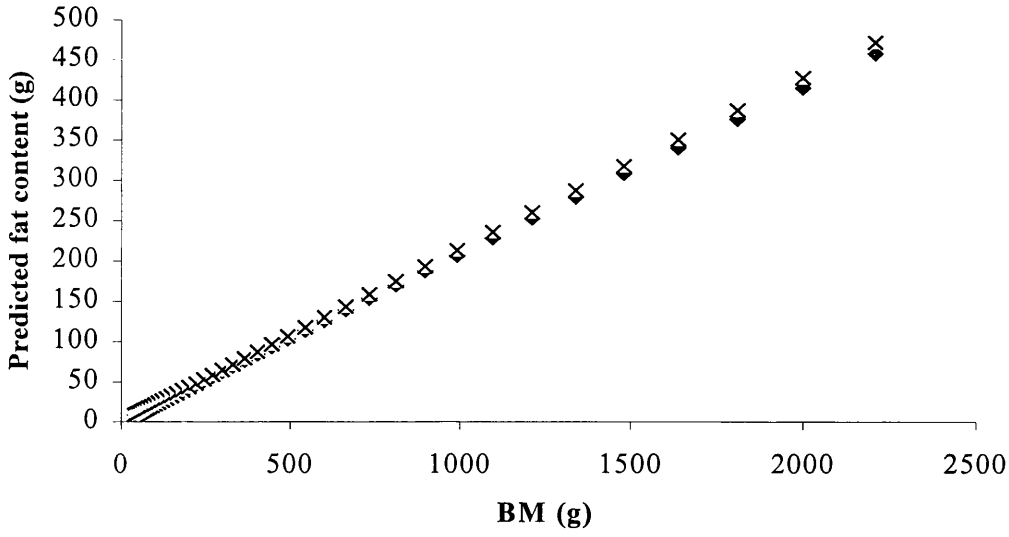


Figure 3.27 The relationship between body mass and fat content in layer chickens fed a broiler (X) and a layer (◆) diet. The predicted fat content was adjusted for age, sex and body mass (BM).

Wet carcass mass was significantly affected by diet ($F_{1,143} = 15.34$, $P \leq 0.001$; BM-OM was a significant covariate). There were also significant BM-OM X age, age X sex and BM-OM X sex interactions. Predicted wet carcass mass was greater in the broiler fed chickens compared to the layer fed chickens (Figure 3.26A).

There was a significant diet effect on lean wet carcass mass ($F_{1,143} = 13.50$, $P \leq 0.001$; LBM-OM and age were significant covariates). There were also significant LBM-OM X age, age X diet and age X sex interactions. The predicted wet carcass mass was greater in the chickens fed a broiler diet compared to the chickens fed a layer diet (Figure 3.26B).

When the carcass composition was analysed on a relative basis the fat content was not significantly affected by diet, but BM-OM and age were significant covariates. There were also significant age X diet and age X sex interactions. Predicted fat content was greater in the chickens fed a broiler diet compared to the chickens fed a layer diet (Figure 3.27).

3.4.8 Summary of Organ Morphology Results

Summary of the Absolute Organ Data

There were no significant differences between genotypes for any of the organs at the 0-500g BM interval. Within the 2000-2500g BM interval, the results indicated that the FB genotype had larger absolute pectoral muscle, intestine and liver mass than either of the other two genotypes and the L genotype had larger absolute leg muscle, carcass and brain mass. At the same BM interval the absolute caeca, gizzard, heart and lung mass were not different between the three genotypes.

Summary of the Relative Organ Data

The FB genotype had a larger relative pectoral muscle mass compared to the other two genotypes when all three covariates were used in the statistical analysis. However, some of the relative organ masses gave contradictory results depending on which covariate was used in the statistical models. For example, the L genotype had a larger carcass mass when the DM-OM covariate was used in the model. When this was replaced with BM-OM the L genotype was equal to the SB genotype and they were both larger than the FB genotype. The whole sequence was then reversed when LBM-OM was used as a covariate with the FB genotype having a larger carcass mass than the other two genotypes. The varying results depending on which covariate was used in the analysis could be a function of the different water or ash contents of the different genotypes. Overall the L genotype had a larger carcass mass compared to the other two genotypes (Table 3.11). When the LBM-OM and DM-OM covariates were used in the statistical model for the relative leg muscle mass it was greater in the FB genotype, whereas, when BM-OM covariate was used there was no difference between the L and FB genotypes but they were both greater than the SB genotype. Overall the FB genotype had a larger leg muscle mass compared to the other two genotypes.

The FB genotype had a larger relative intestine mass compared to the other two genotypes when all three covariates were used in the statistical analysis. However,

the L genotype had larger relative gizzard mass and the SB genotype had a larger caeca mass compared to the other two genotypes with all three covariates (Table 3.11). However, when the LBM-OM and DM-OM covariates were used in the statistical model for the relative liver mass it was greater in the FB genotype, whereas, when BM-OM covariate was used there was no difference between the L and FB genotypes but they were both greater than the SB genotype. Overall the FB genotype had a larger liver mass compared to the other two genotypes.

Table 3.11 A summary of the effect of genotype on the relative organ masses for each covariate used in the analyses.

	Covariate		
Organ	[1]BM-OM	[2]LBM-OM	[3]DM-OM
Pectoral	[4]FB>[5]SB>[6]L	FB>SB>L	FB>L>SB
Leg	L=FB>SB	FB>SB>L	FB>L=SB
Carcass	L=SB>FB	FB>SB>L	L>SB>FB
Intestine	FB>SB>L	FB>SB>L	FB>SB>L
Caeca	SB>L>FB	SB>FB>L	SB>FB=L
Gizzard	L>SB>FB	L=SB>FB	L>SB>FB
Liver	L=FB>SB	FB>SB>L	FB>L>SB
Heart	Prediction unclear	FB>SB>L	FB>SB>L
Lung	FB>SB>L	FB>SB>L	FB>SB>L
Brain	L>SB>FB	L>SB>FB	L>SB>FB

Where; [1]BM-OM-corrected body mass, [2]LBM-OM-corrected lean body mass, [3]DM-OM-corrected dry body mass, [4]FB-fast broiler, [5]SB-slow broiler, [6]L-layer.

The FB genotype had a larger relative lung mass compared to the other two genotypes when all three covariates were used in the statistical analysis. However, the L genotype had larger relative brain mass compared to the other two genotypes with all three covariates (Table 3.11). However, similar results were evident for relative heart mass when both LBM-OM and DM-OM were used as covariates with

the FB genotype having a larger heart mass than the SB and the L genotypes. Unfortunately the prediction model results were unclear when BM-OM was used in the model, but due to the consistency of the other results it can be accepted that the FB genotype had a larger heart mass overall (Table 3.11).

The analysis of M:O ratio, using BM-OM and DM-OM as covariates, indicated that the FB genotype had larger relative total muscle mass and total organ mass compared to the other two genotypes, but there was no difference between the genotypes for relative wet M:O ratio. However, the dry M:O ratio was greater in the FB and SB genotypes compared to the L genotype. The water content of the total muscle mass was also greater in the FB genotype compared to the other two genotypes.

Summary of the Diet Results

The L birds fed a broiler diet had significantly larger relative leg muscle, carcass mass and total fat content compared to those fed a layer diet. In contrast, the birds fed a layer diet had significantly larger relative intestine and liver masses compared to those birds fed a broiler diet.

3.5 Discussion

In the present study, the effect of genetic selection for one primary criterion, namely fast growth rate in broiler chickens, as well as other secondary criteria e.g. improved feed efficiency, reduced fat content, was assessed on a number of morphological variables in one species of bird. However, it must also be noted that within this comparison the modern broiler was compared with a slower growing broiler which had been selected for increased growth rate until 1972 and then artificial selection ceased and also compared to the layer chicken, which has also been subjected to intensive selective pressures. The selection criteria in this case have mainly consisted of reproductive pressures, i.e. increased egg production, increased egg size, improved egg quality etc. (Gowe & Fairfull, 1995). Therefore, it is possible that the selective pressures placed on the layer chicken may also have had consequences on

organ mass. The layer may have directed more of its resources in producing its reproductive organs and therefore it may have to sacrifice the size of other organs to compensate.

Not unsurprisingly, it was established in the present study that intensive genetic selection has had a profound impact on internal organ morphology of the modern broiler. A comparison of growth rates in the three genotypes (Figure 3.4A,B) indicated that where growth rate was maximum (this is shown for male FB's in Figure 3.4B), it was reached earlier in the FB genotype followed by the SB genotype and L genotype at 35, 49 and 63 days respectively. These data are entirely expected since such changes have been the focus of intensive genetic selection for several decades.

3.5.1 Comparative Morphology in Absolute Terms

As previously reported in the results section, there were a significant difference in the growth rates and therefore the BM's at a given age, between the three genotypes. Due to the large difference in BM of the genotypes at different ages and the high degree of correlation between the two, this clearly shows that when the organ data is analysed the BM and age must be taken into account as covariates. Although the absolute organ data does not take either of these covariates into account it does give an indication of the trend that is occurring for each organ within each genotype.

When the absolute data for each organ was compared between genotypes at a given age, for example 42 days of age, the FB genotype had greater mass for all the different tissues (Table 7.14-7.17). This was expected since the FB genotype had reached a large BM by this age whereas, the SB and L genotypes had only reached a mid-point or in the early stages of their growth and had not attained their potential mature BM. Mitchell & Smith (1991) compared the small intestines of three strains of chicken similar to those used in the present study at six weeks of age. They illustrated that the absolute total wet and dry small intestine mass was doubled in the relaxed line (SB) compared to the unselected line (L) and almost doubled again in

the highly selected line (FB). Nir *et al.* (1993) also demonstrated that broilers had larger absolute intestine, liver and pancreas mass when compared to layers during the first two weeks posthatch. Most studies comparing the size of organs in various sized animals do not analyse their data in absolute terms but only in relative terms i.e. taking BM in account in the analysis either correctly using BM as a covariate or as percentages or ratios of BM.

The absolute data for the three genotypes were also compared at similar BM's, namely a BM interval of 0-500 g and 2000-2500 g. At the 0-500g BM interval there were no significant differences between the genotypes for any of the organs removed in this study. Within the 2000-2500 g BM interval, the results indicated that the FB genotype had larger pectoral muscle, intestine and liver mass than either of the other two genotypes and the L genotype had larger leg muscle, carcass and brain mass. At the same BM interval the caeca, gizzard, heart and lung mass were not different between the three genotypes. Therefore at a target BM of approximately 2 kg the FB chicken would have larger pectoral muscle with larger intestines and liver mass to absorb and breakdown the increased nutrients required for this larger muscle. Although the cardio-pulmonary system has not increased in mass in line with the other organs therefore this may be a possible breakdown in the physiological systems. Other organs such as the gizzard and caeca have also not increased in mass, this may be due to the high quality, easily digested diets now fed to broilers which reduces the necessity for a gizzard and caeca. Some of the results shown in the absolute data also appeared when these organs were analysed relative to BM and age.

The correlation statistics between the absolute muscle masses and the masses of the individual supply tissues also gave an indication of which organs were affected by the selection for increased performance. It should be noted, however, that due to the small sample size within an age category these statistics are less robust and therefore should only be taken as an indication of what may be happening biologically. Correlation statistics were produced for each genotype separately and there were significant positive correlations between every muscle mass and every supply tissue for each genotype (Table 3.4-3.5). The results indicate that as muscle mass increased so did the mass of each of the other organs within each genotype. There were

significant correlations for both the wet and dry absolute data at the BM interval of 2000-2500 g. The wet intestine, liver and brain mass all correlated with pectoral muscle mass and carcass mass correlated with leg muscle mass. Similarly the dry intestine, liver and brain mass correlated well but not necessarily significantly with pectoral muscle mass, and liver and lung mass correlated with leg muscle mass. These results indicate that the mass of the intestine, liver and brain are intrinsically linked to the increase in muscle mass and therefore these organs have also been affected by the genetic selection for increased muscle mass. Therefore, as muscle mass has increased as a consequence of genetic selection the intestine and liver masses have also increased and the brain mass has decreased. However, the absolute heart mass did not correlate with either the pectoral or leg muscle masses, thus indicating as genetic selection continues there will not be a corresponding increase in heart mass as well as many of the other necessary supply organs.

3.5.2 The Relative Mass of the Demand Tissues

The genetic selection for larger pectoral muscle mass has been the focus of many of today's broiler breeding companies. Therefore, it would be unlikely that an unselected layer genotype would produce more muscle mass than either of the broiler genotypes. As expected, the relative pectoral mass in the present study was greater in the FB genotype compared to the other genotypes when each of the three covariates (BM-OM, LBM-OM and DM-OM) were used in the analysis (Figure 3.7). This shows that the FB genotype was depositing more protein as pectoral muscle compared to the unselected L genotype, as has been previously recorded (Katanbaf *et al.*, 1988b; Remignon *et al.*, 1994; Dunnington & Siegel, 1995). Katanbaf *et al.* (1988b) compared the progeny of a broiler genotype selected for high (HH) and low (LL) 8 week BM at a common BM and age and showed that a larger relative breast muscle mass was evident in the HH progeny at a common age of 56 days. Similarly Dunnington & Siegel (1995) used the same selection criteria, but fed the progeny a high or low protein and energy diet. The HH progeny fed the high protein diet had significantly larger relative breast muscle mass compared to the LL progeny and the birds fed the low protein diet. Remignon *et al.* (1994) compared the skeletal muscle

of fast and slow growing broiler lines at 11 and 55 weeks of age. The relative muscle mass was greater in the fast growing line compared to the slow growing line at both ages. The larger relative skeletal muscle mass in the faster growing broiler line was in agreement with the results from the present study.

There was a significant gender effect on relative pectoral muscle mass, as was expected. However, the females had a larger quantity of pectoral mass compared to the male birds, for each genotype. This result is surprising; it was expected that the male would have a larger muscle mass compared to the female, at a similar age or BM. When the absolute pectoral mass was plotted against age for each genotype, a larger pectoral mass was evident in the males (Figure 7.1). When the absolute data were plotted on a BM basis (wet weight), there was no difference between genders in the FB or SB genotypes but within the L genotype the females had a larger muscle mass (Figure 7.9A). This result was not repeated when the absolute pectoral muscle mass was plotted on a dry BM basis. There was no clear difference between genders within any of the genotypes (Figure 7.9B). If the difference between wet and dry muscle mass is equal to the water content of the muscle then the higher water content of the female muscle does imply that it is less mature than that of the males (Ricklefs *et al.*, 1994). Both sexes within each of the three genotypes compared did not all reach the same BM. However, unlike some supply tissues where most growth occurs in the first two weeks of the bird's life (Katanbaf *et al.*, 1988a cited in Dunnington & Siegel, 1995), the pectoral muscle, as a demand organ, continuously grows throughout the bird's life, but will eventually reach a plateau. These results may imply that pectoral muscles of the two sexes were growing at different rates. Ideally we would want to compare the two sexes of these genotypes at similar BM's at the end of their muscle growth. Thus it may be fair to assume that if the birds from all three genotypes were allowed to reach equivalent BM's the males would have greater muscle mass than females.

Relative leg muscle mass was larger in the L genotype compared to the FB or SB genotypes when BM-OM was used as a covariate in the statistical model (Figure 3.8A). This result was reversed when LBM-OM and DM-OM were used as covariates in the model. Consequently removing either the fat content from the BM

or the water content from the BM and organ mass would result in only the muscle itself remaining. Therefore the results from the analyses using LBM-OM and DM-OM as covariates clarified what the effect of genotype had been on just the muscle without other factors (fat and water) influencing the result. Although there has not been the same focus of genetic selection for leg muscle mass as there has been for pectoral muscle, an increase in relative leg mass in the broiler genotype was expected. This result is consistent with that reported by Katanbaf *et al.* (1988b), where chickens selected for high and low 8 week BM were compared. When the lines were compared at a similar BM (180 g) there was no difference in relative leg muscle mass, whereas at a similar age (56 days) the line selected for higher BM had larger relative leg muscle mass compared to the line selected for low BM.

The difference in pectoral and leg muscle observed in the present study may be explained by differences in water content of the muscle. The water content of muscle has frequently been used as an inverse index of functional maturity in many avian species, specifically precocial birds (Ricklefs, 1985; Ricklefs *et al.*, 1994; Dietz & Ricklefs, 1997). A high water content of muscle generally indicates that it is relatively undifferentiated and therefore less mature. Ricklefs (1985) compared the growth performance and developmental maturity of muscle between selected and unselected lines of both broiler and quail. An increase in the rate of proliferation of cell nuclei in skeletal muscle of the broiler and quail during the early posthatch period was observed in response to selection for increased BM. Dietz & Ricklefs (1997) also compared the relationship between growth rate and the developmental function of the muscle in four precocial species of bird (Japanese quail, Northern Bobwhite, guinea fowl and the domestic turkey) and an altricial avian species (European Starling). The water content of the muscle was used as an inverse index of maturity and a rapid increase in the maturity index with age in all species for both leg and pectoral muscle was demonstrated. After the first week posthatch the maturity index for both leg and pectoral muscle were highest in the larger species (turkey and guinea fowl) than the smaller species (quail and bobwhite) and the maturity indices of muscles in the starling were substantially lower than the precocial species.

The FB chickens, in the present study, had a higher level of hydration compared to the unselected genotypes (Figure 3.20) which indicates that the muscle of fast growing broilers was less mature than the other two genotypes. The level of hydration in the muscle is important with respect to broiler production as the level of water can affect the quality (Voller *et al.*, 1996; Voller-Reasonover *et al.*, 1997) and possibly the palatability of the meat. Although the consumer views these effects on meat quality as an important issue, the water content of muscle does not appear to be a selection criterion used by the broiler breeder industry.

There has been a 43% increase in poultry meat production worldwide in the last 5 years (Berri, 2000). Poultry meat can be sold as a whole product e.g. a piece of breast meat or can be further processed e.g. chicken burger, chicken Kiev etc. Berri (2000) stated that the decrease in age of slaughter of the modern broiler chicken has reduced the intensity of flavour of the meat and that the meat of younger birds exhibit increased tenderness and juiciness. This could be due to the increased water content of the muscle. A common problem associated with pork meat is pale, soft and exudative meat (PSE) i.e. meat which is pale in colour, soft in texture and exudes water when touched (Mahon, 1999). This syndrome is caused by many factors including high temperature and humidity, exhaustion and stress of the animal during transport to the abattoir and at slaughter (Swatland, 1999). All of these factors hasten the drop in pH by increasing muscle glyoclytic activity, the accelerated rigor mortis enhances protein and pigment denaturation leading to PSE (Berri, 2000). A high volume of free water within the muscle may accelerate the rate of glycolysis, therefore a large water content within the muscle could be detrimental in the storage of meat. Pale, soft and exudative meat has recently been reported in the poultry industry (Mahon, 1999; Berri, 2000). Therefore the increase in water content of broiler muscle, shown in the present study, could be an indication of possible future problems in meat quality.

In the present study the carcass, which mainly comprised of the skeleton, skin, feathers and some residual organs like the pancreas and the kidneys, was significantly larger in the L genotype compared to the FB genotype relative to wet and dry BM (Figure 3.9). Relative to LBM, carcass mass was greatest in the FB

genotype compared to the SB and L genotypes. Plavnik & Hurwitz (1982) demonstrated that feather mass alone was significantly greater in a light Leghorn cross compared to a broiler genotype, and was consistently heavier in females compared to males. Williams *et al.* (2000) demonstrated that the cortical bone of the tibiotarsus was less well mineralised and more porous in selected broilers than that of a slow growing broiler genotype. The two genotypes showed many similarities in the development of the tibiotarsus, and the selected genotype produced bone of the correct dimensions to support the greater weights now attained by these birds. However, the quality of bone in this selected genotype was relatively poor in terms of porosity and the mineral content which is likely to reduce effective breaking strength of the tibiotarsus (Williams *et al.*, 2000). Therefore, the results of the current study indicate that the FB genotype was partitioning the nutrients available to them, i.e. the FB birds were preferentially directing their nutrient resources into building some specific tissues i.e. muscle growth, at the apparent expense of the skeleton and feather growth.

3.5.3 Relative Mass of the Supply Tissues

In considering the impact of genetic selection for fast growth rate on the mass of components of the GIT, it is worth noting that improved feed conversion ratio (FCR) has also been included as a key selection criterion in the modern broiler and also the layer genotype. It was not until the 1980's that most large breeding companies began to select for feed conversion as an independent characteristic (Lilburn, 1988). Therefore it must be assumed that in the present study the FB and L genotypes have been selected for improved FCR but that the unselected broiler genotype (SB) has not. Due to this selection pressure one would expect larger GIT components in either the FB or L genotypes compared to the SB genotype. However, although the mass of the intestines were greater in the FB compared to the SB, those of the L genotype were actually smaller than those of the SB. The relative gizzard mass showed a reverse of this relationship, the L genotype gizzard mass was greater than the SB, which in turn was greater than the FB genotype. In addition, the relative mass of the caeca was greater in the SB compared to both the FB and the L

genotypes (Figure 3.11). These data suggest that the selection for improved FCR as well as improved growth rate, has resulted in a decrease in caecal mass relative to BM.

This selection for FCR has undoubtedly influenced, directly or indirectly, the mass of the components of the GIT. These components are of great importance as supply tissues because this is where the intake, digestion and absorption of all nutrients occurs. The size of the GIT has been implicated as a limiting factor in food intake, and subsequent growth in the broiler chicken (Mahagna & Nir, 1996). Shortly after hatching (approximately 3 days), chicks change from relying on an endogenous (yolk sac) to an exogenous (from the diet) food supply. During this transitional period the GIT must develop to accommodate food storage and to conduct the various processes, such as nutrient and water absorption and enzymatic digestion, required to permit intestinal digestion (Dunnington & Siegel, 1995).

It has been demonstrated in some mammalian species that the different components of the GIT have great plasticity and can increase or decrease their size when different stresses are placed upon the animal. Hammond & Diamond (1992) demonstrated this when female mice were compared at different stages during reproduction. The results indicated that during peak lactation and weaning the length of the intestine increased by 17% compared to virgin mice and the wet intestine mass increased by 95% over the same comparison. The plasticity of the GIT has also been observed in avian species. Savory & Gentle (1976 a,b) demonstrated in Japanese quail that the lengths of the small intestine, caeca and colo-rectum were significantly larger in birds fed a high fibre diet compared to a low fibre diet.

The components of the GIT differ considerably in their respective functions. The gizzard, which mechanically grinds ingested food to reduce its particle size and increase its surface area (Klasing, 1998), was larger in the L genotype compared to the FB genotype with SB intermediate, in relative terms in the present study (Figure 3.12). This is consistent with that reported by Nitsan *et al.* (1991) and Plavnik & Hurwitz (1982). Nitsan *et al* (1991) compared gizzard size during fifteen days posthatch of three lines of chicken that varied divergently with regard to BM. The

relative gizzard mass was largest in the fast growing line at day 0 and smallest on Day 15, compared to the slow growing line which had the largest relative gizzard mass at day 15. A similar result was demonstrated by Plavnik & Hurwitz (1982), in a comparison of a broiler and a layer genotype between 3 and 10 weeks of age. The relative gizzard mass was consistently larger in the layer compared to the broiler line. The lack of an increase in gizzard mass in line with BM could possibly be due to the improved nutritional value of the feed that is now used for broilers. As selection has been occurring, the type of feed given to chickens has also improved, this has possibly led to the broiler chicken developing smaller gizzards i.e. a reduced amount of mechanical work done to release the maximum amount of nutrients.

Rapid changes in the morphology of the small intestine occur in the immediate period posthatch - the villi increase in size and number to increase the absorptive surface area of the intestine (Uni *et al.*, 1995; Noy & Sklan, 1996). In the present study the FB genotype had a significantly larger empty intestine mass than the other two genotypes (Figure 3.10). These data agrees with previous studies (Katanbaf *et al.*, 1988b; Mitchell & Smith, 1991; Nir *et al.*, 1993; Dunnington & Siegel, 1995; Jackson & Diamond, 1996; Mahagna & Nir, 1996). Nir *et al.* (1993) compared the organ masses of a broiler and a layer genotype form 0-15 days of age. Their results indicated that the broiler chicken had a larger relative intestine mass than the layer genotype only on day 8 of the study. Mahagna & Nir (1996) demonstrated that broiler-type chickens had a larger small intestine compared to egg-type chickens over a three-week period posthatch. Although it must be noted that the statistical analysis of these data was performed using ANOVA and BM was not used as a percentage or covariate. Jackson & Diamond (1996) revealed that the relative small intestine wet mass was larger by a factor of 2.8 in a broiler chicken compared to its predecessor, the Red Jungle Fowl, at an equivalent BM. There has been a requirement to increase the intestine mass in broilers to cope with the increased food intake in order to sustain a larger, faster growing BM. Food intake is greater in the broiler chicken than layer chickens (Bedford, 1996) although it was not measured in the present study. Therefore the FB birds would require more digestive and absorptive tissue mass to process all the nutrients from a feed which has high levels of protein and energy.

The caeca are the most important site for microbial digestion for most avian species, including chickens (Klasing, 1998). It is here that the digestion of fibre and long-chain polysaccharides occurs, and also where some water reabsorption takes place (McDonald *et al.*, 1995; Klasing, 1998). The SB genotype had a larger relative caecal mass compared to the FB and L genotypes, and there was very little difference between these two genotypes (Figure 3.11). These results indicate that the caecum is not an essential tissue for the intensively selected broiler or its unselected predecessors. This is despite improvements in the nutritive quality of feed, including the use of endogenous enzymes and pro-biotics, which suggest that smaller caeca could be just as effective. Majumadar & Panda (1990) compared the growth of caecectomized broiler chickens to broilers with their caeca intact. They demonstrated that the removal of either one or both caeca did not influence body weight gain. Chaplin (1989) stated that the effects of caecectomy on water intake and output are transitory and that compensatory adjustments are made within 2 to 3 weeks postsurgery allowing the caecectomized birds to eat and gain weight normally. This evidence suggests that the caeca may be an expensive metabolic organ that the modern broiler can afford to sacrifice in order to increase the development and function of other, more essential tissues. It may also suggest that a sufficient population of microbes could be maintained in smaller caeca.

The caeca are evacuated only about once a day and therefore provide relatively stable conditions for microbial proliferation (Mead, 2000). This results in a large and diverse microbial population, occurring at up to 10^{11}/g of caecal content (Mead, 2000). The ingested feed of a bird can significantly influence the microbial populations by either providing fermentable material, i.e. substrate, or changing the environment in which they live, e.g. increasing viscosity (Bedford, 1996). Since bacterial species have different substrate preferences and growth requirements, the chemical composition of the digesta largely determines the composition of the microbial community within the caeca (Apajalahti & Bedford, 2000). If a diet is of poorer quality, nutrients may evade digestion and absorption in the intestine of the bird, which results in the bacterial populations flourishing using these unabsorbed substrates. Therefore, the addition of enzymes to diets can aid in the reduction of the

microbial population by substrate limitation. The release of nutrients from the diet by enzymatic action means that more of the nutrients can be digested and absorbed by the bird prior to the establishment of an environment favourable for bacterial growth (Bedford, 2000). Therefore, it may also be the case that the reduction in relative caecal mass evident in the FB genotype seen in the present study may be due to the improved quality of the diets now fed to the modern broiler. This improvement in the nutritional quality of the diet has led to a more efficient small intestine, increasing its digestive and absorptive capacity, which was also detected by the larger relative intestine mass in the FB genotype. This in turn has led to a possible decrease in substrate matter reaching the caeca and the microbial population within, therefore the caeca has decreased in size accordingly.

The liver is involved in the metabolism of a range of absorbed nutrients, from the deamination of protein to the breakdown of carbohydrates (Stryer, 1988). The results of the present study show that the FB and L genotypes have a larger relative liver mass compared to the SB genotype when BM-OM or DM-OM were used as covariates in the statistical model (Figure 3.13). However when LBM-OM was used as a covariate the FB genotype had a greater liver mass, but there was no difference between the L and SB genotypes. Consequently removing the water content from the BM and organ mass resulted in only the liver itself remaining. Therefore the results from the analyses using DM-OM as a covariate clarified what the effect of genotype had been on just the liver without other factors such as water content influencing the result. The results indicate that the selected broilers have a similar sized liver to the unselected layer which were similar to the findings of Jackson & Diamond (1996). In contrast, Katanbaf *et al.* (1988b) showed that at 56 days of age, chickens with a low BM had significantly larger livers than birds with a high BM. One would have expected a larger liver mass in the FB genotype to match the larger intestinal mass and greater feed intake expected in the FB. This positive relationship between the intestine and liver mass was also confirmed by the significant positive correlations in the absolute dry data (Figure 7.13) and tending towards significance within the wet data (Figure 7.11). Since a larger intestine would be expected to absorb more nutrients, then one would expect a larger liver would also be required to deal with the by-products and metabolism of increased nutrients processed by the

larger intestine. One explanation as to why the L genotype has a similar relative liver mass as the FB genotype is that the L genotype is responding to the higher protein and energy content of the broiler type diet that has been fed to them. Therefore the liver has increased in mass to cope with the increased nutrients from the improved quality of the diet.

Both organs of the cardio-pulmonary system (heart and lungs), which are responsible for delivering oxygenated and nutrient rich blood to the various tissues in the body and removing carbon dioxide and other waste, were larger in the FB genotype compared to the SB and L genotypes (Figure 3.14-3.15). This is almost certainly due to the fact that the larger, faster growing modern broilers with a larger oxygen requirement for their increased growth require a larger heart and lung capacity and this is reflected in the relative mass of these organs in the present study. These results contrast with Katanbaf *et al.* (1988b) who found that at 56 days of age, chickens with a low BM had significantly larger hearts than birds with a high BM. However, the inappropriate use of simple ratios in the analysis of the data by Katanbaf *et al.* (1988b), could account for this discrepancy between the two data sets.

The increased mass of the heart and lungs in the FB genotype compared to the other genotypes implies that during the genetic selection for improved growth rate there has been a reciprocal increase in the cardio-pulmonary system. However, this increase in mass has not been sufficient to reduce the mortality and morbidity due to metabolic disorders such as ascites and sudden death syndrome (SDS). As previously stated (section 1.2.1) the onset of ascites is due to the imbalance between increased growth rate and the pressure placed upon the cardio-pulmonary system to deliver oxygen at an ever increasing rate to the various tissues in the body. There have been various methods introduced to quantify this imbalance including the ascites heart index (AHI), electrocardiograms and neural network analysis (Roush *et al.*, 1997). Each method was produced with the aim of genetically selecting for broiler chickens resistant to ascites. These methods have been successful in their aim when used in research but they have not been established within the commercial context.

The larger relative heart mass shown in the FB genotype may not reduce the risk of the fast growing broilers producing ascites or SDS, since it is not the whole heart that is under pressure during the onset of ascites but mainly the right ventricle of the heart. The increased oxygen requirement of the fast growing broiler causes an increase in its blood flow, which causes an increase in blood pressure required to drive blood through the capillaries in the lung. The right ventricle is thin walled and when pulmonary hypertension occurs, the right ventricle enlarges rapidly in response to the increased workload. If hypertension continues, the right ventricle has to pump against the pressure and the heart muscle wall will thicken and enlarge, thus causing the onset of ascites. The increase in relative heart mass in the broiler genotype, observed in the present study, is an indirect consequence of the genetic selection process. It may improve the liveability of some broiler chickens but overall the increase in total heart mass and more specifically the right ventricle has not been sufficient to overcome the risk of ascites or SDS.

The striking difference between genotypes with regard to brain mass is consistent with that found by Jackson & Diamond (1996), who compared the modern broiler to the original chicken, the Red Jungle Fowl, which has not undergone any artificial selection for fast growth rate, FCR or carcass yield. The larger brain mass shown in the L genotype compared to the other two genotypes indicates that brain mass has presumably been sacrificed in broilers during the selection for increased growth rate (Figure 3.16). Previous research has indicated that for specific brain adaptations there is a link to the number of cells and volume in the brain (Finlay & Darlington, 1995). Therefore this sacrifice could be due to the modern broiler not requiring the same level of sensory equipment to escape predators as its predecessors, due to living in an artificial environment of a poultry house compared to the natural environment of its ancestor the Red Jungle Fowl. The brain is a metabolically expensive organ that may have become unessential in poultry breeding (Jackson & Diamond, 1996), therefore broiler genotypes have preferentially directed resources towards the growth of other tissues.

3.5.4 Link Between Muscle and Organ Masses

Symmorphosis describes the balance in mass and activity between the different components of an animal's physiology (Taylor & Weibel, 1981; Weibel *et al.*, 1991). There is the suggestion that a breakdown in symmorphosis has taken place in the selected broiler chicken and this may explain the emergence of metabolic disorders. This breakdown in balance between tissues may in turn lead to the development of metabolic disorders, due to the imbalance between the oxygen requirement of a larger mass of demand tissues and the ability of a smaller mass of supply tissues to provide oxygen to them.

In the present study the changes in relative organ mass have been genotypically compared system by system, but these changes could either be linked to the demand or supply tissues or indeed both. Therefore to compare this link of M:O ratio one had to statistically compare each side of this ratio i.e. the total muscle and total organ masses as well as the M:O ratio itself. The imbalance between supply and demand tissues may have occurred in the FB genotype compared to the other genotypes. In the present study the gap between supply and demand tissues is wider in the FB genotype than the L genotype indicating that possibly symmorphosis has broken down (Figure 3.19).

The predicted dry M:O values show that the L chickens had a larger dry M:O ratio at a lighter body mass (DM-OM) but that this result was reversed at the heavier body mass where SB and FB chickens had greater dry M:O ratio than the L chickens. Genotype had a significant effect on the total muscle and total organ masses when they were considered separately. The difference in total organ mass was largest in the FB compared to the SB or L genotypes, although this effect of genotype was not consistent for each organ but varied between genotypes for each individual organ (Figure 3.18). A similar result could be seen for the wet M:O ratio, where the FB genotype had a larger total muscle and total organ mass, but no genotype difference was evident for the wet M:O ratio (Figure 3.17).

The organs which appear to have been either sacrificed or are not required to the

same extent in the selected broiler compared to the other genotypes are; the brain, gizzard, caeca and carcass (which mainly comprised the skeleton and feathers). These organs do not increase in mass relative to BM to the same extent as the other supply and demand organs in the FB genotype. The absolute and relative muscle and organ mass of the FB genotype increase in size more rapidly and reach a larger overall mass compared to the unselected genotypes (Figure 3.17-3.19).

In assessing the impact of organ allometry on BM, growth rate and carcass yield, both in the present study and in previous studies, two issues have to be considered. Firstly, how to generate relative data in order to compare organ size in animals of differing BM. The rate or intensity of a physiological process is usually higher in large individuals of a species than in small ones, when compared on a whole animal basis. So part of the variation in most physiological data results from variation in size of the animals being studied (Packard & Boardman, 1999). Therefore it is important to correctly remove the effect of body mass statistically from the data set so that the correct conclusion of the influence of physiological variable being studied can be made (Poehlman & Toth, 1995). The inappropriate use of ratios and percentages in the statistical analysis of many morphological studies causes a lack of consistency and problems when comparing these studies. A large proportion of the previous work carried out in this area have used either ratios, organ mass as a proportion of BM (Katanbaf *et al.*, 1988b; Nitsan *et al.*, 1991; Mitchell & Smith, 1991; Mahagna & Nir, 1996) or organ masses as a percentage of BM (Dunnington & Siegel, 1995) in their analysis, some of which have been referred to in this thesis. It has to be noted that forming a ratio to scale physiological data for variation in body size will not always lead the investigator to arrive at an incorrect conclusion, but without validation using multiple regression or ANCOVA, such results should be accepted with caution. Therefore, the M:O data in the present study has been validated as it was statistically analysed using ANCOVA

The second assumption that has to be considered is that organ size is not always a true reflection of its activity level or capacity. Organ size has been widely accepted as an indicator of organ performance by many studies and in different species; house sparrows (Chappell, *et* al. 1999), mice (Hammond & Janes, 1998) and chickens

(Jackson & Diamond, 1996). The universal assumption is that the larger the organ the larger its capacity (the quantity or volume an organ can contain) and/or activity (the amount of work done by an organ).

The intestine is possibly one of the organs which is more readily used to investigate this relationship between the capacity and/or activity of an organ and its mass, due to the amount of enzymatic activity available for analysis. Jackson & Diamond (1996) investigated the nutrient uptake capacity of the small intestine in broilers, and observed that nutrient capacity increased as a result of an increase in the mass of the entire small intestine, thereby increasing the absorption and digestion area of the intestine. In a similar study by Jackson & Diamond (1995) on the Red Jungle Fowl, there was a significant, positive relationship between the mass and capacity of the intestine, when the relative uptake capacities were plotted against the relative intestinal and liver masses. The plots indicated that they all had similar developmental trajectories i.e. they reached their peak rates at similar timepoints, showing that one is reliant on the other, i.e. mass and capacity are interrelated. Also, Smith *et al.* (1990) showed that a 40% increase in growth rate in a selected genotype of broiler chicken had a 40% increase in villus area. Therefore, an increase in intestine mass could mean a corresponding increase in villi number and size, which in turn could mean an increase in the digestive absorption capacity of the intestine resulting in an increased nutrient supply to other tissues, including demand tissues such as the muscle. Hammond & Janes (1998) demonstrated that kidney mass, as well as liver and stomach mass, increased linearly with protein intake rate in rodents. Thus, organ mass can be a good indicator of the capacity of an organ. Most studies that measure organ mass simply infer that a difference in mass is indicative of a change in capacity or activity of that organ without actually stating it as a fact (Plavnik & Hurwitz, 1982; Katanbaf *et al.*, 1988b; Mitchell & Smith, 1991; Nitsan *et al.*, 1991; Fontana *et al.*, 1993; Dunnington & Siegel, 1995; Jackson & Diamond, 1995; Mahagna & Nir, 1996). As with the present study any changes in mass observed are interpreted as changes in that organs capacity. However, some studies also urge caution in inferring a change in mass being equal to a change in an organ's capacity. Starck *et al.* (2000) indicated that in any research studying the digestive function or the functional plasticity of the intestine a pivotal measurement in any

animal were the intestinal brush-border uptake rates for various nutrients. Noy & Sklan (1996) also indicated the importance of nutrient uptake measurements for examining intestinal capacity.

3.5.5 Limits to Performance

The responses of the different organs to artificial genetic selection in the present study gives an indication of what organs, if any, are placing a constraint on further growth potential. The correlation results of the absolute data indicate that as muscle mass, specifically the pectoral muscle mass, has increased as a consequence of genetic selection the intestine and liver masses have also increased and the brain mass has decreased. Breeders may have indirectly selected broilers for less noticeable traits such as smaller brains, which are of no direct service to broilers but are of indirect service in freeing up biosynthetic energy for the selected traits (Jackson & Diamond, 1996). Due to the significant correlation between absolute pectoral muscle and intestine and liver mass, it appears that the intestine is able to increase its size in response to a selection pressure for increased growth and meat yield (Figure 3.10). Therefore it is unlikely that the digestive capacity of the gut or the breakdown of these nutrients in the liver are the limiting factor in the growth rate and tissue maturation of the modern broiler chicken.

The absolute heart mass correlated with neither the pectoral or leg muscle mass. This implies that during the selection for increased breast meat yield there has not been a respective increase in heart mass. Julian (1997) postulated that this mismatch between the metabolic demands of rapid growth and a bird's cardiopulmonary performance has resulted in the appearance of ascites. Although the relative mass of the heart has increased in the broiler compared to the other genotypes (Figure 3.14) it may not have been enough to cope with the increased workload placed upon it compared to its predecessors. Hence, this will most likely contribute to the increase of metabolic disorders, such as ascites and SDS, in the modern broiler chicken (Julian, 1997).

Even today the broiler breeder industry is still selecting for increased growth rate as well as other traits, but there must be a limit for the broiler to respond to these selection pressures. A relatively new trait that the breeding companies are selecting for is greater saleable meat yield, which is the selection for increased eviscerated carcass mass and reduced eviscera mass (Pollock, 1997). This new selection pressure can only add to the likelihood of the breakdown in symmorphosis. The present study has demonstrated that some of the supply tissues have reduced in size at the expense of producing more muscle. Eventually this sacrifice of tissues will have implications for the health of the broiler and its susceptibility to diseases, and the potential for improved performance (Rauw *et al.*, 1998).

3.5.6 Carcass Chemical Composition

The predicted relative fat content of the chickens was greater in the FB genotype and in the females compared to the males, which is in agreement with Plavnik & Hurwitz (1982) and Havenstein *et al.* (1994b) (Figure 3.21B). In contrast, the ash and CP content were greater in the L chickens compared to both broiler genotypes (Figure 3.21A,C). The greater fat content of the FB chickens is quite unexpected since the genetic selection for leaner chickens is a criterion used in the broiler breeding industry (Mallard & Douaire, 1988).

There tends to be an inverse relationship between the fat and protein content of a carcass (Edwards *et al.*, 1973 cited in Plavnik & Hurwitz, 1982). The greatest CP content was evident in the L genotype males and the least in the FB females. Feather mass is known to be significantly greater in a layer genotype compared to a broiler genotype (Plavnik & Hurwitz, 1982). Therefore, an increase in feathers, which have a high protein content, could also have resulted in the larger CP levels in the L genotype.

The ash content of the carcasses was greater in the L genotype compared to both broiler genotypes which is in agreement with results by Havenstein *et al.* (1994b). This could be due to the larger carcass mass also evident in the L genotype, since the

carcass mass was largely made up of bone. Williams *et al.* (2000) also demonstrated that a selected broiler genotype had lower bone mineralisation, and therefore ash content, compared to a slower growing broiler genotype. The L genotype chickens were reared to an age of 16 weeks. At this age the females would have been ready to lay eggs and therefore these chickens would have been depositing minerals such as calcium in their skeleton in preparation for laying. The larger carcass mass and therefore skeletal mass and the possible high calcium levels could explain the larger ash content shown in the L genotype chickens.

3.5.7 Dietary Effects on Organ Composition

All of the genotypes were fed a standard broiler ration but a second group of the L genotype was also fed a typical layer diet. One group was fed rations identical in protein and energy content to those fed to the broiler genotypes (22.8-19.8 % protein and 12.7-13.5 MJ/kg energy content) and the second group was fed commercial replacement pullet ration (20.5-16.4 % protein and 11.5-11.8 MJ/kg energy content). There was no significant difference in BM between the L birds fed the two different dietary regimes, but the male birds had larger BM than the female birds. The comparison of the two diets fed to the L genotype only resulted in a few significant differences in the morphology results. Overall the birds fed a broiler diet had significantly larger relative leg muscle (Figure 3.23), carcass mass (Figure 3.26) and total fat content (Figure 3.27), whereas the birds fed a layer diet had larger relative intestine (Figure 3.24) and liver masses (Figure 3.25).

As with most layer genotypes, the L chickens used in the present study have been placed under certain genetic selective pressures but not for fast growth rate, rather they have been actively selected for small BM. This lack of selection for growth rate may explain why these birds, when fed the broiler diet, were not stimulated into increasing their digestive capacities by increasing their intestine or liver masses. Or it may also have been the case that the diet was of a better quality (higher nutritive value) and the birds were able to retrieve the nutrients without increasing their digestive capacity. If L chickens were fed a diet lower in both CP and energy (layer

ration), then it would be reasonable to assume that they would have had a higher feed intake (unmeasured) compared to the L chickens fed a broiler ration. The layers fed a CP and energy rich diet would have to consume less feed and therefore their intestines and liver would be smaller.

Although the L genotype has undergone some genetic selection, the selection for increased breast meat yield is not one of the criteria used. Therefore, the L birds fed the broiler diet, which had an increased CP and energy content, they had a greater leg muscle, cacass mass and fat content. Also the increase in carcass fat evident in the L genotype fed a broiler diet is not unexpected due to the higher fat content of the broiler diet compared to the layer diet (Table 7.2-7.3). Havenstein *et al.* (1994b) compared two genotypes of chicken on an old style diet (1957) to a modern style diet (1991). The 1991 diet contained a higher level of protein (22.5-19.0 %) and energy (13.0-13.2 MJ/kg) compared to the 1957 diet (21.7-16.9 % protein and 12.0-11.9 MJ/kg energy). They reported that the carcass fat of the birds was larger in the chickens fed a 1991 diet than a 1957 diet, therefore similar to the findings in the present study. However, Dunnington & Siegel (1995) compared two genotypes of chicken on a low (20% protein and 11.3 MJ/kg energy) and high (24% CP and 13.56MJ/kg energy) quality diets and they demonstrated no difference in abdominal fat in birds fed the two diets. Although birds fed the lower quality diet did have larger relative intestine mass, which was also demonstrated in the present study. Boa-Amponsem *et al.* (1991) also compared two genotypes of chicken on high (24% protein and 13.2 MJ/kg energy) and low (20% protein and 11.3 MJ/kg energy) quality diets. At 35 days of age the relative leg and carcass mass were significantly greater in birds fed the high quality diet. In contrast, the relative gizzard, small intestine and caeca-colon were larger in the birds fed the low quality diet, probably because the birds consumed more of the low quality diet. Some of these results were in agreement with those in the present study.

3.5.8 Summary

The genetic selection of future generations of broilers is aimed towards increased breast muscle as well as increased growth rate, decreased fat and a decrease in eviscera mass (Pollock, 1997; Le Bihan-Duval *et al.*, 1998). Already breeding companies are assuring the industry that growth will continue to progress at a rate of half a day per year (Pollock, 1997; Urrutia, 1997). This will cause a further increase in the pressure on the supply tissues to deliver oxygen and nutrients to the growing demand tissues. This selection has already had a profound effect on the chicken's gross morphology illustrated by the difference in mass of the various organs between the different genotypes. The present study has shown that the FB had more relative muscle mass, both breast and leg, they also had a larger relative intestine, heart and lung mass. Whereas the layer had a larger relative carcass, gizzard and brain mass and the SB had a larger caeca mass. Therefore the selected broiler has sacrificed the relative size of the brain, gizzard, caeca and carcass, indicating a breakdown in symmorphosis. There must be a limit to the ability of the broiler to respond to the selection pressure on the supply tissues by reducing the size of certain organs to produce more muscle. This in turn will have implications on the health of the broiler and its susceptibility to metabolic diseases, and the potential for improved performance.

CHAPTER 4

4. THE EFFICACY OF USING NEAR-INFRARED REFLECTANCE SPECTROSCOPY (NIRS) FOR THE DETERMINATION OF POULTRY CARCASS TRAITS

4.1 Introduction

4.1.1 The Benefits of Near-Infrared Spectroscopy

The conventional way of analysing feed, carcass and excreta samples is by wet chemical analysis, such as fat by ether extraction, protein by Kjeldahl and ash by burning in a muffle furnace. Even with automated or semi-automated equipment this can be a very time consuming and expensive process. An alternative approach is to use near-infrared reflectance spectroscopy (NIRS) which is less labour intensive once a spectral database of results is produced. Initially NIRS was used to evaluate the nutritional attributes of animal foodstuffs such as cereals, grass and grass silage (Murray, 1993), e.g. by using NIRS one could predict the carbon content of pine needles with 99% certainty (Table 4.1). Today NIRS is also used on animal excreta (Neumeister *et al.*, 1997), animal and plant tissues (Bolster *et al.*, 1996; Young *et al.*, 1997; Flinn *et al.*, 1998; Gillon *et al.*, 1999) and composite feed mixtures (Murray, 1993).

The development of NIRS has occurred in the latter half of the twentieth century. It was first developed by Dr Karl Norris as part of a selection programme to improve cereal grain moisture in 1964 (Norris, 1964 cited in Murray, 1993). This rapid development of NIRS as a technique to measure the chemical composition of agricultural foodstuffs and other agricultural products, has mainly been due to NIRS being a rapid, cheap and nonhazardous tool to implement, compared to conventional methods. It takes a matter of minutes to analyse a sample using NIRS compared to

hours or days in a laboratory. Minimal sample preparation is required and there are no expensive reagents to buy. In addition, there are no hazardous procedures associated with the NIRS technique compared to conventional laboratory methods (e.g. acid digestion, use of heating blocks, ether evaporation).

Table 4.1 Published statistics for near-infrared reflectance spectroscopy calibration of various plant materials and prediction of descriptor in the crop material.

Crop	[1]*n*	[2]r^2	Descriptor	Reference
Pine needles	84	0.99	Carbon	Gillion *et al.*, 1999
Pine needles	84	0.94	Nitrogen	Gillion *et al.*, 1999
Chickpeas (whole)	248	0.94	[3]CP	Flinn *et al.*, 1998
Field peas (whole)	322	0.92	CP	Flinn *et al.*, 1998
Oat	103	0.98	CP	Bruno-Soares *et al.*, 1998
Barley	104	0.98	CP	Bruno-Soares *et al.*, 1998
Wheat	115	0.97	CP	Bruno-Soares *et al.*, 1998
Ryegrass	126	0.98	CP	Bruno-Soares *et al.*, 1998
Sorghum	112	0.98	CP	Bruno-Soares *et al.*, 1998
Legumes	59	0.95	Nitrogen	Ruano-Ramos *et al.*, 1999
Forbs	57	0.96	Nitrogen	Ruano-Ramos *et al.*, 1999

Where; [1] the number of samples scanned by NIRS, [2] the coefficient of determination i.e. the certainty of the NIRS prediction, [3]CP is crude protein.

4.1.2 Theoretical Background of Near-Infrared Spectroscopy

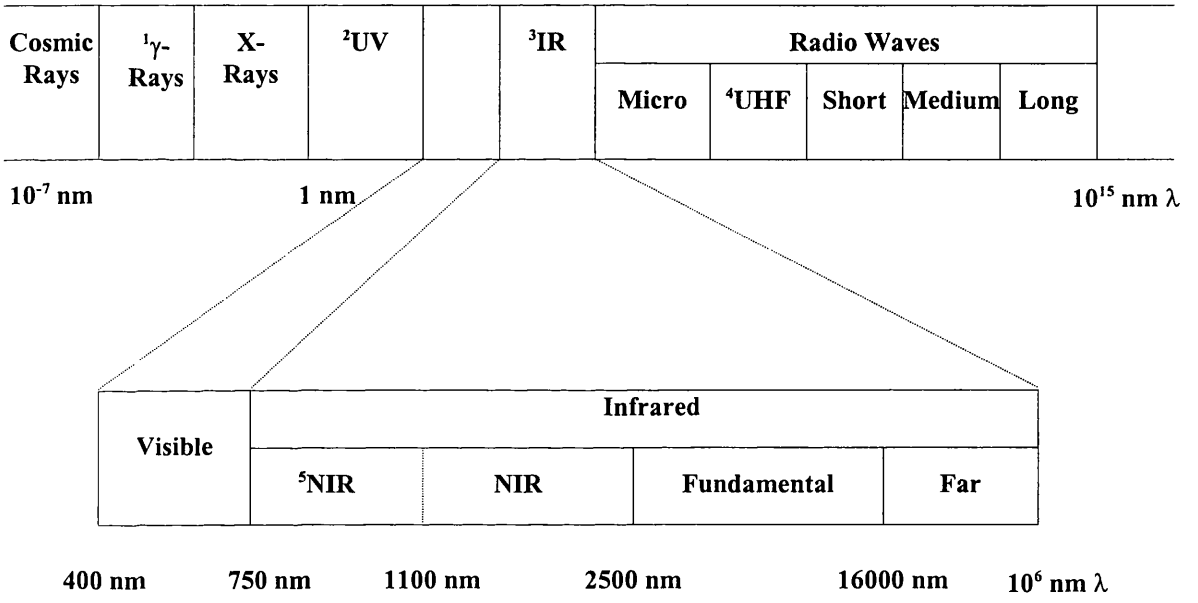
The near-infrared region of the electromagnetic spectrum is located between the visible and the infrared regions of the spectrum (Figure 4.1). Near-infrared reflectance spectroscopy uses a beam of light passed through a monochromator which travels through the sample. The beam of light is either absorbed, transmitted or reflected by the sample, detectors measure the light reflected and a spectrum is produced. The reflectance signals resulting from bending and stretching vibrations of hydrogen (H) covalently bonded to either carbon (C), nitrogen (N) or oxygen (O) atoms. The NIRS then uses these reflectance signals to measure the concentration of

major classes of chemical compounds in organic materials (Bolster *et al.*, 1996).

The reflected, transmitted and absorbed radiation must sum to the incident radiation as follows;

$$I_O = I_A + I_R + I_T$$

Where I_O is the intensity of the incident radiation on a sample, some of the photons may be transmitted through the sample (I_T), reflected from the sample (I_R) or absorbed (I_A) by the resonating covalent bonds within the sample (Murray, 1993).



[1]gamma rays, [2]UV-ultra-violet, [3]IR-infrared, [4]UHF-ultra high frequency, [5]NIR-near-infrared.

Figure 4.1 Electromagnetic spectrum with infrared region amplified (from Ferraz de Oliveira, 1998).

The reflectance spectrometer is able to measure the I_O using a reference tile (a reflecting white tile having no absorption features). The sample, which is an opaque pack, will cause I_T to be zero, the detectors can measure I_R, and I_A can be obtained by the difference (Figure 4.2).

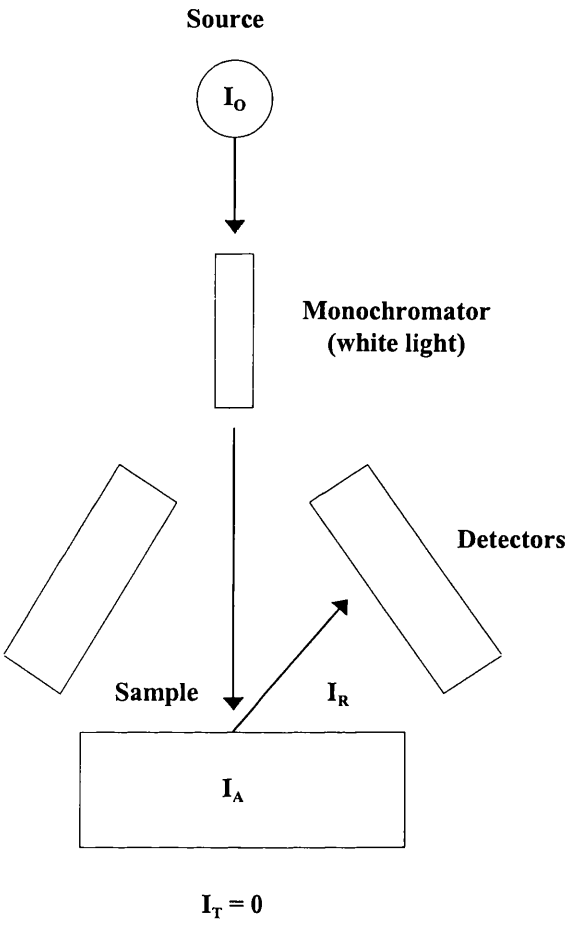


Figure 4.2 Diagram of near-infrared reflectance instrument (from Murray, 1993).

When the vibrations of the NIR radiation are at the same frequency as a molecular bond in the sample scanned, due to a displacement charge produced by the molecule, an absorption band is generated in the spectrum. Therefore, the more molecules of a certain type present in a sample, the more energy will be absorbed at wavelengths specific to those molecules. The NIRS region consists of overtone (between 1000 and 1900 nm) and combination (between 1900 to 2500 nm) bands. For each absorption band there are a series of overtones, which are bands where the same

molecular bonds are being detected, which become less intensive over the region. Each absorption band in the spectrum may be a composite of several bands containing information on more than one type of molecular vibration.

The general location of generic functional groups vibrational information in the NIRS spectra has been intensively studied (Shenk *et al.*, 1992 cited in Ferraz de Oliveira, 1998). There are certain absorption peaks within the NIRS spectrum which are prominent and it is known which bonds are vibrating at these wavelengths; these include H_2O, with two peaks at 1940 and 1445 nm; aliphatic -CH peaks (lipids) at 2310, 1725, 1400 and 1210 nm; -OH peaks (carbohydrates) around 2100 and 1600 nm (Cozzolino, 1998).

It is necessary to collect the spectra of a large number of analysed samples and to use mathematical modelling to extract useful information from the spectra. This correlation transform process is called calibration (Murray, 1993). The determinations of chemical composition using NIRS are based on a calibration that relates the NIRS spectral data to the analytical composition of samples obtained by reference methods (Berzaghi *et al.*, 1997). A condition of NIRS used for this purpose is that the accuracy and precision of the reference values for the calibration data set inpart determines the quality of the predictions made by NIRS (Foley *et al.*, 1998).

A database of the chemical composition of carcasses from three different chicken genotypes over a long growth period was generated using conventional wet chemistry. The objective of the present study was to produce calibration data and predictive equations for crude protein, fat and ash content of these chicken carcasses using NIRS and to thus evaluate the robustness of NIRS as a predictive tool. The ability of NIRS to discriminate between the different chicken carcasses on the basis of different carcass descriptors was also evaluated. In essence, the database and the NIRS spectra would allow one to identify to which genotype an unknown carcass would belong.

4.2 Materials and Methods

4.2.1 Study Animals

Three genotypes of chicken (*Gallus gallus domesticus*) which differed in growth rate and body composition were used in this study. All three genotypes were fed a standard current commercial broiler ration (22.8-19.8 % protein and 12.7-13.5 MJ/kg energy content). In order to ensure that any inter-genotype differences in morphology were not simply due to differences in the energy and protein contents of the diet, one of the genotypes, the layer chickens, were split into two groups and fed two different rations. One group was fed the standard broiler ration and the second group was fed commercial replacement pullet ration (20.5-16.4 % protein and 11.5-11.8 MJ/kg energy content) (Table 3.1). Water and food were available *ad libitum*. The full management details are in Section 3.2.

After dissection, each carcass (the full carcass including feathers, feet, skin, etc. but excluding gut contents) was dried to a constant mass in an oven at 70 °C ± 5 °C, milled and proximate analysis performed. The ash content was determined by combustion in a muffle furnace for 24 h at 500 °C ± 50 °C, the lipid content by the Soxtec petroleum ether extraction method and the nitrogen and CP content by the Kjeltec or Leco analyser method (N X 6.25) (Proximate analysis methods described in full in Appendix 1).

4.2.2 Near-Infrared Spectroscopy Method

The same carcass samples were then scanned with a NIRSystems 6500 visible-NIR spectrophotometer (Foss Electric, UK) (400-2500 nm). A dried, milled, homogenous sub-sample from each carcass was scanned as a packed powder in a 55mm diameter quartz covered reflectance cuvette. The NIRS reflectance measurements were transformed into the log of the inverse of reflectance (log 1/R).

The parameters examined were: sex, age, genotype, body mass (BM), growth rate (GR), and body composition; CP, fat and ash (g/kg).

The NIRS instrumentation was allowed to warm up before scanning any samples. Diagnostic tests were performed on the equipment to check the photometric repeatability, the wavelength accuracy and the instrument response. Once a day, before the NIRS was used, an internal ceramic reference tile was scanned to check the photometric repeatability or "noise" level. The wavelength accuracy was verified by scanning an internal polystyrene standard paddle. Lastly, in order to test the instrument response, the measurement of the absolute reflectance from the ceramic tile was checked (Cozzolino, 1998).

4.2.3 Statistical Analysis

Each sample that was scanned produced its own individual spectrum. Principal component analysis (PCA) was carried out on the NIRS spectra. Principal component analysis uses the best linear vector to explain the variance between the NIRS spectra and this first vector can explain almost 40% of the variance. Most of this first PC variance arises from the baseline offset resulting from particle size effects in reflectance mode. The NIRS then chooses the next orthogonal vector i.e. the next perpendicular line that is completly different to any other line, to explain the residual variance, this continues until most of the variance is explained. In all, 32 PC's were used to explain the variance within the current data set. The data were corrected using standard normal variate (SNV) and detrend transformations. These are mathematical treatments of the data to minimize the effects of scatter. The standard normal variate effectively removes the multiplicative interferences of scatter and particle size (Barnes *et al.*, 1989). The SNV was calculated on the log 1/R spectra at each wavelength, this transformation removes slope variation on an individual sample basis using the following equation,

$$SNV_{(1\text{-}700)} = \frac{(y_{(1-700)} - \bar{y})}{\sqrt{\frac{\sum (y_{(1-700)} - \bar{y})^2}{n-1}}}$$

where $SNV_{(1\text{-}700)}$ are the individual standard normal variations for 700 wavelengths, y is the 700-wavelength log 1/R values, and $\bar{y}$ is the mean of the 700-wavelength log 1/R values (Barnes *et al.*, 1989).

The PCA of the SNV spectra of a given material produces an almost linear slope, but it becomes curvilinear for the spectra of densely packed samples, since the curvature varies with particle size and packing density. A second-degree polynomial transformation was then used to standardise the variation in curvilinearity, which is known as the de-trending of NIR spectra (Barnes *et al.*, 1989).

The use of derivatives in spectroscopy refers to algebraic differences between data at closely spaced wavelengths to approximate derivatives of various orders. A first derivative calculates the slope of a region of the spectrum whilst the second derivative calculates the change in slope (Cozzolino, 1998). A 1,4,4,1 mathematical treatment was used in this study where the numbers refer to the following: the first number refers to the order of the derivative 0 = log 1/R; 1 is the first derivative of log 1/R; 2 is the second derivative of log 1/R; the second number refers to the gap in nm over which the derivative is calulated; the third number refers to the number of nm used in the first smoothing; the fourth number refers to the number of nm over which the second smoothing is applied (Cozzolino, 1998).

The robustness of the NIRS calibration equations developed using Partial Least Squares (PLS) for predicting carcass descriptors was tested using internal cross-validation. Partial least squares chooses the vector that minimises the predicted error sum of squares of the reference data. It calculates for each principal component the loadings or weights for each wavelength, using information on chemical concentrations, so that the variation relevant to the modelling of the chemical variation in the data is described in the first PLS factors (Bolster *et al.*, 1996). Within each analysis the software offers the option of removing any outliers from the

database. The more variability in the data set, the more outliers are removed in "passes". There are drawbacks to removing outliers that should be ackowledged since removing outliers reduces the natural variability within the data set. Also, the outlier may not be an error, which is assumed in the removal process, but an important data point within the data set. In our analysis two passes was the option chosen to eliminate any outliers from the reference data (t) or the NIRS spectra (H), and 259 variables were used between the 400-2500 wavelengths which were also used for the calibration. It should be noted that two types of outliers exist; t-statistic outliers in which the predicted values do not accord with the chemical reference analysis data (such t-statistic outliers should be subjected to a repeat reference analysis) and H-statistic outliers in which the multivariate H-statistic (Mahalanobis distance) suggests that the spectra of this sample are atypical of those which compose the entire sample population.

The standard error of calibration (SEC) can be defined as:

$$\mathrm{SEC} = \frac{\sqrt{\sum (Y - M)^2}}{n - 1 - p}$$

where, Y is the laboratory reference value, M is the NIRS measured value, n is the number of samples and p is the number of terms in the model (Murray, 1993).

The coefficient of determination (r^2), or total explained variation, depends on the spread of the reference values as much as it does on the goodness of fit, and it measures the predictive ability of the equation only relative to the range of reference values in the calibration set (Gillion *et al.*, 1999). When values of r^2 are less than 0.75, regression modelling becomes increasingly unreliable, more samples are required and NIRS calibration will be difficult to validate (Murray, 1993).

The standard error of cross validation (SECV) and the coefficient of determination of the cross validation (1-VR) statistics are the same as SEC and r^2 but apply to the cross validation. So, the closer 1-VR is to 1.00, the more robust is the prediction. The cross validation involved selecting random sub-sets of data and comparing these

against the full data set using modified partial least-squares (MPLS) regression and this produced an estimate of the resulting coefficient (1-VR). The closer 1-VR is to one the stronger the relationship between the reference data and the NIRS predictions within the cross validation.

All of these statistics were used to characterise the different equations obtained and to determine the best calibration equation to fit the data (ISI software Infrasoft International, Port Matilda, PA, USA).

4.3 Results

4.3.1 Reference Data

The means, standard deviations (SD) were calulated using the reference data (Table 4.2). Since the birds were grown over a long time period this caused a considerably large SD within the data set (Table 4.2). Also the standard error of laboratories (SEL) were calculated using the reference data (Table 4.3). The calculation of the SEL checks for accuracy in the duplication of laboratory analyses.

Table 4.2 Carcass descriptors in the pooled data set, using reference methods.

Descriptor	[1]n	Min	Max	Mean	[2]SD
Age (d)	258	4.00	111	39.8	31.2
[3]BM (g)	258	39.9	2907	852	712
[4]GR (g/d)	258	1.95	69.7	19.3	13.3
[5]CP (g/kg)	258	415	781	599	58.9
Fat (g/kg)	258	96.1	438	255	67.2
Ash (g/kg)	258	52.3	144	96.5	15.0

Where; [1]number of samples, [2]standard deviation, [3]body mass, [4]growth rate, [5]crude protein.

The SEL was calculated for the different genotypes of chicken as follows,

$$SEL = \sqrt{\frac{\sum(y_1 - y_2)^2}{2n}}$$

where, y_1 and y_2 are duplicates of a sample and n is the number of samples in the data set. The closer the SEL value is to zero the stronger the laboratory replication. If the SEL is too large then the explained variance r^2 will decrease (Murray, 1993). Within the current study the best SEL was for CP and the worst was for fat with ash being intermediate.

Table 4.3 The standard error of laboratory of the reference data for each genotype of chicken.

Carcass composition	Genotype		
	[1]FB	[2]SB	[3]L
[4]CP (g/kg)	0.70	2.87	2.30
Fat (g/kg)	14.1	12.6	10.3
Ash (g/kg)	4.37	3.87	5.04

Where; [1]fast broilers, [2]slow broilers, [3]layers, [4]crude protein.

4.3.2 Correlation Statistics

There was a high degree of correlation between a number of carcass descriptors (Table 4.4). Body mass was positively correlated to age (R^2= 0.782), GR (R^2= 0.658) and fat (R^2= 0.432) and negatively correlated to ash (R^2= -0.215), but was not correlated to CP. In contrast, age correlates well with CP (R^2= 0.204). As would be expected, fat was negatively correlated with CP (R^2= -0.787) and ash (R^2= -0.656), and ash was positively correlated to CP (R^2= 0.647).

Sex and genotype were statistically analysed using one-way ANOVA. There was a significant effect of genotype on CP ($P \leq 0.05$), fat ($P \leq 0.01$) and ash ($P \leq 0.01$)

content of the carcass. The fat content was not affected by sex but there was a significant effect on CP ($P \leq 0.01$) and ash ($P \leq 0.01$) content.

Table 4.4 Intercorrelation between carcass descriptors in the pooled data set.

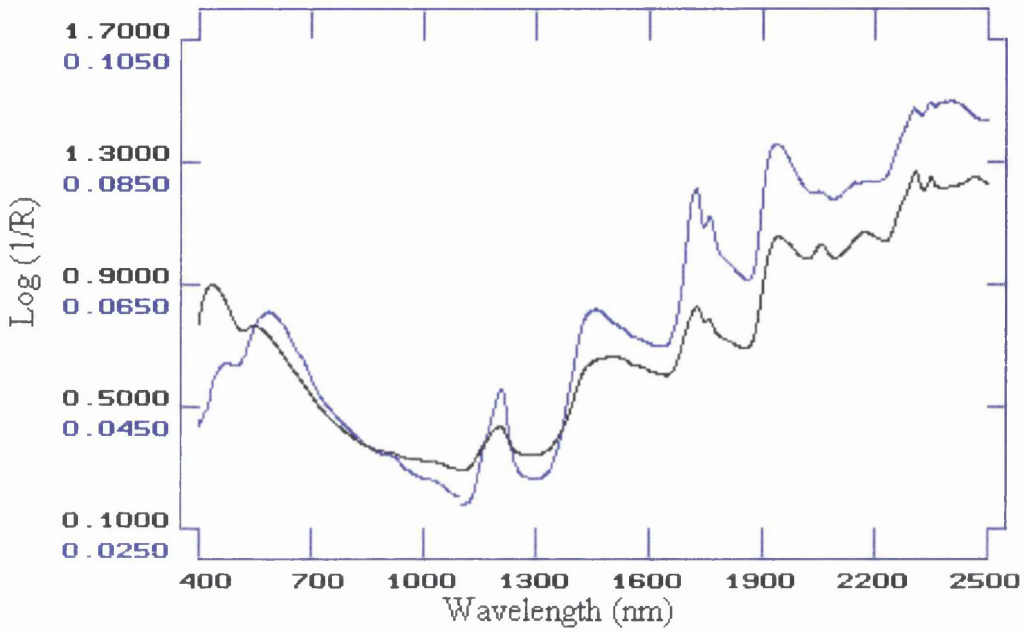
	Age	[1]BM (g)	[2]GR (g/d)	[3]CP (g/kg)	Fat (g/kg)	Ash (g/kg)
Age	1	0.782	0.118	0.204	0.156	0.118
[1]BM (g)		1	0.658	-0.169	0.432	-0.215
[2]GR (g/d)			1	-0.606	0.637	-0.531
[3]CP (g/kg)				1	-0.787	0.647
Fat (g/kg)					1	-0.656
Ash (g/kg)						1

Where; [1]body mass, [2]growth rate, [3]crude protein.

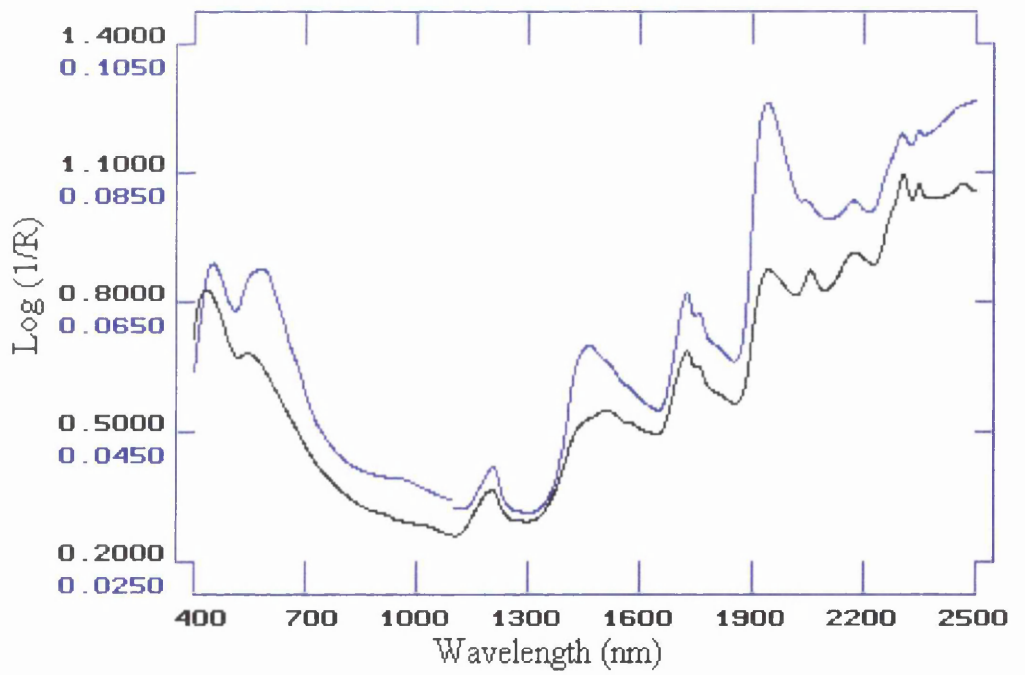
4.3.3 Genotype Comparison

All carcass samples were scanned and individual spectra produced. A mean spectrum and the SD of that spectrum were generated for each genotype of chicken (Figure 4.3A-C) and for all genotypes combined (Figure 4.3D). The NIRS region shows absorption bands around 2350 nm due to -CH and -CH combination, around 2200 nm due to -NH and -OH combination, around 1950 nm due to H_2O first overtone, around 1800 nm due to -CH first overtone, around 1500 nm due to the -NH first overtone and around 1200 nm related to the -CH second overtone (Murray, 1993). The different absorption bands reflect the appearance of different bonds for example, in Figure 4.3d the labels 1, 2 and 3 are peaks which reflect the appearance of -NH, -OH and -CH bonds respectively.

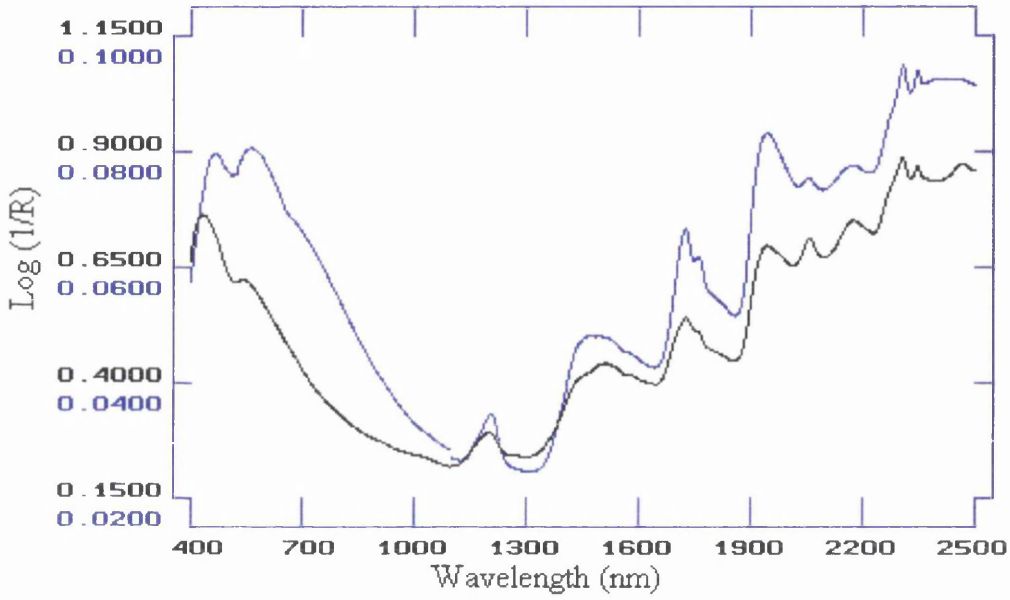
(A)


1.7000
0.1050
1.3000
0.0850
0.9000
0.0650
0.5000
0.0450
0.1000
0.0250
Log (1/R)
400
700
1000
1300
1600
1900
2200
2500
Wavelength (nm)

(B)


1.4000
0.1050
1.1000
0.0850
0.8000
0.0650
0.5000
0.0450
0.2000
0.0250
Log (1/R)
400
700
1000
1300
1600
1900
2200
2500
Wavelength (nm)

(C)



(D)

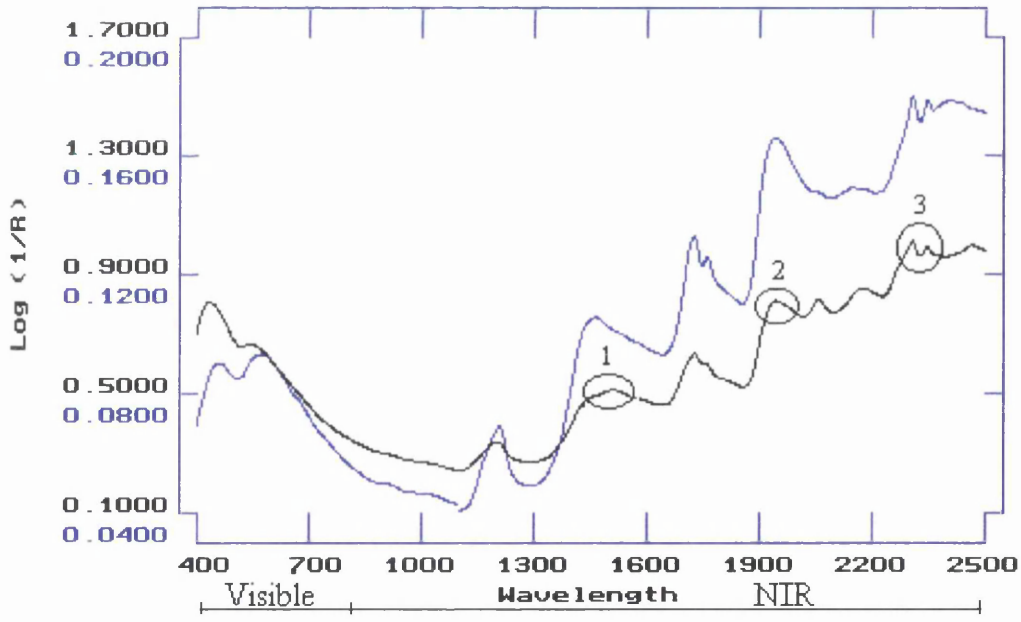


Figure 4.3 Near-infrared reflectance mean absorption spectrum (black) and the standard deviation (blue) of the (A) fast broiler, (B) slow broiler and (C) layer genotypes of chicken. The three genotypes of chicken were combined (D) and a mean and standard deviation produced. In panel D, the numbers circled, 1, 2 and 3, are peaks which reflect the appearance of -NH, -OH and -CH bonds respectively.

4.3.4 Near-Infrared Spectroscopy Statistics of the Pooled Data Set

Although 32 PC factors were used initially to explain the variance in the data set, the first three principal component factors were sufficient to discriminate and cluster the different genotypes of chicken (Figure 4.4). The distance of any one sample from the centroid of a cluster characterises its closeness to the average of its kind, while distances to its nearest neighbours determines if the sample is unique or if there are others showing the same trait (Murray, 1996).

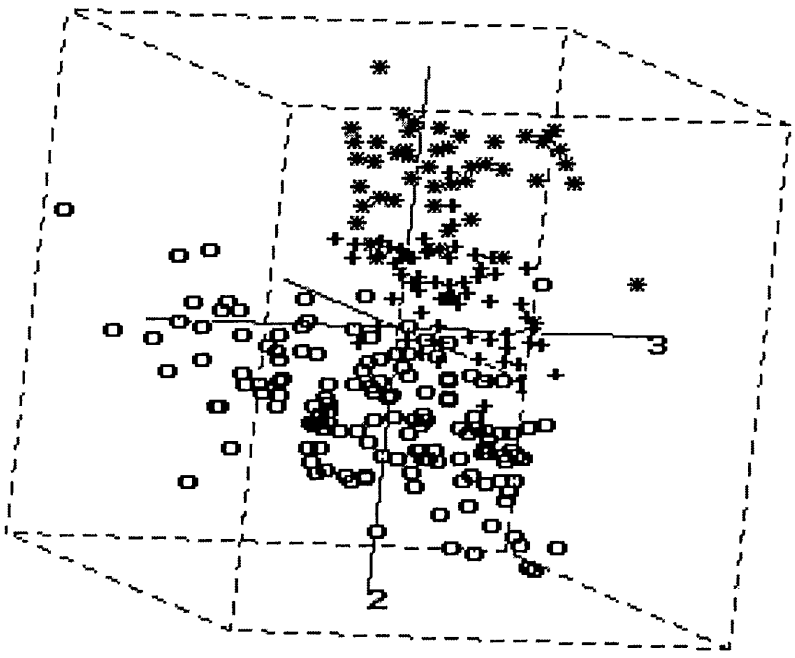


Figure 4.4 Discrimination of three different chicken genotypes using the first three principal components of near-infrared reflectance spectra on the whole data set of chemical composition. Chickens of the same genotype are clustered into groups as follows; fast broiler (*), slow broiler (+) and layer (O). Where 1, 2 and 3 are the principal component factors used in the analysis.

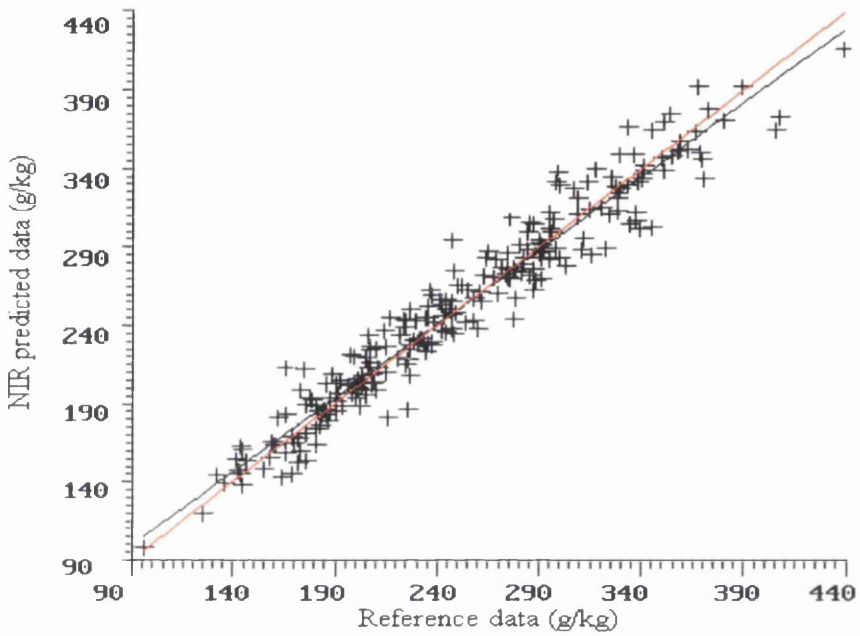
The high r^2 and 1-VR values in the NIRS calibration statistics indicate that both the calibration and cross validation models were strong for all carcass descriptors (Table 4.5). The strength of the calibration equations to predict carcass composition are shown as high r^2 values of 0.93 for fat, 0.84 for CP and 0.65 for ash (Figure 4.5a-c). These figures show the relationship between the chemical reference data and the NIRS predicted data for each of the main chemical components of the carcasses. Each figure shows the line of best fit or regression line, and the line showing the closeness to a perfect fit by NIRS. The other carcass descriptors, such as genotype, age, BM and GR could also be predicted very well using NIRS (r^2= 0.91 - 0.96). However, NIRS was poor at discriminating between chicken carcasses of either sex (r^2= 0.33). The internal cross validations also followed the same trends as the calibration equations.

Table 4.5 The near-infrared reflectance spectroscopy calibration and cross validation statistics for carcass descriptors in the pooled data set of three chicken genotypes. The NIR statistics for the effect of diet apply to the layer genotype data set only.

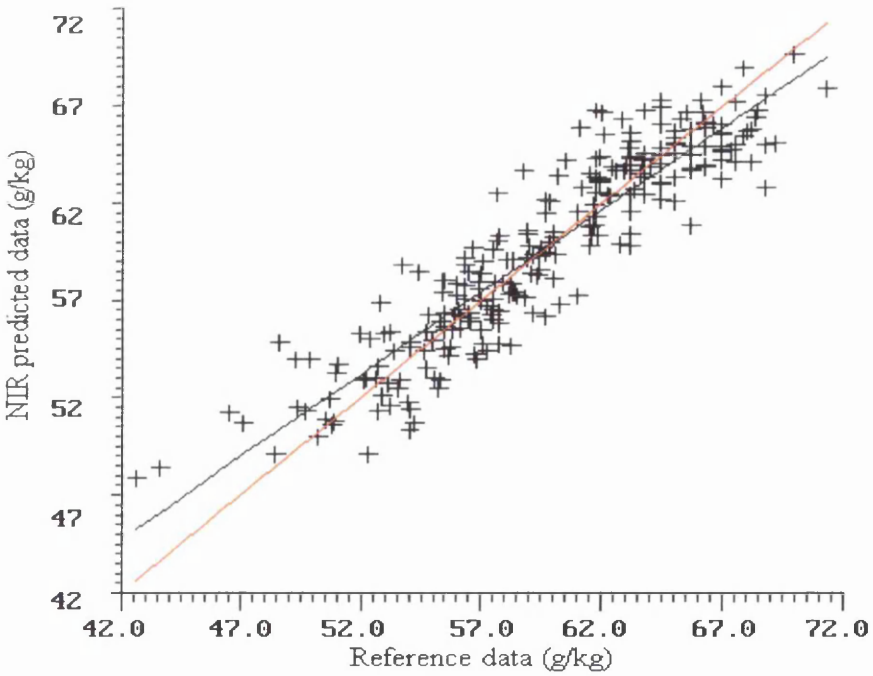
Descriptors	[1]n	[2]SEC	[3]r^2	[4]SECV	[5]1-VR	[6]T	[7]OLLN
Sex	255	0.41	0.33	0.42	0.31	2	3
Age (d)	254	6.40	0.96	7.12	0.95	8	4
Genotype	251	0.20	0.94	0.22	0.92	7	7
[8]BM (g)	247	201.1	0.91	228.8	0.88	7	11
[9]GR (g/d)	250	3.52	0.91	4.11	0.87	7	8
[10]CP (g/kg)	249	21.46	0.84	21.95	0.83	2	9
Fat (g/kg)	242	15.92	0.94	16.39	0.94	3	16
Ash (g/kg)	245	8.04	0.66	8.18	0.65	2	13
Diet	139	0.21	0.82	0.28	0.69	9	5

Where; [1]number of samples, [2]standard error of calibration, [3]coefficient of multideterminationin calibration, [4]standard error of cross validation, [5]coefficient of determination in cross validation, [6]number of PLS factors used to perform the calibration models, [7]number of outliers removed to perform the calibration models, [8]body mass, [9]growth rate, [10]crude protein.

(A)


NIR predicted data (g/kg)
440
390
340
290
240
190
140
90
90
140
190
240
290
340
390
440
Reference data (g/kg)

(B)


NIR predicted data (g/kg)
72
67
62
57
52
47
42
42.0
47.0
52.0
57.0
62.0
67.0
72.0
Reference data (g/kg)

(C)

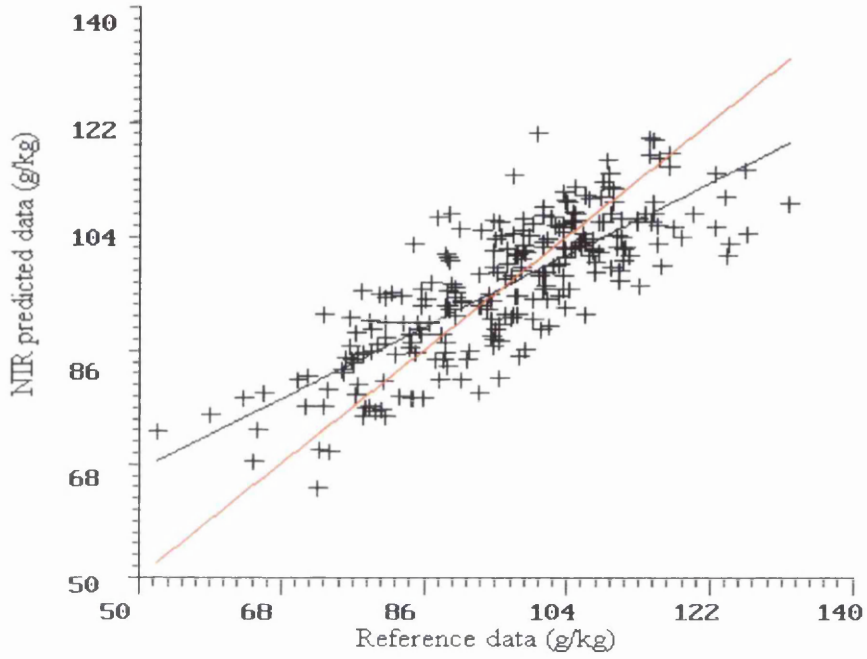


Figure 4.5 The relationship between the chemical reference data and the near-infrared reflectance spectrometry predicted data for (A) fat, (B) crude protein and (C) ash content (g/kg) of chicken carcasses from the pooled data set. The regression line of best fit for the data (black) and a line showing the closeness to a perfect fit by near-infrared reflectance spectrometry (red) are shown.

Cozzolino (1998) stated that the suitability of the reference methods for NIRS calibration can be classified according to the relationship between the error in analysis and the spread in composition. If a parameter shows a narrow range in composition, or if the error in estimation is large compared with the spread (SD) in composition, then regression finds increasing difficulty in finding stable NIRS calibrations. Therefore, the ratio between the SECV and the SD of the pooled data set indicates the strength of the calibration model. When the error exceeds $^1/_3$ or 0.33 of the SD of the population, regression can be misleading (Cozzolino, 1998). The SECV/SD ratios calculated here confirmed that age, genotype, GR and fat had strong calibrations, whereas, BM, CP and ash were poorer and sex did not calibrate at all well (Table 4.6).

Table 4.6 Suitability of the calibration models for the pooled data set.

Descriptors	[1]SD	[2]SECV	SECV/SD
Sex	0.50	0.42	0.84
Age (d)	31.4	7.12	0.23
Genotype	0.78	0.22	0.28
[3]BM (g)	673	229	0.34
[4]GR (g/d)	13.3	4.11	0.31
[5]CP (g/kg)	45.5	21.6	0.48
Fat (g/kg)	65.1	16.4	0.25
Ash (g/kg)	13.8	8.18	0.59

Where; [1]standard deviation, [2]standard error of cross validation, [3]body mass, [4]growth rate, [5]crude protein.

4.3.5 The Effect of Diet on Carcass Composition

Half of the layer genotype (n=72) were fed a typical broiler ration during the rearing period, and the other half of the birds (n=72) were fed a typical layer ration. The layer ration had a lower CP and energy content compared to the broiler ration. The data were analysed to see if diet could be predicted from carcasses. The statistics from the NIRS calibrations for the effect of diet are presented in Table 4.5. The results indicated that NIRS was good at predicting diet (r^2= 0.82), but the internal cross validation for diet was not as strong as the calibration (1-VR= 0.69). The NIRS was unable to clearly discriminate and cluster carcasses from birds fed the two diets using only the first three principal components (Figure 4.6).

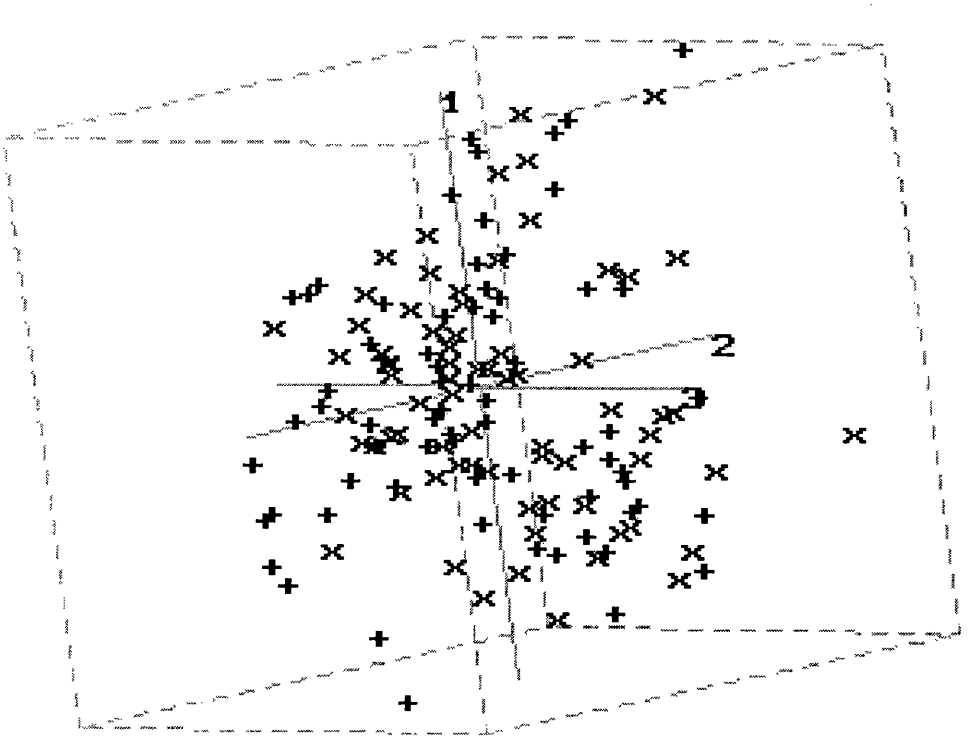


Figure 4.6 Discrimination of the layer birds on the basis of dietary treatment, using the first three principal components of near-infrared reflectance spectra. The layer genotype of chicken were fed either a layer diet (X) or a broiler diet (+). Where 1, 2 and 3 are the three principal component factors used in the analysis.

4.4 Discussion

Most carcass descriptors had a high degree of variation as indicated by the large SD (Table 4.2). The large SD was probably due to the large variation in BM of the birds used in the study, since the birds were reared from posthatch (about 35 g) until maximum growth rate was achieved, and weighed as much as 2.7 kg. A more accurate indication of the variation or error involved in the chemical analyses are shown by the SEL's which range from 0.70 - 2.87 g/kg, 10.34 - 14.05 g/kg and 3.87 - 5.04 g/kg for CP, fat and ash respectively.

Near-infrared reflectance spectroscopy was able to distinguish between carcasses of birds fed two diets and was good at predicting to which dietary treatment group the birds belonged (r^2= 0.82), although the internal cross validation was not as strong as the calibration (1-VR= 0.69). This may indicate that a larger sample size of each diet would strengthen the cross validation.

The reason why NIRS was able to distinguish between the two groups may have been due to the differences within the different carcass descriptors such as BM, GR, CP or fat content. There was a 2-3% difference in protein and a 1-2% difference in energy content of the two diets fed to the L genotype. The ANCOVA statistical model, which has been described previously (section 3.3.3), was used to detect any differences between the two diets fed to the layer chickens. There was a significant age X diet interaction on fat content of the carcasses, but there was no evidence of a diet effect or interaction for CP or ash content. These results imply that the NIRS was discriminating between the diets on the basis of fat content of the chickens.

The results of the current study have demonstrated that NIRS is a powerful technique for discriminating between carcasses from different chicken genotypes and also in predicting the body composition and other characteristics of chicken carcasses. It provides a rapid, reliable and nonhazardous method of determining genotype, age, BM, GR, and both CP and fat content of whole milled chicken carcasses. The suitability of the NIRS calibrations to produce predictive equations were strong for most descriptors, specifically for age, genotype, GR and fat (Table 4.5). Valdes & Summers (1986) observed strong calibration models and predictive equations for CP and fat in whole chicken carcasses in broilers (r^2= 0.98 and 0.91) and layers (r^2= 0.92 and 0.85) as well as chicken breast muscle (r^2= 0.85 and 0.78). They reported that the accuracy of the predictions of CP and fat was similar or better than conventional chemical methods. Good NIRS predictions were also shown by Valdes *et al.* (1989) for CP, fat and gross energy in 18 month old layer chicken whole carcasses and in rainbow trout whole carcasses. The regression values between laboratory values and corresponding values predicted by NIRS were 0.96, 0.95 and 0.90 for chicken and 0.76, 0.78 and 0.75 for fish carcasses for CP, fat and gross energy respectively. The lower r^2 values in the fish samples may have been due to the narrower range of

values from which predictions were calculated (Valdes *et al.*, 1989).

Cozzolino (1998) used NIRS to predict the chemical composition in various meat-type animals. The NIRS r^2 values for raw minced fish were 0.96, 0.99 and 1.00 for total volatile nitrogen, oil and moisture respectively. These NIRS values were much stronger than those previously shown by Valdes *et al.* (1989). This could be a reflection of the strength and accuracy of the initial reference methods. The r^2 values for CP, fat and moisture in lamb meat were 0.71, 0.34 and 0.55 for intact lamb meat and 0.82, 0.73 and 0.76 for minced lamb meat. Overall the predictions were not strong but the NIRS was more robust in predicting chemical composition of the mince compared to the intact meat values. Chicken meat was also compared in a similar way, intact chicken meat had r^2 values for CP, fat and moisture of 0.73, 0.45 and 0.45 compared to 0.97, 0.95 and 0.99 for minced chicken meat. The difference in r^2 values between the intact and minced meat samples indicates that the homogenisation of the sample causes the release of lipids, moisture and proteins from within the meat fibres (Cozzolino, 1998). It also clearly shows the importance of homogenisation of a sample to receive a realistic prediction and to improve that prediction of the chemical composition of any sample.

However, NIRS is poor in the present study at predicting the ash content of carcasses. Ash is the inorganic part of the sample and contains minerals, these do not create absorption bands in the NIRS region (as there are no C, N or O atoms), although associations may happen between minerals and certain organic constituents which absorb in the NIRS region (Ferraz de Oliveira, 1998), hence why ash is not strongly predicted by NIRS. Due to this lack of a strong prediction, the analysis and prediction of ash is not included in many NIRS studies. The chemical composition studies which do include ash in their analysis are plant-based research, and these have varied from r^2 values of 0.77 in ground chickpeas (Flinn *et al.*, 1998) to 0.94 in oats, wheat and sorghum (Bruno-Soares *et al.*, 1998). Most animal-based NIRS research mainly concentrates on the levels of nitrogen or CP, fat and moisture in their meat samples. This is probably due to the importance of protein and fat content of meat for today's consumer.

Near-infrared reflectance spectroscopy is also poor at discriminating between chicken carcasses of either sex. Due to the large difference in fat content of male and female chickens, one would have expected to see an obvious sex difference within the current data set. However, on evaluation of the original data, the reason that no difference was apparent is not surprising since sex was poorly related to all other carcass descriptors (Table 4.4). Also, the carcasses used in this study came from birds that ranged from day-old to 112 days old and as none of these birds were sexually mature, it may not be unexpected that NIRS could not discriminate between them.

Other studies of both plant and animal products have also shown the strength and robustness of NIRS as a predictive tool. A range of materials have been tested using NIRS and most studies were able to successfully predict moisture, CP and fat content using NIRS (Gillion *et al.*, 1999; Ruano-Ramos *et al*,. 1999; Bruno-Soares *et al.*, 1998; Flinn *et al.*, 1998; Neumeister *et al.*, 1997; Young *et al.*, 1997). Bruno-Soares *et al.* (1998) compared the chemical composition of green crop cereals (oats, barley, wheat, ryegrass, sorghum and triticale) with their NIRS spectra. The NIRS calibrations indicated good correlations with all chemical components (ash, CP, crude fibre, neutral detergent fibre, acid detergent fibre, acid detergent lignin) showing r^2 values higher than 0.73. In each case, Ruano-Ramos *et al.* (1999) only examined the nitrogen and ash content of grassland samples (total herbage, grasses, legumes and forbs). The correlations for these samples were strong for both nitrogen (r^2= 0.93 - 0.99) and ash (r^2= 0.82 - 0.95). Ground chickpeas also had strong calibrations for CP, moisture, fat, ash and total dietary fibre when their NIRS spectra were compared to chemical reference values (r^2= 0.77 - 0.99) (Flinn *et al.*, 1998).

4.4.1 Alternative Methods of Measuring Body Composition

There are a number of other methods of analysing the body composition of an animal. The most commonly used are total body electrical conductivity or bioimpedence and dual-energy x-ray absorptiometry (DXA). Both of these methods have the advantage of being able to work with live subjects and the ability to do

repetitive measurements on the same subject as it grows and develops, and in response to dietary manipulations. Both are non-invasive procedures that can predict the chemical composition of an animal and both have also been validated in human subjects (Peppler & Mazess, 1981 cited in Mitchell *et al.*, 1997; Segal *et al.*, 1988 cited in Fuller & Elia, 1989).

The bioimpedence method, like all electrical conductance methodologies, is based on the non-resonant absorption of an electromagnetic field in tissues. The lean body mass and total body water can be calculated from the conductivity absorbed by the animal, since it is known that the electrical conductivity of lipids is less than 5% compared to lean tissues, body fluid or bone (Piasecki *et al.*, 1995). Scott *et al.* (1991) used bioimpedence to predict the total lean mass in a number of different bird species over a range of BM (40-160 g). They demonstrated that the bioimpedence index of a bird could be related to its total lean mass calculated by laboratory analysis. There was an inverse linear relationship within each species of bird between the reference data and the bioimpedence data, as the bioimpedence index decreased the total lean mass increased. The accuracy of their predictive equations for total lean body mass in starlings was quite good, with a percentage error of between 0.32 - 4.45.

Dual-energy photon absorptiometry was originally developed to measure the mineral mass and bone density in humans by Peppler & Mazess (1981) (cited in Mitchell *et al.*, 1997), and this technique later evolved into DXA. The DXA method uses a low radiation dose to conduct a whole body scan of a subject and detects the differential attenuation of the radiation by bone, fat and lean tissue (Mitchell *et al.*, 1997). Mitchell *et al.* (1997) evaluated the use of DXA for the measurement of body composition in growing broiler chickens. The birds were scanned by DXA at BM's ranging from 403 to 3,289 g, and the birds were then analysed for water, protein and lipid content by conventional laboratory methods. The r^2 values indicated the correlation between the chemical data and the predicted DXA data were good. The r^2 values for BM were 0.98 - 1.00, for lean body mass were 0.90 - 0.97 and for total body water were 0.90 - 0.99. This indicated that DXA was very strong at predicting BM, lean body mass and total body water. However, body fat was poorly predicted

by DXA (r^2 = 0.40 - 0.81). This contrasts with Geers *et al.* (1998), who found strong predictive equations using DXA for fat (0.98), lean (0.99) and ash (0.93) content of growing pigs.

Both of these methods appear to have potential in predicting the body composition of animals, although the DXA method appears to be less robust and more dependent on the weight of the animal being scanned rather than the accuracy of the prediction. The main advantage of these methods is that the animal need not be sacrificed in order to measure its body composition, but there are many disadvantages. These included the time required to scan each animal and the need to anaesthetise the animal to ensure that it remains motionless during the scanning procedure (Mitchell *et al.*, 1997). When using the bioimpedence method, the body temperature of the animal, the position of the animal in the scanning chamber and any metal tags on the animal can all affect the prediction results (Scott *et al.*, 1991).

Near-infrared reflectance spectroscopy does not have the same problems as bioimpedence or DXA and therefore it makes a more robust, fast and reliable tool to analyse both plant or animal tissue, or even animal by-products e.g. excreta or digesta. There is also a larger scope of what NIRS can determine, not just lean and fat mass but also ash, crude fibre, neutral detergent fibre and acid detergent fibre can all be predicted using NIRS spectra.

Near-infrared reflectance spectroscopy has many potential implications in animal-based industries. For example feed producers or mills could analyse feed at all different stages of production, from the individual ingredients to the final product. This gives the producer the ability to check the nitrogen and energy values of the feed, as long as reference data had previously been collected on similar samples and is in itself reliable. Near-infrared reflectance spectroscopy also has the ability of checking for potential contaminants within the feed. The Bovine Spongiform Encephalopathy (Amendment) Order 1996 (SI 1996 No 962) prohibits the sale or supply of any mammalian meat and bone meal, or any feeding stuff known to include mammalian meat and bone meal, for the purpose of feeding to farm animals in the United Kingdom. To determine whether the contaminant was present or not,

the feed sample is scanned using NIRS and this information could be checked rapidly and accurately. Meat producers and processing plants could also potentially use NIRS to analyse the quality of the meat products i.e. to check the protein, fat or water content of the meat. The main advantage of using NIRS is the speed in which information about a sample can be determined compared to conventional methods. To analyse a set of 30 samples for nitrogen, fat and ash using conventional methods of Kjeltec, Soxtec and a muffle furnace would take approximately 7 hours for nitrogens, 20 hours for fats and 24 hours for ash and that includes no replication of samples. Using NIRS the chemical compostion of the samples could be determined in less than a couple of hours. The time taken to do analyse samples must be an important consideration when looking at the implications of the use of NIRS both in laboratories and within industry.

CHAPTER 5

5. GENERAL DISCUSSION, CONCLUSIONS AND FUTURE PLANS

The genetic selection for growth rate, FCR and breast meat yield of the broiler chicken over the past 50 years has had a large effect on the reduction in the age of the chicken to reach an optimal slaughter weight of 1.82 kg. In 1950 it took the broiler chicken 12 weeks to reach 1.82 kg (Gyles, 1989), whereas this was reduced to just 5 weeks in 1999 (Ross Breeders, 1999).

This large reduction in time for the broiler to grow to its optimal slaughter weight may have had serious consequences on the broiler's ability to maintain symmorphosis. That is, the ability to balance all the systems of it's body, so that each system is tuned to one another and the functional capacity of a system does not exceed that of any other (Taylor & Weibel, 1981; Weibel, *et al.*, 1991). There is already evidence to suggest that the broiler chicken is not coping with the intensive genetic selection for improved growth rate, feed efficiency and greater meat yield that it has undergone. This evidence is that there has been an unexpected increase in mortality, which has largely been attributable to metabolic disorders (Groves, 1997; Rauw *et al.*, 1998).

The most common metabolic disorders afflicting poultry are ascites, SDS and skeletal disorders and these have had a very important impact on the poultry industry. One of these metabolic disorders, ascites, is a major cause of death within the broiler industry (Julian, 1997). Maxwell & Robertson (2000) indicated that the incidence of mortality due to ascites was as many as 1.4% in Britain and there is a worldwide incidence of 4.7%. It is estimated that the production loss attributable to ascites costs almost one billion American dollars annually, on a worldwide basis (Maxwell & Robertson, 1997). There is no doubt, therefore, that ascites is a great financial loss to the broiler industry.

The factors that predispose a flock of broilers to ascites are generally associated with the circulatory and respiratory systems (Odum, 1993; Julian, 1997). Therefore, the size of these and other systems compared to the overall muscle deposition, would be an indication of whether the broiler has been able to maintain symmorphosis throughout the period of genetic selection.

The primary objective of this project was to determine the effects that the genetic selection which the modern broiler has undergone have had on the gross morphology, metabolic rates (MR) and carcass composition of the broiler chicken, when compared to other genotypes of chicken. The chicken is a good model to examine symmorphosis due to the wide range of intra-specific genetic variation due to artificial selection pressures found within one species.

5.1 Resting and Peak Metabolic Rates in Different Chicken Genotypes

Due to the dramatic increase in the growth rate and breast meat yield of the broiler a subsequent increase in its resting metabolic rate (RMR) was also anticipated, since larger animals usually have larger maintenance costs and therefore larger RMR. Even though the ANCOVA model adjusted the birds to the same BM one would still expect the different genotypes to have different maintenance and growth costs at a similar BM. Surprisingly there was no difference in RMR between the FB and L genotypes when the data were corrected for BM. Correcting the metabolic rate data for LBM resulted in the FB genotype having a larger RMR in birds above 80g body mass (see Figure 2.4). This contrasts with previous research that found that the layer chicken had a larger RMR than the broiler chicken (Kuenzel & Kuenzel, 1977; Visser & van Kampen, 1991). However, Jackson & Diamond (1996) did not find that genotype had an effect on RMR when they compared a modern broiler with the Red Jungle Fowl. Most of the previous research on MR in the domestic fowl has occurred in birds of more than 21 days of age (see Table 2.1). The current study only determined the MR of birds younger than 14 days of age, therefore it is hard to

compare MR between the different studies. Also, previous literature shows that there is great variation in the RMR of the domestic fowl (see Table 2.1), it is therefore difficult to make any assumptions of what would occur in the genotypes studied in the current work beyond two weeks of age. Jackson & Diamond (1996), who compared the RMR of the broiler and the Red Jungle Fowl until both genotypes were over 1kg BM, recorded no relationship between RMR and genotype even at the heavier weight range. However, when the three genotypes within the current study were grown beyond two weeks of age their organ morphology was very divergent. The mass of many of the supply and demand tissues of the FB genotype were larger compared to the other two genotypes. One would expect that such a large increase in tissue mass would require a subsequent increase in the cost of maintenance. The RMR results presented in the current study also generally showed no relationship with genotype. One could therefore speculate that this same relationship would initially continue after the chickens were two weeks of age, but as the chickens increased in age and BM there would be a subsequent change in the RMR. One would expect that the FB genotype would have a larger RMR than either of the other two genotypes in older and heavier birds.

After a short-term metabolic load was placed on the birds, in this case reduced temperature, peak MR (PMR) was greater in selected broilers compared to the layer genotype (see Figure 2.5). This was in contrast to Visser & van Kampen (1991) who showed no significant difference in PMR between broiler and layer chickens, but in that study the PMR was only measured chicks immediately post-hatch that weighed 45 g. The results of the current study indicate that the broiler chicken was able to maintain a higher metabolic rate and consequently a larger metabolic scope. That is, retained a greater capacity to generate heat in response to a thermal challenge. The larger MS could be attributed to the broilers greater total muscle mass and their ability to increase their PMR when required.

5.2 Intra-specific Variation in Morphological Data

The morphology of the three genotypes in the first study only related to the first two

weeks of life. In contrast, the second study characterised the differences in gross morphology between the genotypes during the time taken for each genotype to reach their maximum growth rate. This was to determine whether the differences in the organ masses found between the different genotypes in the first two weeks of life were consistent throughout the entire growing period or whether they had changed with continued development of the birds.

Some of the differences in organ masses between the different genotypes observed for the first two weeks of life remained consistent in the older birds. For example, the relative mass of the brain was consistently larger in the L genotype and the relative intestine mass was consistently larger in the FB genotype throughout the period of development studied. A larger intestine mass in broilers compared to other genotypes has been seen in previous research (Katanbaf *et al.*, 1988b; Mitchell & Smith, 1991; Nir *et al.*, 1993; Dunnington & Siegel, 1995; Jackson & Diamond, 1996; Mahagna & Nir, 1996). Jackson & Diamond (1996) supported the results shown for brain mass. The current research gives little support to the existence of growth constraints residing in the digestive capacity of the gut as suggested by Mahagna & Nir (1996), since the intestine mass, both in absolute and relative terms, was shown to be consistently larger in the fast growing broiler genotype. This gives an indication of the bird's ability to increase its intestine mass during the pressure of genetic selection.

Many of the other organ masses described in the present study did not show a consistent relationship between the genotypes within the two studies e.g. the caeca mass. The relative caeca mass was greater in the FB genotype in the younger birds, whereas in the older birds the SB genotype had larger relative caeca mass. In some cases, the organ mass results were inconsistent when different covariates were used in the statistical analyses e.g. the liver and leg muscle mass. The FB and L genotypes had a larger relative liver mass than the SB genotype when BM-OM or DM-OM were used as covariates in the statistical model. However when LBM-OM was used as a covariate the FB genotype had a greater liver mass, but there was no difference between the L and SB genotypes. Similarly, the relative leg muscle mass was larger in the L genotype compared to the FB or SB genotypes when BM-OM

was used as a covariate in the statistical model. This result was reversed when LBM-OM and DM-OM were used as covariates in the model. Due to the large fat and water content of the bird, which often confounds the data, the most appropriate covariates to use when analysising morphological data would be either dry body mass or lean body mass.

The relative gizzard mass was larger in the L genotype in the birds below 80g and across the full growing period of the birds, which was consistent with previous research (Plavnik & Hurwitz, 1982; Nitsan *et al.*, 1991), but this result was not repeated in the young birds above 80g grown to 14 days. The relative heart mass was larger in the FB genotype compared to the SB or L genotypes in the young and older birds, except when BM-OM was used as a covariate in the statistical model. The results for relative heart mass in the current study were contrasted with those reported by Katanbaf *et al.* (1988b). There was generally no significant effect of genotype on liver mass in the younger birds, and the genotype difference observed in the older birds appeared to be between the SB and the other two genotypes. Therefore the results indicate that the selected broilers have a similar sized liver as the unselected layer, which was similar to the findings of Jackson & Diamond (1996). The relative caeca mass was shown to be larger in the SB compared to the other two genotypes in the older birds grown over the whole developmental period. This possibly indicates that the caeca mass alone may not be a good indicator of their potential activity levels as it is not the caecum itself that is the functional unit but rather the microbial population contained within.

The relative pectoral muscle mass was also larger in the FB genotype in both the young and older birds. This was expected since breast meat yield has been an actively selected criterion in the broiler breeding industry. The effect of genotype on the relative leg muscle mass gave conflicting results between younger and older birds and between the different covariates being used in the model. In the complete growth study, the L genotype had more leg muscle when BM-OM was used as a covariate whereas FB had more leg muscle than the other two genotypes when LBM-OM was used as a covariate. This suggests that the fat content of the chicken may be confounding the results. The water content of the muscle mass was larger in the FB

genotype compared to either the SB or L genotypes. A high water content of muscle generally indicates that it is relatively undifferentiated and less mature (Ricklefs, 1985; Ricklefs *et al.*, 1994; Dietz & Ricklefs, 1997). Therefore, although the FB has more muscle, this muscle is less mature and is possibly of a poorer quality (Voller *et al.*, 1996; Voller-Reasonover *et al.*, 1997).

In addition to the organs already discussed, the lung and carcass masses were measured in all three genotypes across the full growing period. The relative carcass mass was larger in the L genotype and the lung mass was consistently larger in the FB genotype. The larger relative lung mass evident in the broiler chickens was consistent with the other cardio-pulmonary organ, the heart, also being larger in the FB genotype. The organ morphology results suggest that the genotype differences observed in the first two weeks of life were not always indicitative of what occurs throughout the full growth period.

Metabolic disorders, specifically ascites, place immense pressure on the cardio-pulmonary system. The results of the present studies indicate that the relative heart and lung mass are greater in the selected broiler genotype. Although the cardio-pulmonary system has increased in size and therefore their capacity has probably also increased, during the selection for growth, it obviously has not increased enough to overcome the problem of ascites.

A key indicator of whether symmorphosis has been maintained in the broiler is whether there has been the same proportion of supply tissues supporting the increased demand tissues, mainly muscle. Therefore, the ratio of muscle mass to organ mass (M:O) was examined. This demonstrated that although the FB genotype has a larger total muscle it also has a larger total organ mass. This indicates that as muscle deposition has increased through active genetic selection, the mass of the supply organs have also increased, but the supply organs have not increased at the same rate (see Figure 3.19). The gap between supply and demand tissues was wider in the FB genotype compared to the L genotype, which indicates that symmorphosis has broken down. This breakdown could have serious consequences in the animals ability to avoid the development of metabolic disorders. Within the current studies

only the mass of the relevant organ has been measured and not the organs activity or capacity. Although this was the case, organ mass is still an indication that a breakdown in symmorphosis has occurred otherwise metabolic disorders would not have developed.

An important aspect of the present study was to explain how possible errors could occur using percentages or proportions of BM when statistically analysing morphological data. As discussed, this has occurred in previous research. The method of statistical analysis used throughout the current research was an analysis of covariance (ANCOVA) model produced in the Genstat 5 statistical package. The final model used was refined after previous versions were tested, as the sequence in which the variables, factors and interactions are entered in the model is of great importance. There were various significant interactions produced from the ANCOVA model for the analysis of the different organ masses and the carcass composition data. The significant interactions limited the way in which one could possibly analyse or interpret the data to compare the genotypes. Alternative methods of analysis were least significant difference which assumes that the data sets being compared are balanced. Paired T-tests also assume that the data is balanced and when produced graphically the two sets of data have different intercepts but a common slopes i.e. that they lie parallel to one another. None of the data sets from the present study fitted either of these statistics because they were both unbalanced and had significant interactions. Therefore the most appropriate way of handling the data sets in the current study was to use the predictive model produced in Genstat 5.

Metabolic age could also have been used as an alternative method of analysing physiological data on a relative basis. When a comparison is made on a physiological variable, the body mass of the animal must be taken into account. Metabolic age uses the relationship between time taken to mature and the mature weight of the animal which can be transformed to provide a metabolic age scale on which differences associated with adult size are eliminated (Taylor, 1968). The derived equation for metabolic age in the chicken is given by:

$$\theta = t/A^{0.24}$$

where t is the number of days from start of growth, in chickens this is the beginning of incubation rather than hatch and is defined as (hatch-21), and A is the mature weight in kg (Laird, 1966 cited in Gordon *et al.*, 2001).

Table 5.1 The metabolic age of each of the three genotypes and both sexes at a number of timepoints. The metabolic age was calculated from mature body masses obtained from the literature.

Genotype	Age from start of incubation (days)	Male			Female		
		Mean [1]BM (kg)	Mature BM (kg)	Metabolic age	Mean BM (kg)	Mature BM (kg)	Metabolic age
[2]FB	41	0.88	6.21	26.4	0.83	4.87	28.0
	62	2.77		40.0	2.35		42.4
	76	-		-	-		-
	104	-		-	-		-
	132	-		-	-		-
[3]SB	41	0.45	4.62	28.4	0.39	3.68	30.0
	62	1.34		42.9	1.08		45.4
	76	2.03		52.6	1.55		55.6
	104	-		-	-		-
	132	-		-	-		-
[4]L	41	0.20	2.80	32.0	0.19	2.06	34.5
	62	0.61		48.4	0.49		52.1
	76	1.03		59.4	0.78		63.9
	104	1.83		81.2	1.25		87.4
	132	2.44		103.1	1.54		110.9

Where; [1]BM-Body mass, [2]FB-fast broiler, [3]SB-slow broiler, [4]L-layer

The metabolic age was calculated at varying timepoints using the data from the current trial (Table 5.1). None of the three genotypes used in the present study reached their mature BM, therefore mature weight was sourced from previous

performance and production data for these genotypes (*not published*). At 41 days post-hatch (62 days from the start of incubation) the male chickens of the FB genotype had a metabolic age of 40 days whereas the SB and L birds were 42.9 and 48.4 days respectively. The mean BM of these same birds were 2.77, 1.34 and 0.61kg. These results indicate that the birds at this timepoint had a similar metabolic age, but had enormously different actual BM's.

Metabolic age would have made an interesting alternative analysis within the present study, whereby the comparison of body composition data could have been made on the three genotypes at the same metabolic ages, and therefore removing the effects of both age and BM. Unfortunately within the current study the FB and SB genotypes were not grown to their body mature weight. This was due to time constraints, for example the FB genotype would have had to be reared for approximately 10 weeks instead of 6 weeks. There would have been the implications on the birds welfare, the risk of metabolic disorders and severe leg abnormalities. Also there was not a large enough data set to robustly statistically compare the data at a single metabolic age, since there were only 4 birds in each genotype and sex that could be compared at a given timepoint.

5.3 Effect of Genotype on Carcass Composition of the Chicken

The effect of genotype on carcass composition in the young birds was not repeated in the older birds, except for the relative ash content results, which were consistently larger in the L genotype. The relative fat content was greater in the SB genotype in the younger chickens (0-14 d), but in the full development period (0-42 d) the FB chickens had a greater fat content, even though reduced fat content is a selection criterion in broilers and has been over the last few decades.

The relative crude protein (CP) content of the chicken carcass was greater in the FB and L chickens compared to the SB genotype in the younger birds, but across the full development period the L genotype had a larger CP content. This larger CP content

in the L genotype could be linked to the larger carcass mass, which would mean a possible larger feather mass in the L genotype, since feathers have a high protein content.

Due to the high protein content of muscle, and that the FB genotype had a larger total muscle mass it had been anticipated that the CP content would be greater in the FB chickens than the other two genotypes. However, this was not the case. The somewhat conflicting results of total muscle mass and CP content may have been attributable to the muscle in the FB having a higher water content compared to the other genotypes. After drying, the total muscle mass was significantly different between the three genotypes, being larger in the FB genotype, but this difference may not have been enough to have an effect on the CP content of the carcass.

5.4 NIRS, an Alternative Predictive Tool of Poultry Carcasses

Near-infrared reflectance spectroscopy was able to successfully discriminate between three different genotypes of chicken and between chickens fed two diets, varying in protein and energy content. The predictive equations produced from the calibrations of the NIRS spectra were strong for age, genotype, BM, GR and fat. However, the CP content was less well predicted and the calibration models obtained for ash content were poor.

The sex of the chicken could not be predicted at all from the NIRS spectra of the dried, milled chicken carcasses. Thus, further work involving a very specific design of animal trial would be necessary to optimise the conditions for discrimination of poultry sex from spectra of the carcass. A more suitable study design would be one that did not include age as a factor but simply compared a large number of each genotype of chicken at one BM, perhaps the broiler slaughter weight of approximately 2 kg body weight would be appropriate.

Overall NIRS was successful as an alternative tool to predict the carcass composition

of chickens. The implications of this are reduced costs in terms of both finance and time for analysing carcass samples in the future.

5.5 Potential for Improvements in Experimental Design

Whilst the study examining the effect of genotype on organ morphology generated a large, comprehensive database and produced interesting results, there were several aspects of the experimental design that could be improved upon. Although each genotype was reared in the same facility, in similar housing and under identical environmental conditions where possible, each of the flocks was reared in consecutive order, rather than simultaneously. This was a reflection of genotype availability and time constraints. Although the flocks were not reared at the same time, one could argue that it is genotype differences that were being observed rather than rearing effects, especially if the birds were reared under similar conditions of temperature, humidity and ventilation etc. According to Havenstein (1994 a,b), genetic selection accounts for approximately 80% of any increased performance of modern broilers and environmental effects only account for 20%. Therefore, any environmental differences occurring within the current studies, will have a minimal effect overall on broiler performance, the majority will be due to genetic selection. All of the environmental factors could have consequential results on the study, although the error should be minimal because the environmental factors are all tightly controlled, therefore, there should be very little house-to-house or room-to-room variation. The variation that may have been caused by the time of year that the experiments were executed could not have been avoided due to the large variation in the timespan required to complete each study i.e. 6 weeks compared to 16 weeks. The design of each phase of the study (i.e. each genotype flock) did not include any blocking or use of replicate pens, although each genotype and sex was replicated at each timepoint. For the purposes of the morphological study, each bird was regarded as the experimental unit, since each of the organs within the animal are influenced by one another, therefore each bird was a replicate.

If the experiment were to be repeated, an improved experimental design would be to

place all three genotypes in one open-span house containing pens, therefore reducing any possible environmental effects. Each pen would contain a smaller number of chicks, approximately 40 birds per pen, and each pen would be replicated a minimum of four to eight times, to check that there was no pen variation. In addition due to the diet effects in the layer genotype that were observed during the present trial, it would be interesting to repeat this part of the study, but instead there would be three diets (a modern broiler diet, an older style broiler diet and a layer pullet diet) to feed to all three genotypes. This would produce a 3 X 3 factorial design in the experiment. The birds would be removed for dissection at a common BM as well as a common age. This is because the statistical analysis within the present study indicated that the BM of the bird was just as important as its age, although this would be logistically more difficult.

It should also be noted that throughout the trials different methods of nitrogen analysis were used. Although to have a lack of consistency when using an analytical method is not ideal, care was taken to ensure that equipment used was always calibrated before use, and blanks used as standards. Each chosen method analysed the samples for total nitrogen content so the different methods should be readily comparable.

5.6 Recommendations for Future Work

Due to time and equipment pressures the measurement of metabolic rates of the chickens beyond two weeks of age was not possible. It would be of great interest to continue with this study and compare the metabolic rates of the same three genotypes of chicken, as in the present study, from hatch until they reached approximately 2 kg body weight. One could then observe how the pressures of genetic selection have affected the chickens ability to maintain or alter its resting and peak metabolic rates right throughout its growing phase.

The larger intestine mass observed in the FB genotype of the chicken implies that the intestine of the modern broiler has a greater activity and/or capacity. Histological

sections of the intestine would indicate the size, number and surface area of the villi present, which would also give an indication of the difference in the possible uptake capacity of the gut between the different genotypes (Smith *et al.*, 1990; Uni *et al.*, 1995; Uni *et al.*, 1999). Therefore, collecting histological sections of the intestines of the different genotypes of chicken used in the present study would help clarify whether an increased relative mass of intestine observed actually means an increased capacity of that organ.

Organ size has been widely accepted as an indicator of organ performance (Jackson & Diamond, 1996; Hammond & Janes, 1998; Chappell, *et* al. 1999). It is assumed that the larger the organ the larger its capacity (the quantity or volume an organ can contain) and/or activity (the amount of work done by an organ). Therefore, the uptake capacity of the gut could also be measured to identify any genotypic differences. Measurement of glucose, proline and oleic acid uptake in the different sections of the small intestine i.e. the duodenum, jejunum and ileum could be compared between the different genotypes using the inverted sleeve method (Karasov *et al.*, 1986; Noy & Sklan, 1996). The measurement of brush-border enzyme activity during the first few weeks post-hatch would also allow one to quantify any genotypic effects, either using the homogenised tissue (Uni *et al.*, 1999) or the digesta contained within the intestine (Nitsan *et al.*, 1991; Dunnington & Siegel, 1995).

The use of NIRS as a tool for successfully assessing the chemical constituents and other biological parameters of poultry carcasses has been clearly demonstrated in the current study. Also the robustness of NIRS in discriminating between different genotypes of chickens and chickens fed different diets has also been demonstrated. Further work needs to be done on the external validation of the NIRS predictions produced in the present study and to build on the database that has now been established. It would also be interesting to establish if NIRS could discriminate between the breast and leg muscle among the different genotypes and whether fresh and frozen meat samples could be discriminated.

5.7 Conclusion

Overall the research presented here show that the relative masses of some of the key supply tissues in broilers have been effected by the intensive genetic selection for fast growth rate, FCR and breast meat yield by reducing the size of the less essential organs (relative brain, gizzard, caeca and carcass mass) to allow the increase of other tissues i.e. the total muscle mass. There is a possibility that the genetic selection for fast growth rate and increased meat yield could continue, but that a limit could soon be reached due to the clear breakdown in symmorphosis that has occurred. If, on the other hand, the current level of intensive selection does continue there will ultimately be a further increase in metabolic disorders, and thus mortalities, since the heart and lungs, as well as other supply organs, are being placed under a much greater metabolic workload. This is especially likely since there appears to be an active selection for decreased eviscera in today's broiler breeder industry. However, inclusion of some of the support organs e.g. the heart and lungs, in the selection criteria may help to alleviate some of these problems. Increasing the size and possibly the capacity of the cardio-pulmonary organs could reduce the levels of some metabolic disorders since it is this system which is placed under the most pressure in the modern broiler chicken.

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7.1 APPENDIX 1 – Proximate Analysis Methods

7.1.1 Determination of Ash

The weight of a crucible, which had been placed in an oven overnight and then allowed to cool for 30 minutes in a desiccator, was taken. Approximately 1 g of sample was weighed into a crucible. The crucible was placed in an ashing oven, and the temperature gradually raised to 500 °C ± 50 °C. The samples were left in the ashing oven overnight. After the oven was left to cool down for 30 minutes, the crucibles were removed and placed in a 60 °C oven and left for an hour to equilibrate to that temperature. After this time the samples were removed from the oven and placed in a desiccator to cool for 30 minutes, after which time they were weighed. The ash content of the sample was then corrected for the weight of the crucible.

7.1.2 Determination of Fat

The fat content was determined using a Soxtec extraction unit (model 1040) the temperature of the water bath was allowed to rise to approximately 90 °C. Hot water was allowed to circulate to heat the hot plates and cold water was passed through the condensers. A 1g sample was weighed into an extraction thimble (26 mm x 60 mm). A piece of oil free cotton wool was placed on top of the sample to prevent loss. An adapter was attached to the top of the thimble and the thimbles inserted into the condensers. A tared cup, containing 50 ml of solvent (Petroleum spirit 40-60 °C, G.P.R., Merck Ltd), was placed beneath the thimble on the hot plate. The handle was lowered so that the cups were firmly joined to the condensers. The recovery knobs above the condensers were all opened and the extraction knob moved to boiling, to allow the solvent to drip through the sample. The samples were left to boil for 40 minutes and before being rinsed for a period of 45 minutes. On completion of rinsing the recovery knobs were closed and the air pump switched on, to collect the solvent as it is evaporated off. The lever was then moved to evaporation for 15 minutes. After this time had elapsed the handle was released and the cups inspected. If a small amount of solvent remained, the cups were left in a fume cupboard until all the solvent had evaporated. The cups were placed in an oven

at 60 °C for 60 minutes, and then removed and cooled in a desiccator for 30 minutes before being weighed. The cups were then placed back in the oven for a further 30 minutes and weighed again to ensure all solvent had been evaporated. The following equation was used to calculate the fat content of the sample,

Fat (g/kg) = ((X-Y)*1000)/ W

where, X = weight of cup + oil (g), Y = weight of cup (g), W = weight of sample (g).

7.1.3 Determination of Nitrogen using Colorimetric Method

Approximately 0.25 g of dried, milled sample was weighed into a digestion tube containing anti-bump granules. To each sample, 7.5 ml of digest acid and three 1 ml aliquots of hydrogen peroxide (100 volumes) was added. Digesting the sample with sulphuric acid and hydrogen peroxide removes the organic fraction of the sample and any nitrogen present is converted to ammonium sulphate.

Once the reaction had subsided the tube was placed in a digest block (340 °C ± 20 °C) in the "up" position for 15 minutes followed by 45 minutes in the "down" position. After this time the tubes were removed from the block and were placed on a rack to cool for at least 15 minutes. When the tubes were cool enough to handle, 69.6 ml of distilled water was added to each tube using a dispenser and mixed thoroughly.

Two reagents, A and C were prepared prior to the analysis. Reagent A was prepared by dissolving 31.26 g of phenol, 3.37 g of sodium hydroxide and 0.156 g of sodium nitroprusside in water, then water was added to make 5 litres. Reagent C was prepared by dissolving 99.4 g of trisodium orthophosphate, 11.69 g of disodium hydrogen orthophosphate and 15.5 g of sodium hydroxide in 1.5 litres of water. To this solution 31.2 ml of sodium hypochorite solution and then water was added to make 2.5 litres.

To each sample solution (0.025 ml) 5.0 ml of reagent A was added and mixed thoroughly, then 2.5 ml of reagent C was added. The solution was mixed thoroughly again and allowed to stand for 60 minutes before it was read on the spectrophotometer at 584 nm. Working standard solutions were prepared at 0, 100 and 200 ppm of nitrogen at the same time as the samples. Absorbances were read for both the standards and the samples. A calibration graph of concentration against absorbance was calculated and each sample concentration read from this graph.

Nitrogen (ppm)= (A x 75)/W

where, A = nitrogen (ppm) read from graph, W = weight (g).

7.1.4 Determination of Nitrogen using the Kjeldahl Method

The Kjeldahl method uses the production of ammonia from the sample being analysed to calculate the nitrogen content of that sample. Approximately 0.3 g of composite, milled carcass sample was weighed out into a digestion tube, two tubes were left empty for use as blanks, and two catalyst tablets added to all tubes along with 3 cm^3 of concentrated sulphuric acid. All the digestion tubes were then placed on a heated block, which was set to 400 °C ± 20 °C. An exhaust manifold was immediately attached to the tubes and this was connected to a filter pump and the mains water supply. The sample was left to digest for one hour after the tubes had become clear. After this time the tubes were removed from the block and the exhaust manifold removed. The tubes were placed on a rack to cool for at least 15 minutes and then 10 cm^3 of water was added to each tube.

The contents of the tubes were then mixed using a rotary mixer to dissolve any crystals that might have formed as the sample cooled. The contents of the digestion tubes were then analysed using a Kjeltec apparatus.

Into a conical flask 25 cm^2 of boric acid was measured and the flask placed on the kjeltec machine with the over-flow pipe placed in it. The blank digestion tube was

then attached to the steam pipe, alkali dispensed and the steam lever turned to the open position. The machine was then left for 4-5 minutes and once the solution in the conical flask had turned green, the steam lever was turned to the closed position and the digestion tube removed. Any nitrogen present in the digestion tube was converted into ammonia and collected in the conical flask, producing the green colour. The contents of the conical flask were then titrated with 0.1 M hydrochloric acid, the quantity of acid required for the titration indicates how much alkali in the form of ammonia was present in the sample. This procedure was then repeated for each of the digestion tubes. The nitrogen content was then calculated using the following equation.

$$\text{Nitrogen} = ((1.4007(V-V_0))/m) \times 0.1$$

Where V is the volume (ml) of the standard volumetric solution of acid used in the determination,

V_0 is the volume (ml) of the standard volumetric solution of acid used in the blank test,

m is the mass (g) of the test portion,

0.1 is the molarity of the hydrochloric acid used,

7.1.5 Determination of Nitrogen using the Leco Method

The Leco method uses the combustion of a sample, which then releases pure nitrogen, to calculate the nitrogen content of the sample. Approximately 0.2 g of dried, milled sample was weighed into a tared tin foil cup that was then folded over to retain the sample but to exclude air. The sample was placed into the Leco analyser and the weight and identification number of the sample recorded. The sample was then combusted in pure oxygen at 950 °C, causing nitrogen to be released as N_2 and No_x (where x=2, 3 or 4). All of the combustion gases, including the excess oxygen, were collected in a ballast volume. An aliquot of this volume of gas was automatically removed and injected into a helium carrier stream, it was then passed through heated copper metal filings to remove oxygen and convert NO_x to N_2.

The gas stream was then passed through carbon dioxide and moisture absorbing reagents to remove any other remaing contaminants so that the remaining gas contained helium and N_2. This gas then entered a thermal conductivity (TC) detector. The nitrogen concentration was measured by the TC difference between pure helium on the reference side of the TC cell and that of both the helium and nitrogen on the measurement side of the TC cell. Using this difference in TC, the analyser calculates the percentage nitrogen (or percentage crude protein) of the sample. This procedure was then repeated for each of the other samples.

7.1.6 Calculation of Crude Protein (CP)

It is assumed that nitrogen is derived from protein containing 16% nitrogen, using the following equation an approximate protein value of the test sample is obtained;

CP = Nitrogen content x 6.25

This is not true protein since the nitrogen determined can be from sources other than protein, such as free amino acids, amines and nucleic acids (McDonald *et al.*, 1995) and is therefore referred to as crude protein (CP).

7.2 APPENDIX 2 – Genstat Programmes for Statistical Analyses of Metabolic and Organ Morphology Data.

The following are the full Genstat programmes used to statistically analyse the metabolic and organ morphology data produced in chapter 2. The analyses started with a full model, which incorporated all possible main effects, covariates and interaction terms,

```
> model response variable
> terms Genotype*Dissector*BM-OM*Age
> fit Age
> add[print=acc,est,m] BM-OM
> add[print=acc,est,m] Genotype
> add[print=acc,est,m] Dissector
> add[print=acc,est,m] Age. BM-OM
> add[print=acc,est,m] Genotype.Age
> add[print=acc,est,m] Genotype. BM-OM
> add[print=acc,est,m] Genotype.Dissector
> add[print=acc,est,m] Dissector.Age
> add[print=acc,est,m;fprob=y] Dissector. BM-OM
> predict[print=d,p,se] Genotype
> stop
```

Where significant interactions were revealed in the ANCOVA model, which occurred mainly with the organ masses, these organ masses were analysed using a stepwise regression model.

```
>print 'STEPWISE'
>model response variate
>terms (Genotype+Dissector+Dissector.Genotype)*(BM-OM+Age)
>fit [print=*]
>rkeep df=d0
>for [ntimes=15]
>step [inratio=4;outratio=4;fprob=y;tprob=y]
(Genotype+Dissector+Dissector.Genotype)*(BM-OM+Age)
>rkeep df=d1
```

>exit d1.eq.d0

>calculate d0=d1

>stop

The significant terms generated from the stepwise regression were then entered into a prediction model in Genstat.

>model response variate

>fit significant terms from stepwise regression

>predict [predictions=predtab] Genotype,BM-OM;levels=!(1,2),!(35,37,39...75)

>print predtab

>variate [42] x,y,z

>equate olds=predtab;news=z

>variate x;values=!(21(1,2))

>variate y;values=!((35,37,39...75)2)

>print x,y,z

where, (1,2) are the genotypes being predicted and (35,37,39...75) are the BM-OM (g) at which the organ masses are being predicted. The organ masses which were then generated were produced graphically to indicate which of the genotypes were greater.

The following are the full Genstat programmes used to statistically analyse the organ morphology data produced in chapter 3. The analyses started with a full model, which incorporated all possible main effects, covariates and interaction terms,

> model response variable

> terms Genotype*Sex*BM-OM*Age

> fit BM-OM

> add[print=acc,est,m] Age

> add[print=acc,est,m] Genotype

> add[print=acc,est,m] Sex

> add[print=acc,est,m] Age.BM-OM

> add[print=acc,est,m] Genotype.BM-OM

> add[print=acc,est,m] Age.Genotype

> add[print=acc,est,m] Genotype.Sex

> add[print=acc,est,m] Age.Sex

> add[print=acc,est,m;fprob=y] Sex.BM-OM

> stop

Where significant interactions were revealed in the ANCOVA model, which occurred mainly with the organ masses, these organ masses were analysed using a stepwise regression model.

>print 'STEPWISE'

>model response variate

>terms (Genotype+Sex+Sex.Genotype)*(BM-OM+Age)

>fit [print=*]

>rkeep df=d0

>for [ntimes=15]

>step[inratio=4;outratio=4;fprob=y;tprob=y](Genotype+Sex+Sex.Genotype)*(BM-OM+Age)

>rkeep df=d1

>exit d1.eq.d0

>calculate d0=d1

>stop

The significant terms generated from the stepwise regression were then entered into a prediction model in Genstat.

In the following model (1,2,3) are the three genotypes which are being predicted for and (4.0,4.1,4.2...7.5) are the logged BM-OM values at which the organ masses are being predicted.

>model response variate

>fit significant terms from stepwise regression

```
>predict [predictions=predtab] Genotype,BM-OM;levels=!(1,2,3),!(4.0,4.1,4.2...7.5)
>print predtab
>variate [108] x,y,z
>equate olds=predtab;news=z
>variate x;values=!(36(1,2,3))
>variate y;values=!((4.0,4.1,4.2...7.5)3)
>print x,y,z
```

7.3 APPENDIX 3 - Dietary Specifications of Diets Used in the Study of Organ Morphology in Three Chicken Genotypes.

Table 7.1 The formulation of the broiler diet fed to fast broiler and slow broiler genotypes from 0-42 d and 0-56 d respectively.

Ingredient	Starter Inclusion Level (%)	Grower Inclusion Level (%)	Finisher Inclusion Level (%)
Wheat Meal	53.6	46.2	51.8
Barley Meal	5.00	5.00	5.00
Highprotein Soya	19.2	23.3	19.2
Extra Protein	10.1	15.0	12.5
Fish Meal	7.48	3.00	2.49
Soya Oil	1.50	1.06	-
Fat	-	3.00	5.50
Limestone	0.86	1.03	0.98
Salt	-	0.06	0.03
Sodium Bicarbonate	0.06	0.11	0.03
Enzyme	-	0.05	0.05
Dicalcium Phosphate	0.84	1.14	1.23
Lysine	0.40	0.21	0.29
Methionine	0.26	0.22	0.26
Vitamin and Mineral Mix	0.70	0.60	0.60

Table 7.2 The formulation of the broiler diet fed to layer genotype from 0-112 d.

Ingredient	Starter Inclusion Level (%)	Grower Inclusion Level (%)	Finisher Inclusion Level (%)
Wheat Meal	55.0	34.0	34.0
Barley Meal	-	29.5	29.5
Maize	-	3.80	3.80
Vegetable Oil	4.00	2.50	2.50
Soyabean Meal	32.5	21.3	21.3
Limestone	1.00	0.70	0.70
Fish Meal	5.70	7.00	7.00
Dicalcium Phosphate	0.75	0.34	0.34
Salt	0.24	0.25	0.25
Choline Chloride	0.03	0.02	0.02
DL-methionine	0.13	0.04	0.04
Vitamin Mix	0.40	0.30	0.30
Mineral Mix	0.25	0.25	0.25

Table 7.3 The formulation of the layer diet fed to layer genotype from 0-112 d.

Ingredient	Starter Inclusion Level (%)	Grower Inclusion Level (%)	Finisher Inclusion Level (%)
Wheat Meal	32.0	45.0	31.0
Barley Meal	30.0	30.0	29.3
Grass Meal	1.50	0.70	5.00
Vegetable Oil	1.00	1.00	3.30
Soyabean Meal	31.0	19.5	20.5
Limestone	0.90	1.00	8.20
Fish Meal	1.00	-	-
Dicalcium Phosphate	1.66	1.90	1.80
Salt	0.30	0.30	0.25
DL-methionine	0.09	0.05	0.10
Vitamin Mix	0.30	0.03	0.30
Mineral Mix	0.25	0.25	0.25

7.4 APPENDIX 4 – The Absolute Mass of the Organs and Carcass Composition of Chickens with the Three Genotypes Shown Separately

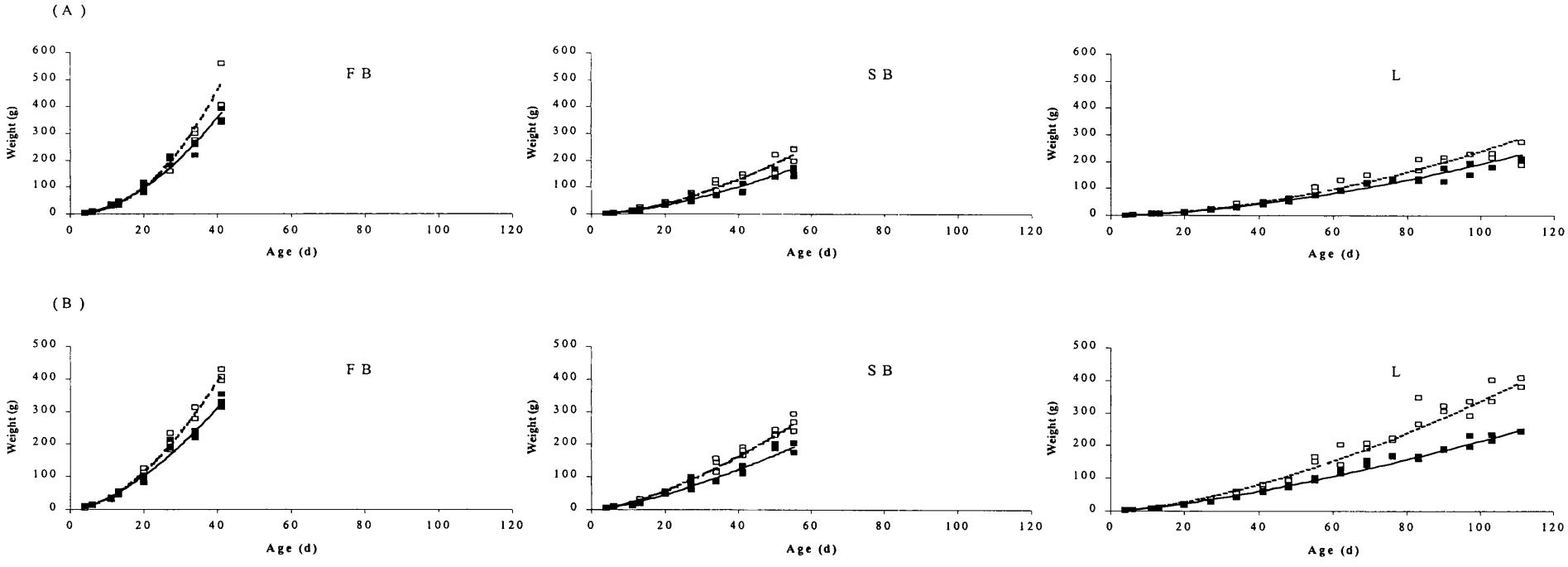

Figure 7.1 The absolute (A) pectoral muscle and (B) leg muscle mass (g) of male (□) and female (■) chickens in fast broiler (FB), slow broiler (SB) and layer (L) chicken genotypes. The data presented are shown for each individual bird.

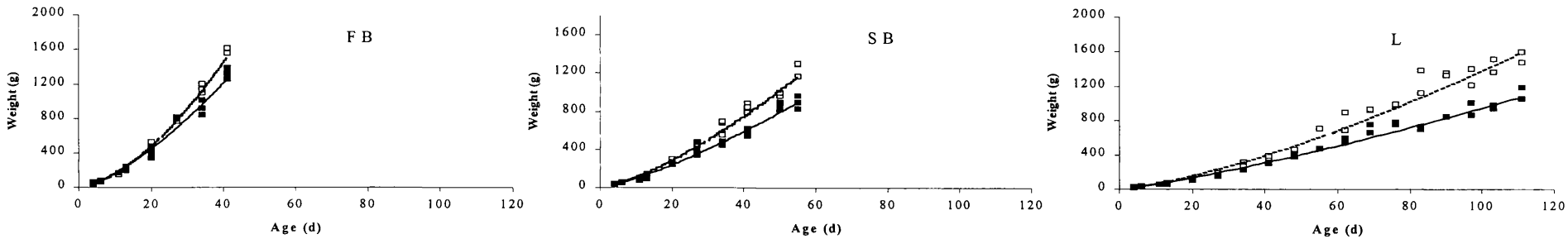

Figure 7.2 The absolute carcass mass (g) of male (□) and female (■) chickens in fast broiler (FB), slow broiler (SB) and layer (L) chicken genotypes. The data presented are shown for each individual bird.

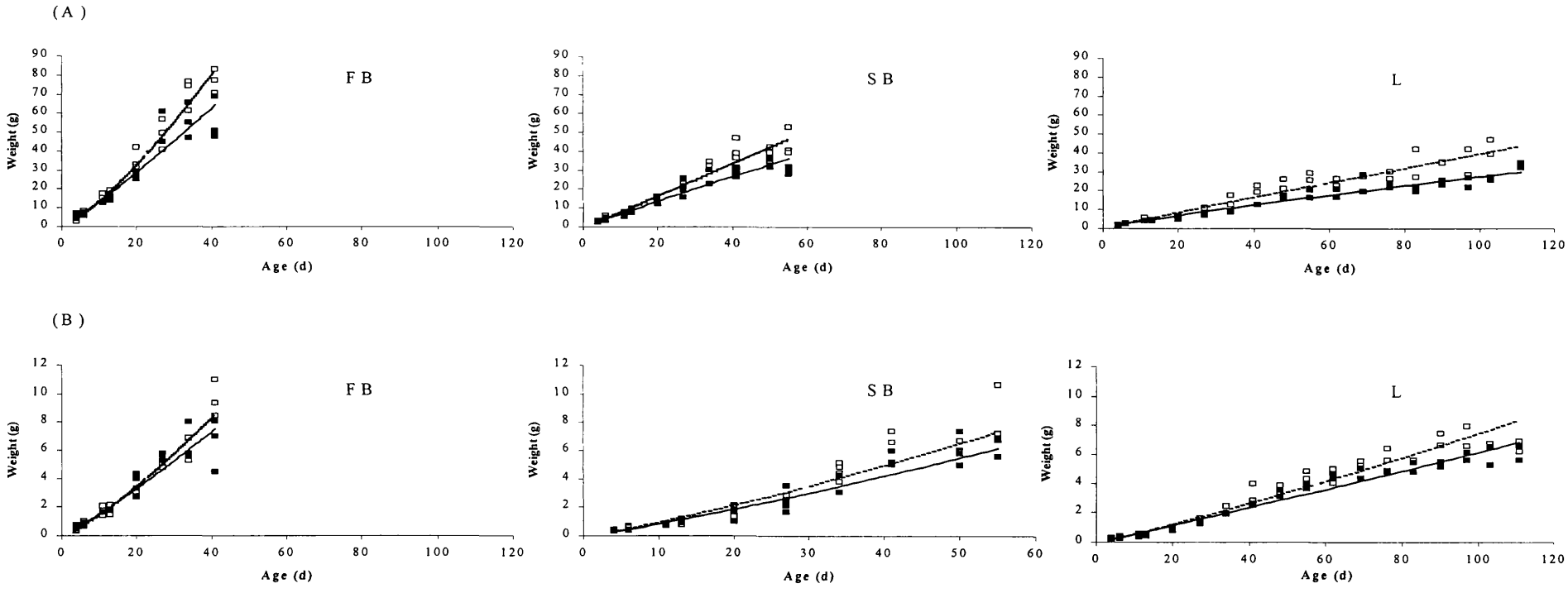

Figure 7.3 The absolute (A) intestine and (B) caeca mass (g) of male (□) and female (■) chickens in fast broiler (FB), slow broiler (SB) and layer (L) chicken genotypes. The data presented are shown for each individual bird.

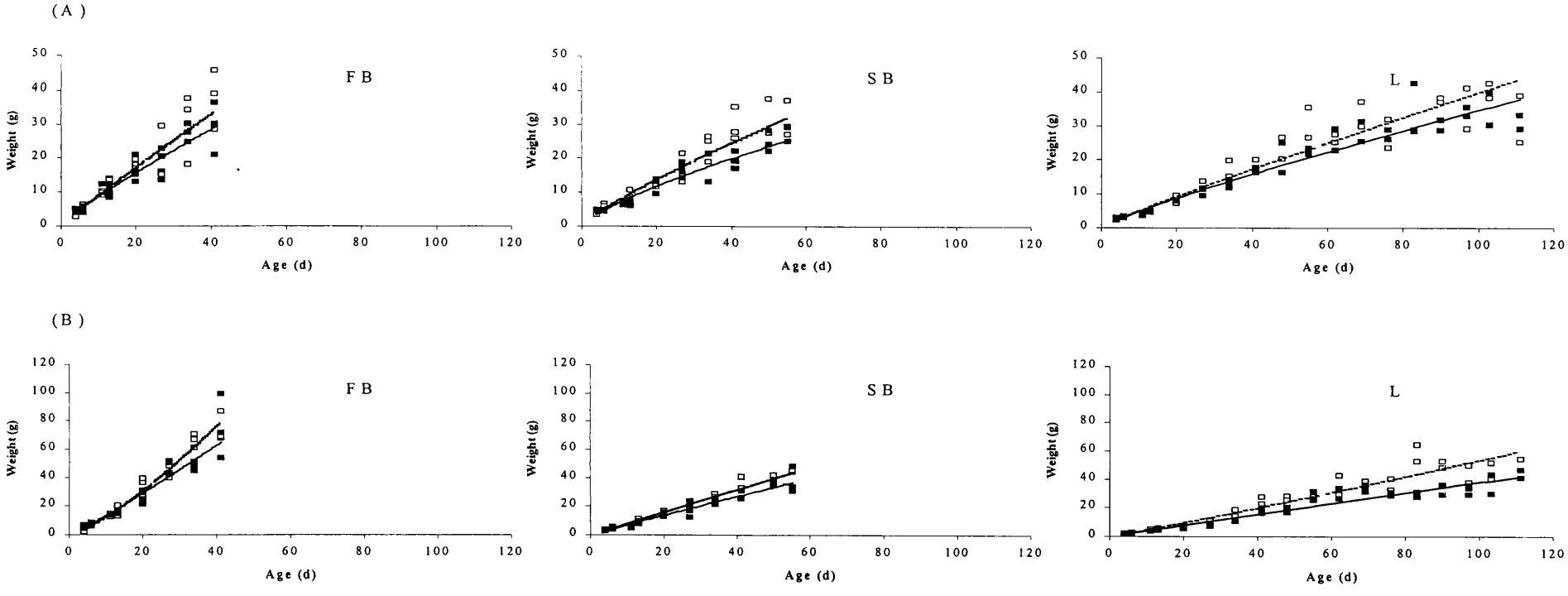

Figure 7.4 The absolute (A) gizzard and (B) liver mass (g) of male (□) and female (■) chickens in fast broiler (FB), slow broiler (SB) and layer (L) chicken genotypes. The data presented are shown for each individual bird.

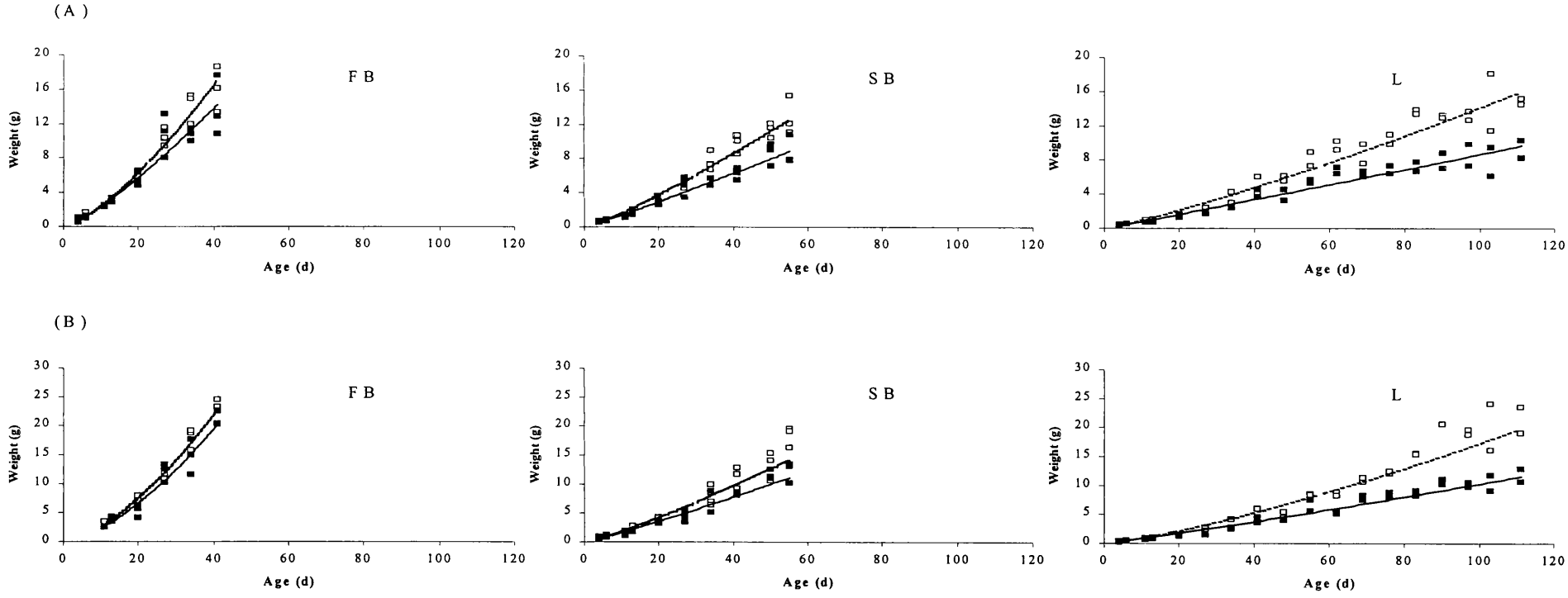

Figure 7.5 The absolute (A) heart and (B) lung mass (g) of male (□) and female (■) chickens in fast broiler (FB), slow broiler (SB) and layer (L) chicken genotypes. The data presented are shown for each individual bird.

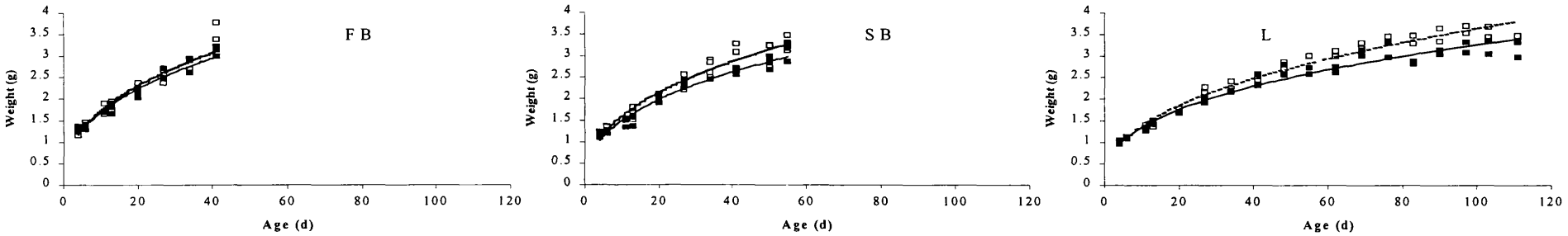

Figure 7.6 The absolute brain mass (g) of male (□) and female (■) chickens in fast broiler (FB), slow broiler (SB) and layer (L) chicken genotypes. The data presented are shown for each individual bird.

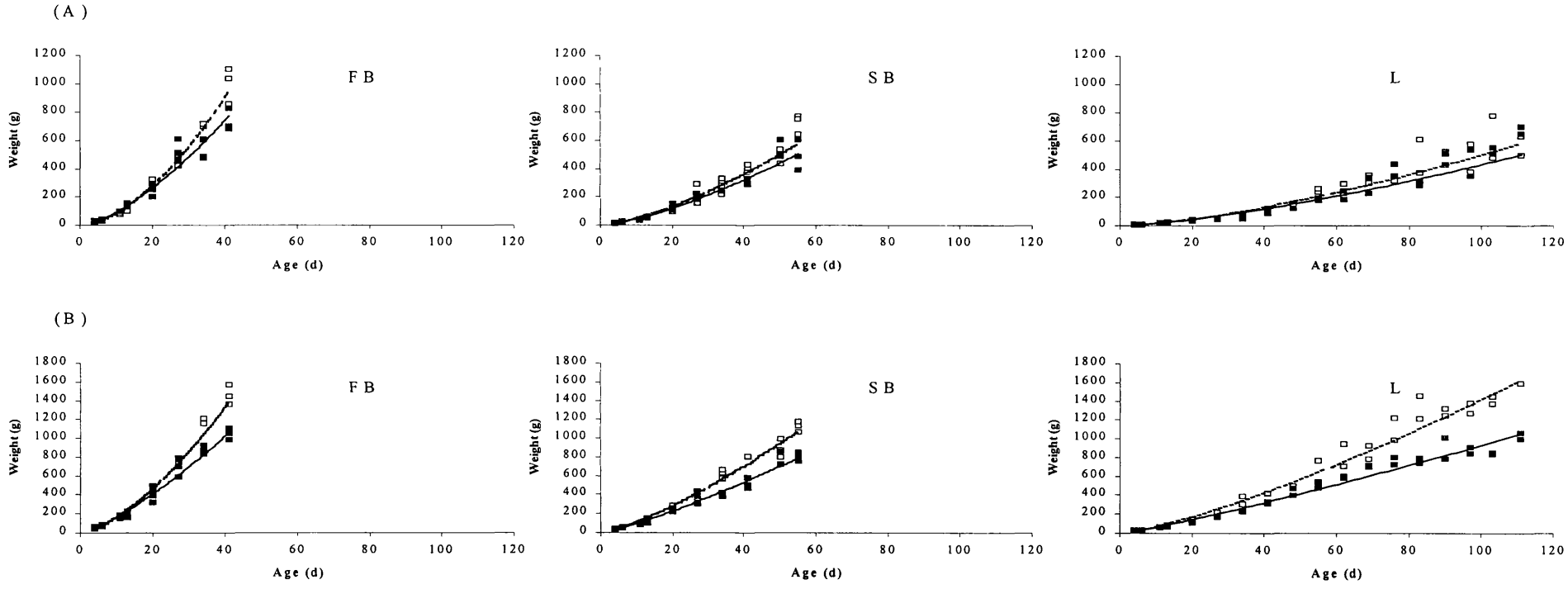

Figure 7.7 The absolute (A) fat and (B) crude protein content (g) of the whole bird carcass of male (□) and female (■) chickens in fast broiler (FB), slow broiler (SB) and layer (L) chicken genotypes. The data presented are shown for each individual bird.

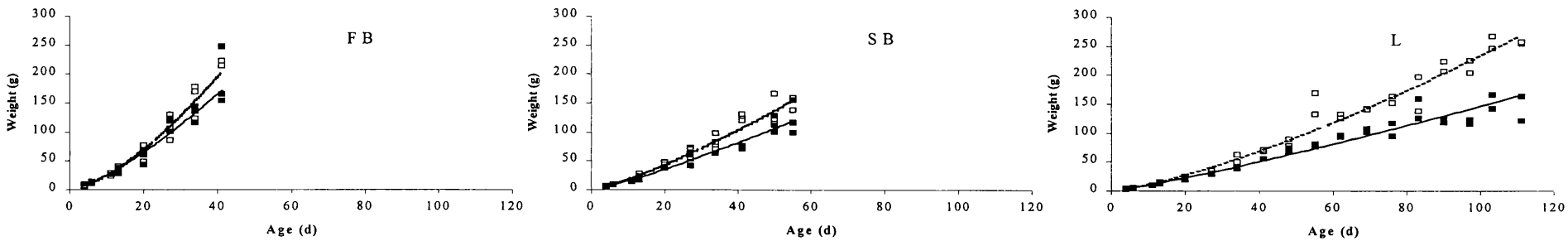

Figure 7.8 The absolute ash content (g) of the whole bird carcass of male (□) and female (■) chickens in fast broiler (FB), slow broiler (SB) and layer (L) chicken genotypes. The data presented are shown for each individual bird.

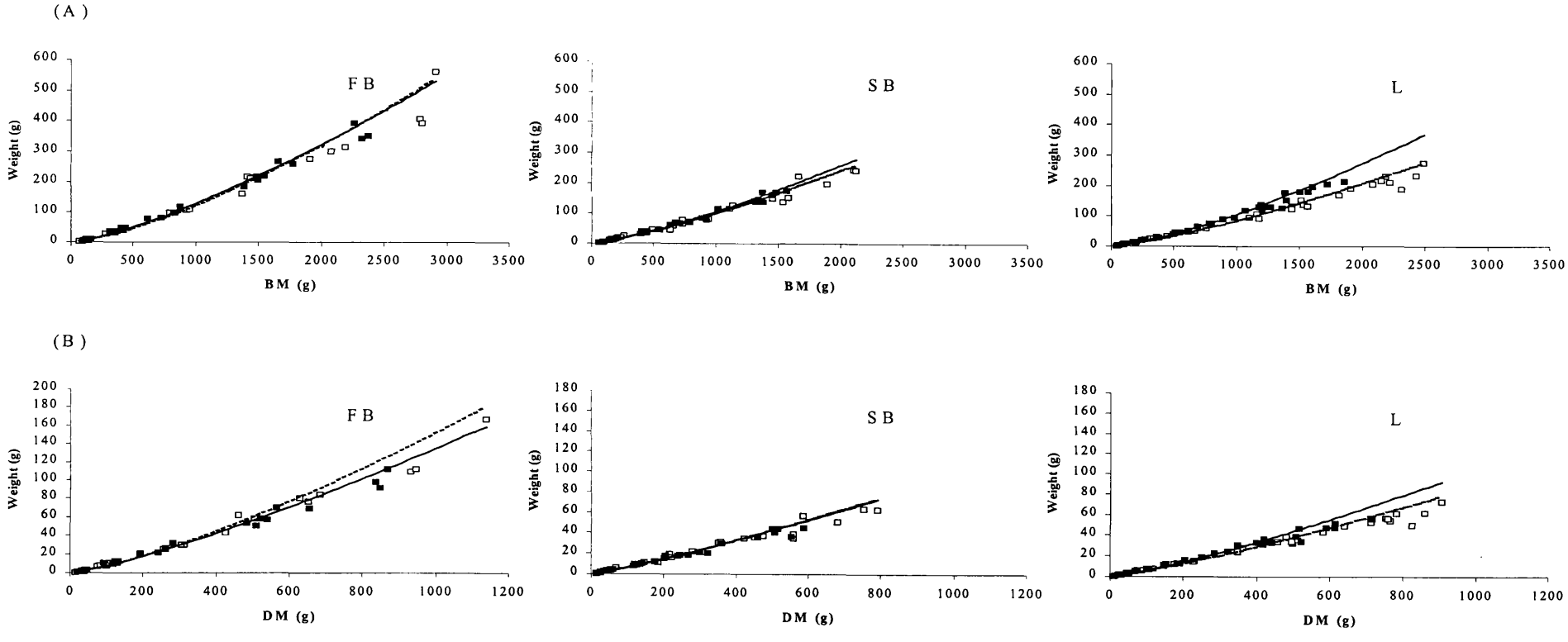

Figure 7.9 The absolute (A) wet and (B) dry pectoral muscle mass (g) of male (□) and female (■) chickens in fast broiler (FB), slow broiler (SB) and layer (L) chicken genotypes. The data presented are shown for each individual bird.

7.5 APPENDIX 5 – Correlation Matrices of the Absolute Wet and Dry Masses (g) of all the Organs of the Three Genotypes

Table 7.4 Correlation matrix of the absolute wet tissue masses of the fast broiler genotype. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

	Pectorals	Legs	Intestine	Caeca	Gizzard	Liver	Heart	Lungs	Brain
Intestine	0.923	0.955							
	(0.000)	(0.000)							
Caeca	0.948	0.953	0.957						
	(0.000)	(0.000)	(0.000)						
Gizzard	0.857	0.903	0.915	0.886					
	(0.000)	(0.000)	(0.000)	(0.000)					
Liver	0.948	0.956	0.941	0.953	0.856				
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)				
Heart	0.955	0.961	0.959	0.965	0.875	0.979			
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)			
Lungs	0.974	0.987	0.959	0.957	0.916	0.963	0.964		
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)		
Brain	0.947	0.960	0.951	0.964	0.899	0.957	0.959	0.967	
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	
Carcass	0.984	0.996	0.954	0.956	0.907	0.957	0.960	0.991	0.965
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)

Table 7.5 Correlation matrix of the absolute wet tissue masses of the slow broiler genotype. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

	Pectorals	Legs	Intestine	Caeca	Gizzard	Liver	Heart	Lungs	Brain
Intestine	0.925	0.950							
	(0.000)	(0.000)							
Caeca	0.949	0.961	0.961						
	(0.000)	(0.000)	(0.000)						
Gizzard	0.914	0.929	0.943	0.921					
	(0.000)	(0.000)	(0.000)	(0.000)					
Liver	0.960	0.972	0.958	0.961	0.959				
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)				
Heart	0.969	0.983	0.970	0.962	0.928	0.972			
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)			
Lungs	0.971	0.971	0.921	0.945	0.924	0.966	0.951		
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)		
Brain	0.928	0.940	0.947	0.922	0.946	0.952	0.942	0.924	
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	
Carcass	0.987	0.993	0.958	0.967	0.943	0.980	0.981	0.978	0.951
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)

Table 7.6 Correlation matrix of the absolute wet tissue masses of the layer genotype. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

	Pectorals	Legs	Intestine	Caeca	Gizzard	Liver	Heart	Lungs	Brain
Intestine	0.934	0.943							
	(0.000)	(0.000)							
Caeca	0.949	0.930	0.944						
	(0.000)	(0.000)	(0.000)						
Gizzard	0.888	0.876	0.903	0.952					
	(0.000)	(0.000)	(0.000)	(0.000)					
Liver	0.938	0.951	0.960	0.943	0.902				
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)				
Heart	0.941	0.974	0.949	0.935	0.895	0.968			
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)			
Lungs	0.948	0.976	0.918	0.913	0.848	0.921	0.961		
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)		
Brain	0.895	0.882	0.938	0.956	0.935	0.922	0.905	0.863	
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	
Carcass	0.984	0.994	0.958	0.956	0.897	0.964	0.975	0.973	0.912
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)

Table 7.7 Correlation matrix of the absolute dry tissue masses of the fast broiler genotype. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

	Pectorals	Legs	Intestine	Caeca	Gizzard	Liver	Heart	Lungs	Brain
Intestine	0.951	0.963							
	(0.000)	(0.000)							
Caeca	0.866	0.874	0.886						
	(0.000)	(0.000)	(0.000)						
Gizzard	0.853	0.888	0.901	0.818					
	(0.000)	(0.000)	(0.000)	(0.000)					
Liver	0.942	0.958	0.944	0.904	0.864				
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)				
Heart	0.887	0.912	0.899	0.888	0.851	0.906			
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)			
Lungs	0.975	0.975	0.970	0.890	0.900	0.962	0.901		
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)		
Brain	0.927	0.945	0.936	0.934	0.879	0.954	0.911	0.949	
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	
Carcass	0.986	0.981	0.961	0.879	0.882	0.954	0.903	0.987	0.950
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)

Table 7.8 Correlation matrix of the absolute dry tissue masses of the slow broiler genotype. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

	Pectorals	Legs	Intestine	Caeca	Gizzard	Liver	Heart	Lungs	Brain
Intestine	0.929	0.933							
	(0.000)	(0.000)							
Caeca	0.951	0.945	0.951						
	(0.000)	(0.000)	(0.000)						
Gizzard	0.928	0.936	0.942	0.924					
	(0.000)	(0.000)	(0.000)	(0.000)					
Liver	0.967	0.972	0.970	0.961	0.968				
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)				
Heart	0.969	0.960	0.938	0.963	0.913	0.959			
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)			
Lungs	0.967	0.967	0.932	0.946	0.939	0.974	0.948		
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)		
Brain	0.926	0.914	0.948	0.911	0.952	0.962	0.902	0.940	
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	
Carcass	0.986	0.995	0.944	0.958	0.942	0.982	0.965	0.978	0.928
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)

Table 7.9 Correlation matrix of the absolute dry tissue masses of the layer genotype. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

	Pectorals	Legs	Intestine	Caeca	Gizzard	Liver	Heart	Lungs	Brain
Intestine	0.948	0.937							
	(0.000)	(0.000)							
Caeca	0.955	0.920	0.930						
	(0.000)	(0.000)	(0.000)						
Gizzard	0.900	0.891	0.892	0.934					
	(0.000)	(0.000)	(0.000)	(0.000)					
Liver	0.941	0.946	0.956	0.933	0.916				
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)				
Heart	0.942	0.965	0.912	0.891	0.881	0.930			
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)			
Lungs	0.958	0.983	0.903	0.896	0.861	0.915	0.950		
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)		
Brain	0.903	0.890	0.918	0.934	0.942	0.933	0.881	0.874	
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	
Carcass	0.992	0.992	0.955	0.949	0.898	0.954	0.957	0.973	0.904
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)

Table 7.10 Correlation matrix of the absolute wet tissue masses of all three genotypes at 0-500 g body mass. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

	Pectorals	Legs	Intestine	Caeca	Gizzard	Liver	Heart	Lungs	Brain
Intestine	0.939	0.918							
	(0.000)	(0.000)							
Caeca	0.929	0.944	0.907						
	(0.000)	(0.000)	(0.000)						
Gizzard	0.883	0.921	0.845	0.913					
	(0.000)	(0.000)	(0.000)	(0.000)					
Liver	0.958	0.952	0.953	0.918	0.880				
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)				
Heart	0.965	0.978	0.930	0.942	0.920	0.972			
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)			
Lungs	0.931	0.926	0.878	0.875	0.856	0.894	0.920		
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)		
Brain	0.860	0.910	0.787	0.907	0.937	0.846	0.895	0.816	
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	
Carcass	0.967	0.992	0.900	0.939	0.934	0.944	0.977	0.920	0.925
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)

Table 7.11 Correlation matrix of the absolute wet tissue masses of all three genotypes at 2000-2500 g body mass. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

	Pectorals	Legs	Intestine	Caeca	Gizzard	Liver	Heart	Lungs	Brain
Intestine	0.633	-0.183							
	(0.011)	(0.515)							
Caeca	0.020	-0.349	0.139						
	(0.943)	(0.202)	(0.622)						
Gizzard	-0.482	0.128	0.030	-0.179					
	(0.069)	(0.650)	(0.916)	(0.523)					
Liver	0.753	-0.018	0.493	0.038	-0.471				
	(0.001)	(0.948)	(0.062)	(0.893)	(0.076)				
Heart	0.189	0.387	0.228	0.397	-0.206	0.487			
	(0.499)	(0.155)	(0.414)	(0.143)	(0.462)	(0.066)			
Lungs	0.317	0.374	-0.004	-0.146	0.119	0.162	0.295		
	(0.250)	(0.169)	(0.988)	(0.604)	(0.672)	(0.563)	(0.286)		
Brain	-0.589	0.325	-0.770	-0.094	0.110	-0.457	0.065	0.215	
	(0.021)	(0.237)	(0.001)	(0.739)	(0.698)	(0.087)	(0.817)	(0.441)	
Carcass	-0.372	0.692	-0.689	-0.266	-0.032	-0.400	0.059	0.417	0.692
	(0.173)	(0.004)	(0.005)	(0.338)	(0.911)	(0.140)	(0.833)	(0.122)	(0.004)

Table 7.12 Correlation matrix of the absolute dry tissue masses of all three genotypes at 0-500 g body mass. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

	Pectorals	Legs	Intestine	Caeca	Gizzard	Liver	Heart	Lungs	Brain
Intestine	0.946	0.944							
	(0.000)	(0.000)							
Caeca	0.942	0.946	0.925						
	(0.000)	(0.000)	(0.000)						
Gizzard	0.921	0.925	0.895	0.927					
	(0.000)	(0.000)	(0.000)	(0.000)					
Liver	0.960	0.963	0.949	0.933	0.929				
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)				
Heart	0.961	0.974	0.940	0.951	0.932	0.970			
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)			
Lungs	0.938	0.936	0.893	0.891	0.885	0.895	0.930		
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)		
Brain	0.821	0.828	0.737	0.856	0.891	0.826	0.842	0.773	
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	
Carcass	0.965	0.980	0.906	0.944	0.939	0.958	0.969	0.917	0.880
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)

Table 7.13 Correlation matrix of the absolute dry tissue masses of all three genotypes at 2000-2500 g body mass. The levels of significance of the correlation coefficients difference from zero, are shown in parentheses.

	Pectorals	Legs	Intestine	Caeca	Gizzard	Liver	Heart	Lungs	Brain
Intestine	0.805	0.408							
	(0.000)	(0.131)							
Caeca	0.063	-0.010	0.017						
	(0.823)	(0.971)	(0.953)						
Gizzard	-0.312	0.025	0.075	-0.473					
	(0.258)	(0.928)	(0.790)	(0.075)					
Liver	0.844	0.533	0.747	0.123	-0.345				
	(0.000)	(0.041)	(0.001)	(0.661)	(0.208)				
Heart	0.112	0.279	0.197	0.271	0.130	0.096			
	(0.691)	(0.314)	(0.481)	(0.329)	(0.644)	(0.734)			
Lungs	0.286	0.640	0.059	-0.068	-0.095	0.292	0.035		
	(0.302)	(0.010)	(0.834)	(0.809)	(0.736)	(0.290)	(0.901)		
Brain	-0.492	-0.109	-0.657	0.067	-0.155	-0.342	-0.068	0.429	
	(0.063)	(0.700)	(0.008)	(0.813)	(0.582)	(0.212)	(0.810)	(0.111)	
Carcass	-0.169	0.340	-0.501	0.123	-0.388	-0.181	-0.163	0.589	0.625
	(0.548)	(0.214)	(0.057)	(0.662)	(0.153)	(0.518)	(0.563)	(0.021)	(0.013)

7.6 APPENDIX 6 – The Mass (Absolute, g) of the Organs and Carcass Composition of the Three Genotypes of Chicken

Table 7.14 The mean (± SEM) wet mass (absolute, g) of the demand organs, for each of three genotypes of chicken at weekly intervals.

Organ		Age (weeks)															
Mass (g)	Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	[1]FB	8.01	41.20	99.14	200.5	271.7	407.7	-	-	-	-	-	-	-	-	-	-
		(0.66)	(2.05)	(4.79)	(8.01)	(13.92)	(32.26)										
Pectoral	[2]SB	4.82	17.15	37.42	61.70	91.03	116.4	164.3	192.3	-	-	-	-	-	-	-	-
		(0.25)	(1.29)	(1.50)	(4.28)	(9.46)	(12.07)	(12.78)	(17.49)								
	[3]L	1.90	7.01	13.27	23.21	33.99	45.89	58.77	86.70	103.1	127.6	132.1	161.5	180.3	191.9	201.4	220.2
		(0.20)	(0.44)	(0.33)	(1.11)	(3.39)	(1.92)	(3.02)	(8.05)	(9.58)	(7.72)	(2.13)	(18.21)	(19.58)	(16.51)	(13.75)	(18.96)
	FB	13.79	49.20	107.3	200.6	267.0	371.1	-	-	-	-	-	-	-	-	-	-
		(0.64)	(1.60)	(5.47)	(6.83)	(16.62)	(18.71)										
Leg	SB	9.19	23.93	51.43	83.25	117.7	150.2	216.2	231.1	-	-	-	-	-	-	-	-
		(0.35)	(1.44)	(1.03)	(4.79)	(12.01)	(14.06)	(10.45)	(18.39)								
	L	4.75	11.10	20.98	33.50	50.10	69.17	86.51	128.9	146.9	174.6	194.9	235.0	251.4	263.4	296.7	319.1
		(0.24)	(0.66)	(0.96)	(2.56)	(4.13)	(6.10)	(5.94)	(17.64)	(19.46)	(15.49)	(14.92)	(44.98)	(36.07)	(31.35)	(44.25)	(43.73)
	FB	73.25	226.1	460.5	800.5	1040	1461	-	-	-	-	-	-	-	-	-	-
		(2.71)	(5.40)	(22.20)	(6.00)	(55.24)	(63.09)										
Carcass	SB	53.08	120.4	262.4	415.9	572.3	714.4	922.4	1077	-	-	-	-	-	-	-	-
		(1.02)	(5.24)	(6.45)	(14.46)	(41.36)	(58.49)	(27.85)	(83.96)								
	L	32.13	65.18	111.8	172.5	255.2	342.0	433.0	593.5	685.7	818.3	881.6	985.1	1095	1123	1199	1327
		(0.73)	(3.37)	(3.04)	(9.78)	(22.96)	(20.17)	(19.74)	(66.68)	(75.75)	(65.87)	(61.84)	(164.1)	(141.6)	(116.8)	(141.4)	(125.7)

Where; [1]FB-fast broiler, [2]SB-slow broiler, [3]L-layer.

Table 7.15 The mean (± SEM) wet mass (absolute, g) of the organs of the gastro-intestinal tract and the liver, for each of three genotypes of chicken at weekly intervals.

Organ		Age (weeks)															
Mass (g)	Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	[1]FB	7.25	16.13	30.88	51.33	63.28	66.56	-	-	-	-	-	-	-	-	-	-
		(0.31)	(0.70)	(1.84)	(3.14)	(4.60)	(5.80)										
Intestine	[2]SB	4.42	8.42	13.90	21.10	30.50	34.98	36.10	37.32	-	-	-	-	-	-	-	-
		(0.30)	(0.26)	(0.52)	(1.06)	(1.64)	(3.10)	(1.68)	(3.77)								
	[3]L	2.70	4.51	5.94	8.90	12.05	16.80	20.33	22.91	21.84	26.35	25.64	27.89	29.89	29.92	34.93	33.62
		(0.15)	(0.12)	(0.29)	(0.94)	(2.00)	(2.49)	(2.19)	(2.91)	(2.03)	(2.14)	(1.76)	(5.00)	(3.11)	(4.31)	(5.12)	(0.62)
	FB	0.81	1.77	3.43	5.26	6.39	8.07	-	-	-	-	-	-	-	-	-	-
		(0.04)	(0.07)	(0.23)	(0.13)	(0.42)	(0.90)										
Caeca	SB	0.49	1.01	1.69	2.50	4.28	6.04	6.16	7.42	-	-	-	-	-	-	-	-
		(0.03)	(0.05)	(0.13)	(0.19)	(0.30)	(0.36)	(0.34)	(0.70)								
	L	0.35	0.54	0.96	1.38	2.07	2.98	3.47	4.18	4.52	5.07	5.44	5.35	6.20	6.58	6.34	6.34
		(0.03)	(0.04)	(0.04)	(0.10)	(0.12)	(0.33)	(0.16)	(0.26)	(0.21)	(0.26)	(0.38)	(0.19)	(0.53)	(0.50)	(0.35)	(0.29)
	FB	5.48	11.13	17.60	19.03	28.76	33.44	-	-	-	-	-	-	-	-	-	-
		(0.30)	(0.78)	(0.98)	(2.13)	(2.83)	(3.56)										
Gizzard	SB	5.12	7.62	12.36	16.94	21.02	24.52	27.29	28.80	-	-	-	-	-	-	-	-
		(0.27)	(0.53)	(0.48)	(1.01)	(1.92)	(2.70)	(2.25)	(1.86)								
	L	3.27	4.86	8.19	11.60	15.13	17.77	22.07	26.78	26.23	30.90	27.65	35.59	34.02	34.67	37.83	31.61
		(0.13)	(0.13)	(0.50)	(0.85)	(1.75)	(0.84)	(2.36)	(3.06)	(1.35)	(2.40)	(1.83)	(3.98)	(2.28)	(2.47)	(2.63)	(2.96)
	FB	7.12	15.88	29.23	46.62	56.96	74.90	-	-	-	-	-	-	-	-	-	-
		(0.34)	(0.84)	(2.19)	(1.77)	(4.29)	(6.56)										
Liver	SB	4.53	9.04	14.61	18.30	25.15	32.69	38.28	41.77	-	-	-	-	-	-	-	-
		(0.18)	(0.36)	(0.51)	(1.16)	(0.97)	(2.80)	(1.22)	(3.12)								
	L	2.46	4.88	6.77	9.46	13.63	21.32	22.48	29.03	33.02	35.37	33.20	43.99	41.47	37.58	41.10	49.00
		(0.21)	(0.27)	(0.33)	(0.98)	(1.81)	(2.37)	(2.55)	(1.35)	(3.49)	(1.44)	(2.59)	(8.88)	(5.44)	(4.51)	(4.57)	(3.33)

Where; [1]FB-fast broiler, [2]SB-slow broiler, [3]L-layer.

Table 7.16 The mean (± SEM) wet mass (absolute, g) of the cardio-pulmonary organs and the brain, for each of three genotypes of chicken at weekly intervals.

Organ		Age (weeks)															
Mass (g)	Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	[1]FB	1.22	3.14	5.99	10.58	12.44	14.93	-	-	-	-	-	-	-	-	-	-
		(0.08)	(0.07)	(0.22)	(0.62)	(0.90)	(1.24)										
Heart	[2]SB	0.84	1.77	3.18	4.92	6.80	8.03	10.04	10.87	-	-	-	-	-	-	-	-
		(0.02)	(0.08)	(0.13)	(0.26)	(0.59)	(0.86)	(0.75)	(1.16)								
	[3]L	0.53	0.89	1.52	2.09	3.04	4.64	4.92	6.83	8.28	7.61	8.70	10.49	10.54	10.92	11.34	12.11
		(0.01)	(0.08)	(0.08)	(0.14)	(0.46)	(0.49)	(0.64)	(0.84)	(0.89)	(0.82)	(4.06)	(1.85)	(1.53)	(1.43)	(2.51)	(1.67)
	FB	-	3.77	6.49	11.52	16.34	22.27	-	-	-	-	-	-	-	-	-	-
			(0.12)	(0.43)	(0.44)	(1.17)	(0.66)										
Lungs	SB	1.04	2.09	3.63	4.56	7.68	9.84	12.55	15.37	-	-	-	-	-	-	-	-
		(0.06)	(0.12)	(0.16)	(0.22)	(0.71)	(0.81)	(0.78)	(1.51)								
	L	0.53	0.96	1.41	2.03	3.06	4.65	4.52	6.90	6.96	9.47	10.33	12.09	15.68	14.70	15.28	16.52
		(0.02)	(0.05)	(0.05)	(0.24)	(0.38)	(0.47)	(0.35)	(0.81)	(0.93)	(0.91)	(1.13)	(1.96)	(2.88)	(2.60)	(3.29)	(2.94)
	FB	1.40	1.84	2.18	2.53	2.83	3.28	-	-	-	-	-	-	-	-	-	-
		(0.02)	(0.03)	(0.04)	(0.04)	(0.06)	(0.11)										
Brain	SB	1.26	1.60	2.02	2.35	2.62	2.87	2.96	3.21	-	-	-	-	-	-	-	-
		(0.02)	(0.05)	(0.03)	(0.04)	(0.08)	(0.12)	(0.10)	(0.08)								
	L	1.10	1.42	1.72	2.10	2.26	2.47	2.73	2.82	2.87	3.16	3.26	3.11	3.29	3.41	3.38	3.28
		(0.01)	(0.03)	(0.02)	(0.08)	(0.06)	(0.06)	(0.07)	(0.10)	(0.12)	(0.06)	(0.10)	(0.16)	(0.14)	(0.13)	(0.13)	(0.11)

Where; [1]FB-fast broiler, [2]SB-slow broiler, [3]L-layer.

Table 7.17 The mean (± SEM) of total wet muscle and organ masses (absolute,g), total muscle water content (g) and the muscle:organ ratio (M:O), for each of the three genotypes of chicken at weekly intervals.

		Age (weeks)															
Organ	Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	[1]FB	21.80	90.40	206.5	401.1	538.7	778.7	-	-	-	-	-	-	-	-	-	-
		(1.11)	(3.54)	(9.49)	(12.64)	(28.85)	(47.38)										
Total	[2]SB	14.01	41.09	88.86	145.0	208.8	266.6	380.5	423.4	-	-	-	-	-	-	-	-
Muscle		(0.52)	(2.72)	(2.34)	(8.68)	(21.31)	(25.92)	(19.54)	(34.56)								
(g)	[3]L	6.65	18.11	34.25	56.70	84.08	115.1	145.3	215.6	250.0	302.2	327.0	396.5	431.7	455.3	498.1	539.3
		(0.39)	(1.06)	(1.19)	(3.63)	(7.34)	(7.86)	(7.94)	(25.00)	(28.89)	(22.22)	(16.61)	(62.99)	(53.81)	(46.51)	(57.96)	(51.37)
	FB	23.27	53.65	95.81	146.9	187.0	223.5	-	-	-	-	-	-	-	-	-	-
		(0.88)	(1.85)	(4.70)	(6.12)	(12.29)	(12.93)										
Total	SB	17.71	31.55	51.40	70.67	98.06	119.0	133.4	144.7	-	-	-	-	-	-	-	-
Organ		(0.51)	(1.11)	(1.57)	(3.34)	(5.27)	(9.83)	(5.64)	(10.06)								
(g)	L	10.94	18.06	26.52	37.56	51.25	70.64	80.52	100.1	103.7	117.9	114.2	138.5	141.1	137.8	150.2	152.5
		(0.44)	(0.55)	(1.06)	(3.04)	(6.52)	(5.81)	(6.88)	(8.06)	(7.81)	(7.38)	(6.20)	(17.34)	(15.51)	(11.92)	(17.16)	(8.84)
	FB	0.94	1.70	2.16	2.76	2.70	3.52	-	-	-	-	-	-	-	-	-	-
		(0.04)	(0.08)	(0.03)	(0.14)	(0.08)	(0.24)										
M:O	SB	0.80	1.30	1.73	2.05	2.12	2.24	2.87	2.92	-	-	-	-	-	-	-	-
		(0.04)	(0.05)	(0.05)	(0.06)	(0.15)	(0.12)	(0.17)	(0.06)								
	L	0.61	1.00	1.30	1.52	1.66	1.64	1.82	2.14	2.40	2.56	2.86	2.84	3.05	3.29	3.32	3.52
		(0.01)	(0.04)	(0.06)	(0.05)	(0.07)	(0.05)	(0.07)	(0.13)	(0.11)	(0.08)	(0.02)	(0.16)	(0.15)	(0.11)	(0.13)	(0.19)
	FB	16.39	65.33	146.0	281.1	389.5	551.1	-	-	-	-	-	-	-	-	-	-
		(0.82)	(2.59)	(6.08)	(7.85)	(20.51)	(31.66)										
Water	SB	10.54	30.59	65.62	107.5	153.6	196.3	276.7	304.4	-	-	-	-	-	-	-	-
Content		(0.37)	(2.05)	(171)	(6.41)	(15.95)	(19.06)	(15.88)	(23.35)								
(g)	L	5.18	13.90	26.24	43.13	64.06	87.07	109.6	160.8	187.6	225.3	244.2	296.6	319.5	338.2	368.5	397.5
		(0.28)	(0.82)	(0.88)	(2.82)	(5.65)	(6.12)	(6.08)	(18.67)	(21.87)	(16.72)	(12.89)	(47.53)	(40.79)	(34.80)	(44.20)	(40.43)

Where; [1]FB-fast broiler, [2]SB-slow broiler, [3]L-layer.

Table 7.18 The carcass composition (mean ± SEM) (absolute, g/bird), for each of the three genotypes of chicken at weekly intervals.

Organ	Genotype	Age (weeks)															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Crude protein	[1]FB	75.31 (3.11)	210.5 (8.11)	425.8 (20.27)	727.1 (26.32)	988.5 (64.17)	1255 (97.63)	-	-	-	-	-	-	-	-	-	-
	[2]SB	53.14 (0.92)	122.8 (5.74)	249.6 (8.62)	386.9 (15.04)	538.2 (46.79)	661.6 (65.44)	828.6 (42.41)	965.8 (73.49)	-	-	-	-	-	-	-	-
	[3]L	35.93 (0.53)	74.68 (4.34)	123.9 (7.94)	190.0 (12.42)	289.8 (36.66)	367.6 (27.04)	464.1 (22.24)	637.6 (75.50)	707.1 (83.46)	779.6 (51.49)	933.6 (111.0)	1051 (172.3)	1087 (121.5)	1100 (132.9)	1124 (165.1)	1308 (162.6)
Fat	FB	36.79 (1.65)	131.1 (5.82)	280.6 (14.36)	498.2 (21.94)	599.3 (41.84)	868.7 (70.21)	-	-	-	-	-	-	-	-	-	-
	SB	23.48 (1.57)	55.01 (1.55)	118.4 (6.46)	201.4 (14.87)	269.9 (17.48)	356.5 (22.90)	513.4 (22.48)	609.3 (60.58)	-	-	-	-	-	-	-	-
	L	8.76 (1.00)	20.10 (2.81)	37.09 (2.63)	49.55 (2.55)	66.42 (4.84)	106.7 (8.99)	138.9 (9.16)	219.8 (19.59)	238.3 (22.55)	307.7 (28.04)	363.9 (25.06)	395.8 (73.82)	495.7 (21.77)	462.1 (56.30)	583.6 (67.72)	619.5 (43.10)
Ash	FB	12.08 (0.42)	34.96 (1.51)	62.86 (4.16)	110.2 (5.89)	144.4 (10.09)	208.6 (16.44)	-	-	-	-	-	-	-	-	-	-
	SB	8.75 (0.20)	21.33 (1.27)	41.56 (1.19)	60.23 (3.53)	75.82 (5.54)	100.0 (11.36)	125.0 (9.17)	131.5 (9.85)	-	-	-	-	-	-	-	-
	L	5.53 (0.14)	13.38 (0.55)	22.84 (1.37)	32.83 (1.65)	48.78 (5.19)	63.24 (4.16)	77.49 (4.46)	114.8 (22.18)	113.1 (10.03)	123.5 (10.67)	132.3 (15.90)	155.7 (15.33)	168.9 (27.25)	167.2 (27.81)	206.4 (30.23)	199.8 (33.65)

[1]FB-fast broiler, [2]SB-slow broiler, [3]L-layer.