AN INVESTIGATION OF FACTORS INFLUENCING RATE OF LAY, EGG WEIGHT AND EMBRYONIC GROWTH IN BROILER BREEDER HENS

by

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ABSTRACT

The laying performance of broiler breeder hens is characteristically poor. Of the eggs that are produced, a large proportion are rejected before setting because they are either too small or excessively big, and of the eggs that are set, hatchability rates are often low, depending on the age of the hen. Since so much is still unknown about broiler breeders, many avenues of research would be fruitful. In this study, four disparate aspects were investigated as a means of improving the number of hatchable eggs per hen.

The effect of linoleic acid intake on egg weight of broiler breeders and laying hens was compared. Analysis of both published and experimental data revealed that egg weight was influenced significantly by this fatty acid. Breeder egg production was affected concomitantly. Increasing linoleic acid intake of young hens would increase early egg size and the number of eggs set, while decreasing the linoleic acid intake of ageing breeders would decrease egg size and production.

The influence of 20 week body weight and nutrient intake on early laying performance of 360 broiler breeders was determined. The excellent performance achieved was independent of 20 week body weight. Laying performance and weight gain increased as food allocation and nutrient density increased. A comparison of theoretical and recommended nutrient intakes revealed that hens are overfed pre-peak and that energy intakes should not decline post-peak as is recommended.

The investigation into the effect of breeder age (egg size) on embryonic and chick growth revealed that exponential embryonic growth was restricted within small eggs due to a lower yolk supply, rate of yolk absorption, and water loss. Chicks from small eggs were light, and grew relatively slowly. However, during times of chick shortage, small eggs could be used successfully if managed correctly.

In the fourth experiment in this series, the response of laying hens to dietary tryptophan was measured successfully, but the objective of comparing this response with that of broiler breeders failed. As a result of overestimating the tryptophan requirement of breeders, the coefficients of response could not be estimated. It was concluded that an additional breeder tryptophan trial should be conducted.

DECLARATION

I hereby declare that the research reported in this thesis does not contain material which has been accepted for the award of any other degree or diploma in another University and to the best of my knowledge, material previously published or written by another person, except where due reference is made in the text.

L. McLoughlin

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CHAPTER 1

GENERAL INTRODUCTION

The depressed laying performance of broiler breeder hens throughout the laying cycle is of major concern, since the production of settable eggs is a critically important component of the poultry industry. Nevertheless, this field of poultry research has received scant attention. Research conducted on commercial laying hens is simply assumed to be directly applicable to broiler breeders. As a consequence, it is uncertain whether the poor laying performance is due to either one, or a combination of factors such as genetics, the environmental conditions and/or nutrition. Therefore, four disparate aspects which include the effect of linoleic acid intake on egg weight, the effect of nutrient intake and 20 week body weight on early laying performance, the influence of broiler breeder age (egg size) on pre- and post-natal growth of broilers, and the response of broiler breeders to tryptophan intake were investigated as a means of increasing the number of settable and hatchable eggs produced by broiler breeder hens.

The use of linoleic acid intake to manipulate egg weight

In comparison to a commercial laying flock with a 52 week production period, broiler breeder hens will produce on average, a staggering 140 fewer eggs per hen housed over their laying cycle of 40 weeks (Nilipour, 1997). Since breeders are housed on the floor, as opposed to being kept in cages, the number of birds per square metre is reduced, and consequently, labour and production costs are elevated. Combined with the fact that broiler breeders consume significantly more food than do commercial layers, the cost of producing a dozen fertile eggs can be two to three times as high as that of table eggs (Nilipour, 1997). Therefore, it is of considerable economic importance to ensure that of the few eggs that are produced, and of the even-fewer that are placed in the incubator, hatchability rates are maintained at a high level to help cover the costs of production.

Hatchability is influenced by factors which include male and female fertility, length of storage of eggs, handling and hygiene and the nutrition of the parent stock

(Hodgetts, 1994). However, egg size (or bird age) has a far more profound effect on hatchability than do the above. As a broiler breeder flock ages, egg size increases rapidly at first from the initial weight of approximately 45g at 24 weeks. The rate of increase then slows down until a maximum egg size of 80g is reached at 64 weeks of age. Small eggs produced by young hens are known to exhibit poor hatchability rates (Sunde and Bird, 1959; Morris *et al.*, 1968; Smith and Bohren, 1975). Hatcheries experience even lower hatchabilities of the large eggs laid by ageing flocks (Reinhart and Hurnik, 1984; Wilson and Harms, 1988); these may be in the region of 70 percent. Peak hatchability of approximately 85 percent occurs in the middle of the laying cycle (Mauldin *et al.*, 1991). There appears to be an optimum egg size for maximum hatch, but the relationship is not clear. It could be speculated that the hatchability of eggs produced at the commencement and towards the end of the laying period could be improved by increasing egg size early in the laying period, and decreasing it as the birds age.

In addition, hatching eggs are three to four times more valuable than commercial eggs. However, the eggs produced within the first two weeks of production are regarded as too small for setting and are usually discarded, as are the excessively large eggs produced at the end of the laying cycle. If the size of the eggs produced by broiler breeders at these times could be altered, the financial benefits to the industry would be substantial.

Egg weight may be manipulated through husbandry procedures, including the use of various lighting programmes and altering nutrient intakes in both the rearing and laying periods. During the rearing period, delaying age at sexual maturity by means of light restriction has been shown to decrease the production of small eggs. However, short photoperiods have an equivalent, although negative effect, on egg production (Morris, 1985). Increasing the energy intakes of young hens appears to increase egg weight (Pearson and Herron, 1982), but can also result in poor laying performance later in the laying cycle. The adoption of ahemeral cycles after the commencement of lay will increase the size of eggs laid by young hens. However, egg production will decline by the same proportion (Morris, 1973). During the laying period, decreasing the weight of eggs produced by ageing broiler breeders can be achieved by decreasing daily energy intakes (Pearson and Herron, 1982). However,

egg production will be adversely affected. Maintaining the birds on a 24 hour ahemeral cycle will also decrease egg weight, while egg production will increase concomitantly (Morris, 1985).

It has been suggested that the main nutritional tool for manipulating egg weight throughout the production period of commercial laying hens is through linoleic acid intake (Wakeman, 1997), since rate of lay is not significantly altered by the content of this fatty acid in the feed (Balnave, 1982; Whitehead, 1981; Scragg *et al.*, 1987; Sell *et al.*, 1987). Since very little research has been conducted on broiler breeders, the objective of the study was to determine whether egg size could be altered by varying the concentration of linoleic acid in the diets of breeders. This would be a viable option if early egg size could be increased and if the size of the eggs laid by the hens towards the end of the laying cycle could be reduced. Therefore, responses in egg weight to linoleic acid intake were compared between broiler breeder and commercial laying hens.

The influence of nutrient intake and 20 week body weight on early laying performance

Of major concern to the broiler breeder industry is the poor performance of broiler breeder hens especially during the initial laying period. This is because, in comparison to the performance objectives outlined by the breeding companies, peak egg production is characteristically depressed, while laying persistency post-peak, is poor. Since very little research has been conducted during the early laying period, it is uncertain whether the depressed performance is due to either one, or a combination of factors such as genetics, the environmental conditions and nutrition.

In an attempt to reach the set production goals, broiler breeders are often fed in excess of the recommended daily feed intakes stipulated by the breeding companies. However, no significant response in egg production has been observed. Therefore, it can be speculated that the poor performance may be due to overfeeding during the early laying period. Research by Hocking (1996) has in fact shown that overfeeding after photostimulation leads to an increase in the number of multiple ovulations and hence, to the rate of internal laying.

20 week body weight is also thought to be an indicator of early laying

performance. The frequently observed flat, depressed peak has been attributed to the variation in body weight at 20 weeks. However, there is no research which substantiates this assumption.

Unfortunately, the mind set has been what Cherry (personal communication) has described as insanity, repeating the same rearing and production procedures flock after flock and hoping for a better resultant performance. It is apparent that an entirely different approach is necessary. Future research should be of an exploratory nature, deviating from typical lighting and nutritional practices in both the rearing and laying periods.

Therefore, the objectives of the present experiment were to investigate the effect of food, or nutrient, intake and 20 week body weight on laying performance during the pre-peak, peak and post-peak periods, and to compare the nutrient (energy and amino acid) requirements recommended by the breeding companies with those determined theoretically.

The influence of broiler breeder age, or egg size, on pre- and post-natal growth of broilers

World wide, broiler breeder hens produce about 40 billion eggs annually. Depending on company standards, approximately two to 12 percent of the eggs are classified as unfit for hatching (Nilipour, 1997). An important factor influencing the number of eggs which are rejected over the laying cycle of a particular flock, is minimal fertile egg weight.

The rule of thumb in the industry is that fertile eggs should weigh at least 50-52g in order to be classified as fit for incubation. A uniform flock with an optimum mean body weight should be able to lay eggs of more than 50g by the time 15 to 20 percent hen day production is achieved, which is approximately two weeks into the laying cycle (Nilipour, 1997). However, if the small eggs laid by young flocks were to be hatched, it may be possible that the production costs incurred throughout rearing and early production, could be decreased by increasing the number of day-old chicks per hen housed.

It has been widely documented that there is a strong positive correlation between pre-incubation egg weight and the weight of the hatchling. The ratio between

the two variables is typically between 0.62 and 0.76 (Halbersleben and Mussehl, 1922; Bray and Iton, 1962; Proudfoot *et al.*, 1982; Yannakopoulous and Tservent-Gousi, 1987). In reviewing the phenomenon in six species, Shanawany (1987) developed the following prediction formula: Hatching weight = 0.96 x Egg weight$^{0.90}$, with consideration for additional variables such as incubator humidity and temperature.

However, the effect of egg weight on subsequent growth of broilers has practical and economic implications worth considering. Throughout the growing period, pre-incubation egg weight has a significant influence on broiler weight (Merrit and Gowe, 1965; Morris *et al.*, 1968; Gardiner, 1973; Proudfoot and Hulan, 1981; Whiting and Pesti, 1984). In fact, Morris *et al.* (1968) determined that the relationship was linear up to 12 weeks of age. In general the consensus is that larger hatching eggs result in larger chicks, which in turn, results in larger broilers at market age (Wilson, 1991).

Even so, Gardiner (1973) has suggested that it may be profitable for broiler producers to grow small chicks hatched from eggs below 56g. Hearn (1986) recommended growing the chicks separately to reduce competition and hence improve variability, growth rate (Bray, 1983; cited by Wilson, 1991) and profitability (Morris *et al.*, 1968; Hearn, 1986).

The ability to successfully hatch and grow chicks from small eggs would have a major impact on the poultry industry. However, it is important to have an in depth knowledge of factors influencing the growth of chicks during both the pre- and post-natal phases of development in order to identify areas of future research which could involve manipulation of environmental conditions and parent stock nutrition. In addition, it is vitally important to ascertain whether smaller chicks have unique nutritional requirements. Identifying differences, if any, would enable the broiler producer to feed the chicks according to their specific daily protein and energy requirements and hence, maximise growth performance. Therefore, the objectives of this study were to investigate the effect of pre-incubation egg size or parental flock age on embryonic growth, yolk-sac utilisation and water loss from eggs throughout incubation, and to investigate the subsequent effect of pre-incubation egg weight on post-hatch growth and residual yolk utilisation of broilers.

A comparison of the response of laying hens and broiler breeders tryptophan intake

The amino acid requirements of laying hens have been exhaustively researched. However, this is not true of the amino acid requirements, particularly the tryptophan requirements, of broiler breeder hens. To date, the parameters predicting optimum tryptophan intakes of laying hens have been applied to broiler breeder flocks in order to maximise egg output. However, inherent differences between the breeds raises doubts as to whether the requirements of both broiler breeders and laying hens can be estimated using the same prediction equations.

Typically, broiler breeders exhibit lower rates of laying than do laying hens. Within a particular flock, a large proportion of breeders lay in open cycles, that is, at a rate of less than 0.5. It has been determined experimentally that the efficiency with which amino acids are deposited into the egg is related to the rate of egg production (Fisher, 1976). Below a rate of lay of 50 eggs/ 100 hen housed, efficiency of amino acid utilisation declines linearly (Fisher, 1994) from a maximum of 0.85 (McDonald and Morris, 1985) to zero. In fact, Bowmaker and Gous (1991) determined that a flock of broiler breeders utilised amino acids for egg production with an efficiency of between 0.47 (in the case of lysine) and 0.5 (for methionine). Excluding those birds not laying in closed cycles increased these values to 0.57 and 0.65, respectively. However, the efficiencies remained considerably lower than the efficiency of utilisation estimated for laying hens. Therefore, it has been suggested (Gous, personal communication) that birds with lower efficiencies of utilisation may require higher dietary amino acid intakes in order to optimise egg output.

In addition, broiler breeders are heavier and their body fat content is considerably greater than that of laying hens (Bowmaker and Gous, 1991). Therefore, their amino acid requirements for maintenance of body weight are expected to be higher in comparison to commercial layers. However, in a response experiment described by Bowmaker and Gous (1991), extrapolation of the egg production response curves to zero gave significantly lower estimates of the lysine and methionine intakes per kilogram of body weight compared to the maintenance coefficients determined on laying hens. Since there is no energy cost in maintaining lipid reserves (Emmans and Oldham, 1989), Emmans and Fisher (1986) suggested that the estimation of maintenance requirements per kilogram body weight should be based

rather on the protein content of the body, especially when hens of different size and body composition are compared.

Because of the differences between broiler breeders and laying hens described above, the objective of this experiment was to quantify and to compare the responses to dietary tryptophan intake, of both laying hens and broiler breeders, on a bird basis, and on a lipid free basis.

CHAPTER 2

THE USE OF LINOLEIC ACID INTAKE TO MANIPULATE EGG WEIGHT

2.1 INTRODUCTION

Jensen *et al.* (1958) and Shutze *et al.* (1958) were among the first to establish that the addition of vegetable oils to laying hen diets enhances egg weight. This effect has been ascribed to a specific property of the oil rather than to its extra-energetic effect (Edwards and Morris, 1967) since replacement of oil by starch to provide the same amount of energy has been shown to depress egg weight (Balnave, 1971).

Vegetable oils are particularly rich in linoleic acid and it is to this unsaturated fatty acid that improvements in egg weight have been attributed (Shutze and Jensen, 1963; Srichai and Balnave, 1981; Scragg *et al.*, 1987). However, on the basis of no treatment differences in egg weight between birds fed different ratios of safflower oil (rich in linoleic acid) to olive oil (rich in oleic acid), Shannon and Whitehead (1974) concluded that another unsaturated fatty acid, oleic acid, was as effective in maintaining egg weight as linoleic acid. Increasing linoleic acid concentrations in excess of the effective fatty acid requirement of 8-10g/kg was not specifically required for maximum egg size. However, it appears that the birds were responsive to the addition of either oil as the authors did not include a low oil treatment. These findings are supported by Whitehead (1981), although he did show that with iso-energetic diets, either maize oil, which has a high concentration of linoleic acid, or olive oil increased egg weight when compared to a diet low in maize oil. Scragg *et al.* (1987) and Mannion *et al.* (1992) subsequently determined that increasing linoleic acid intake increases mean egg weight while a similar increase in oleic acid intake does not.

It appears that the effects of dietary fatty acids on individual egg components are age dependent. In young layers, yolk and albumen contents increase with increasing linoleic acid concentration; yolk weight being relatively more responsive to low inclusion rates and albumen weight to high (Sell *et al.*, 1987; Whitehead *et al.*, 1993). With old layers, the increase in egg weight has been attributed solely to an

increase in albumen (Whitehead *et al.*, 1991).

Responses to either linoleic acid supplementation or depletion are rapid (Balnave, 1982; 1987; Whitehead *et al.*, 1991). Balnave (1987) found that increasing dietary linoleic acid concentration through the use of linoleate-rich sunflower oil and rice pollard gave positive gains in egg weight which were reversed by feeding low-linoleate coconut oil and wheat. Responses to these alternative diets, beginning at 48 weeks of age, were rapid in that egg weight responded to cyclical four week alterations in dietary linoleic acid intake. Young layers also showed a beneficial response within three weeks (Balnave, 1982).

The economic implications for the egg industry are significant. A change in linoleic acid intake of laying hens could be used to alter the proportion of eggs in a desired egg weight grade within a few weeks. Increasing the egg weight of young broiler breeders by increasing linoleic acid intake would significantly increase the number of settable eggs produced and a decreased intake at the end of the laying cycle would decrease egg weight, and hence improve hatchability.

The objective of the present study was to quantify and compare the relationship between egg weight and linoleic acid intake, both experimentally and by means of a quantitative analysis of published data. In addition, response differences between laying hens and broiler breeders were compared. In order to ascertain further the mechanisms behind the egg weight response, the response in egg component weights to linoleic acid intake were investigated.

2.2 MATERIALS AND METHODS

Literature review

A literature search for the years 1958-1997 was conducted in order to obtain suitable data on egg weight and linoleic acid intake for various strains of laying hens and broiler breeders from 22-70 weeks of age. All dietary fat and linoleic acid trials were included if they met the following criteria:

- Diets were iso-energetic and iso-nitrogenous to ensure that egg weight responses were solely due to increasing linoleic acid concentration.
- Food intake and an analysis of linoleic acid concentration was included for each particular age group.
- There were at least three dietary treatments.

The search yielded five papers, none of which dealt with broiler breeders or reported any significant influence on rate of lay. The sources are listed in Table 2.1.

TABLE 2.1

Sources of data

Author	Age group (weeks)	Strain
Whitehead (1981)	21 - 45 (a) 45 - 73 (b)	White Leghorn
Balnave (1982)	20 - 48	Hazletts Tinted[1] Hazletts Brown[1]
Scragg *et al.* (1987)	22 - 45 (a) 45 - 69 (b)	Babcock B380
Sell *et al.* (1987)	24 - 36	White Leghorn
Mannion *et al.* (1992)	30 - 45 (a) 54 - 70 (b)	Leach QSA[1]

[1] Australian strains

Statistical analyses. The relationship between egg weight and linoleic acid content was determined by means of regression analysis (Minitab Rel. 10.5Xtra, 1995), and by fitting different intercepts to each of the eight data sets. Both linear and quadratic terms were fitted initially. Terms that were not statistically significant ($P<0.05$) were then dropped from the analysis.

Experiment

Birds and management. 24 Ross broiler breeder hens aged 49 weeks and 48 commercial laying hens of two age groups, 27 (Amberlink) and 54 weeks of age (Hyline-Brown) respectively, were placed in an open sided house (9m x 8m). The broiler breeders and laying hens were kept individually in wire cages equipped with a nipple drinker and feeders of dimensions 8 x 25 x 9cm and 28 x 7.5 x 16cm, respectively. The cages were arranged in two banks, each bank consisting of 48 cages, made up as six rows (three tiers, back to back) of eight cages per row. The laying hens were randomly allocated to one of the banks and the breeders to one side of the second bank for ease of management. Since the broiler breeders had been maintained previously on 18 hours of light, and the laying hens on 16 hours, the lighting regime used throughout the experiment was 18L:6D. The mean daily temperature throughout the trial fluctuated between a minimum of 16C and a maximum of 26C.

Treatments and feeds. During the experimental period of six weeks, the hens were offered one of six diets varying in linoleic acid concentration. Essentially, therefore, the trial consisted of 18 treatments, with each treatment being replicated four times. The six dietary treatments were randomly assigned within four blocks of six broiler breeders and 12 layers respectively. The diets were mixed by diluting a summit feed, high in linoleic acid content, with a dilution feed low in linoleic acid concentration (Table 2.2) in the proportions shown in Table 2.3. Sunflower oil was replaced by starch and sugar, respectively, to form the dilution feed. Replacement with a non-linoleic acid containing oil was decided against as this would have confounded the experiment with oils of different fatty acid proportions. Both the summit and dilution diets were formulated to be iso-nitrogenous and iso-energetic and these were analysed for energy, protein, amino acid and calcium content.

TABLE 2 .2

Composition (g/kg) of the summit and dilution diets

Ingredient	Summit	Dilution
Maize meal	266.91	266.91
Soybean oilcake meal 50	134.96	134.96
Wheat bran	122.00	121.18
Fish meal 65	59.98	60.00
Sugar	64.37	150.00
Starch	64.37	150.00
Sunflower husks	57.23	6.85
Sand	57.23	6.85
L-lysine HCL	2.09	2.09
DL-Methionine	2.29	2.29
Limestone	84.01	84.29
Monocalcium phosphate	7.69	7.69
Salt	4.37	4.37
Vitamin and mineral	2.50	2.50
Sunflower oil	70.00	-

Analysis	Calculated	Actual	Calculated	Actual
AME (MJ/kg)	11.3	11.6	11.3	11.8
Protein	149.5	149.4	149.7	144.8
Linoleic acid	44.1	55.6	7.7	8.1
Lysine	10.0	8.6	10.0	9.3
Methionine	4.9	3.8	4.9	4.0
Methionine + Cystine	7.0	-	7.0	-
Tryptophan	1.7	-	1.7	-
Arginine	9.4	7.6	9.4	7.3
Threonine	5.5	4.6	5.5	4.5
Isoleucine	6.3	5.7	6.3	5.8
Phe + Tyr	11.8	9.7	11.8	9.1
Valine	7.3	6.8	7.3	6.8
Calcium	35.0	33.5	35.0	37.8

[1] *Vitamin premix provided/kg diet*: 13000 IU vitamin A, 3000 IU vitamin D_3, 30mg $\propto$-tocopheryl acetate, 3mg menadione, 2mg thiamin, 6mg riboflavin, 5mg pyridoxine, 15 μg cyanocobalamin, 2mg folic acid, 100μg biotin, 15mg pantothenic acid, 60mg nicotinic acid. *Mineral premix provided/kg diet*: 20mg Cu, 3mg I, 40mg Fe, 70mg Mn, 100mg Zn, 0.2mg Se, 0.5mgCo, 0.5mg Mo

TABLE 2.3

Blending proportions of the summit and dilution diets and the measured content of linoleic acid

Linoleic acid content (g/kg)	Summit	Dilution
8.10	0	100
12.2	20	80
28.1	40	60
36.6	60	40
45.7	80	20
55.6	100	0

The linoleic acid concentration of each diet was determined by subjecting them to a continuous extraction with diethyl ether in a Soxhlet apparatus for 24 hours, wherein fresh solvent condensate was allowed to percolate continuously. Following evaporation of the solvent, the extracted matter was quantified and a sample of the extract was subjected to treatment with BF_3/methanol in order to convert the extracted triglycerides/free fatty acids to fatty acid methyl esters for gas chromatographic analysis.

Allocation of feeds. Daily food allocation (180g/ bird) was measured in plastic bags up to seven days before being fed to the broiler breeders. Feeding took place at the same time each morning. Food remaining in the troughs at the end of each day was not removed, but the total food remaining at the end of each week was measured and discarded. The laying hens were given *ad libitum* access to the diets.

Measurements. Body weight was recorded at the beginning of the trial, after three weeks and finally at the end of the trial (after six weeks).

Weekly food intake of the broiler breeders was calculated by subtracting the amount remaining at the end of each week from the amount fed. Weekly food intake

of the laying hens was calculated by subtracting the weight of the trough at the end of the week from the weight of the full trough at the beginning of the week.

Egg weight was recorded twice daily on three days of each week, and egg production was recorded on each day of the week.

Yolk, albumen and shell weight of one egg from each bird was recorded initially, and thereafter, at two week intervals throughout the duration of the trial. Albumen weights were determined by subtraction of the yolk weight and dry shell weight from the egg weight. Therefore, the number of eggs used to produce an average response per treatment over the six week experimental period was four.

Statistical analyses. The design of the experiment was a 6 x 3 factorial, randomised block design. However, the design was unbalanced since a few birds died or went into moult during the course of the six week trial. Therefore, the mean responses and standard errors of variables for the three strains, six diets and strain x diet interactions were determined using the general linear model of Minitab Rel. 10Xtra (1995). Regression analyses related the variables either to linoleic acid intake, or when it became necessary to reduce error due to variation in food intake, to dietary linoleic acid concentration . Differences between strains were determined by means of dummy variables, while differences in response between levels of linoleic acid, within and between strains, were accounted for by fitting the appropriate regression models. Since the response of variates is important in a response trial, the slopes, and not the constant terms, are reported.

2.3 RESULTS AND DISCUSSION

Egg weight

Literature review. Linoleic acid intakes used in previously published papers ranged from 0.5 to 3.25g/ bird d. Initially, egg weight was regressed against linoleic acid intake using strain and the age groupings reported by the authors (Table 2.1) as dummy variables. The slopes were found neither to differ from linearity ($P<0.05$) by fitting a quadratic term to the data, nor from one another ($P<0.05$). As a result, a

common linear regression term (0.78 ± 0.11), representing all the previous experiments, was fitted to the data (R^2 0.98). The constant terms, representing effects of both strain and age, were identified by means of dummy variables. It is evident (Fig. 2.1) that egg weight and linoleic acid intake express a positive linear relationship. Therefore, in order to alter egg weight by 5g, it would appear that the linoleic acid intake would have to change by approximately 8g/bird d.

Experiment. A wide range of linoleic acid intakes was achieved in the experiment: intake by the broiler breeders ranged from 1.26 to 8.88g/ bird d, and in the two laying strains from 0.8 to 6.4g linoleic acid/ bird d. The response in mean egg weight for each strain and dietary linoleic acid content over the final three week period, the period when response differences between treatments were at their greatest, are detailed in Table 2.4. Linoleic acid contents, rather than intakes, are reported due to the widely different intakes of food by the three strains on the six dietary treatments. The interaction between strain and dietary linoleic acid content was not statistically significant. Egg weight expressed a linear relationship with dietary linoleic acid intake in all three strains (Fig. 2.2). Details of the linear regression coefficients representing the response differences between broiler breeders, Amberlink and Hyline-Brown laying hens are given in Table 2 4. The slopes, which differed significantly ($P<0.05$) from zero, were similar between strains. Again, there was no evidence of a significant quadratic response.

In order to compare the egg weight responses, the three data sets from the present experiment were added to the eight used in the review of the literature, and the statistical analyses used previously were repeated, this time with 11 data sets. Regression analysis of the individual experiments revealed that the slopes did not differ significantly from one another, and a common regression term (0.78 ± 0.08) was therefore justified. The model fitted the data well with an R^2 value of 0.97 being obtained.

Therefore, over a range of linoleic acid intakes from 0.5 to 8.88g/ bird d, egg weight is linearly related to linoleic acid intake, increasing by approximately 0.8g for each gram increase in linoleic acid intake, regardless of age or strain of hen.

TABLE 2.4

Response in mean egg weight (g) to dietary linoleic acid content among three groups of laying strains over the final three week period of the trial

Linoleic acid content (g/kg)	Broiler Breeders[1]	Amberlink[2]	Hyline- Brown[3]	Mean
8.10	65.71	56.32	58.02	60.01 ± 0.92
12.2	69.19	56.18	57.99	61.12 ± 0.80
28.1	70.88	60.99	59.16	63.68 ± 0.84
36.6	71.69	58.63	63.17	64.50 ± 0.76
45.7	70.79	58.91	59.98	63.23 ± 0.88
55.6	74.09	60.80	61.57	65.48 ± 0.95
Mean	70.39 ± 0.64	58.64 ± 0.55	59.98 ± 0.64	
Regression coefficient[4]	0.76 ± 0.31[a]	0.64 ± 0.23[a]	0.79 ± 0.28[a]	
P	0.007	0.006	0.017	R^2 0.83

Residual S.E. (42 df) 2.64
Strain x diet interaction NS ($P<0.05$)
[1]52-54 weeks of age, [2] 30-32 weeks of age, [3]57-59 weeks of age.
[4]Linear response to linoleic acid intake.
[a-b]Linear regression coefficients with superscripts in common are not significantly different ($P<0.05$)

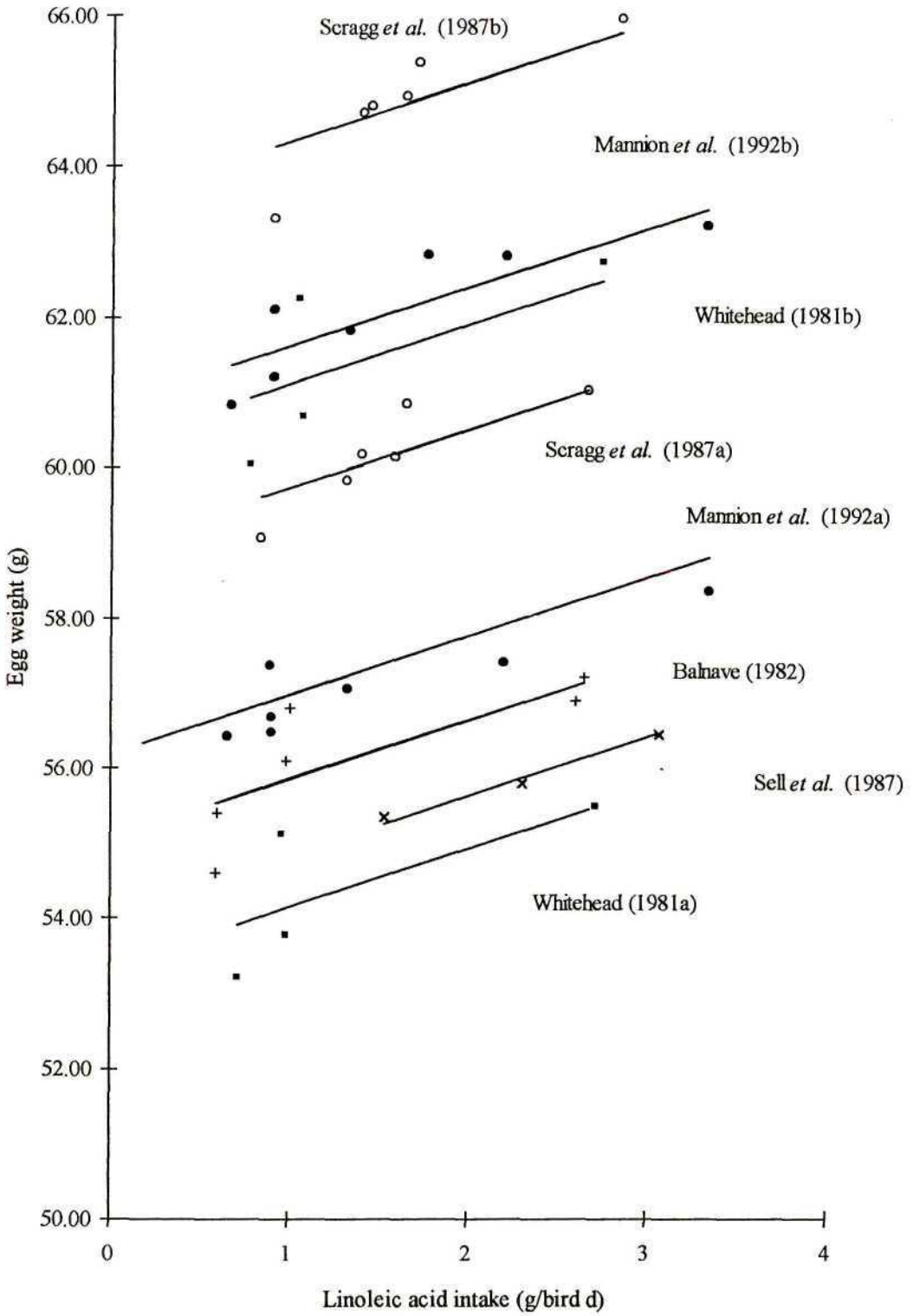

FIG. 2.1 —*The effect of linoleic acid intake on egg weight as summarised from the literature (1958 - 1997). A common slope has been fitted to each data set*

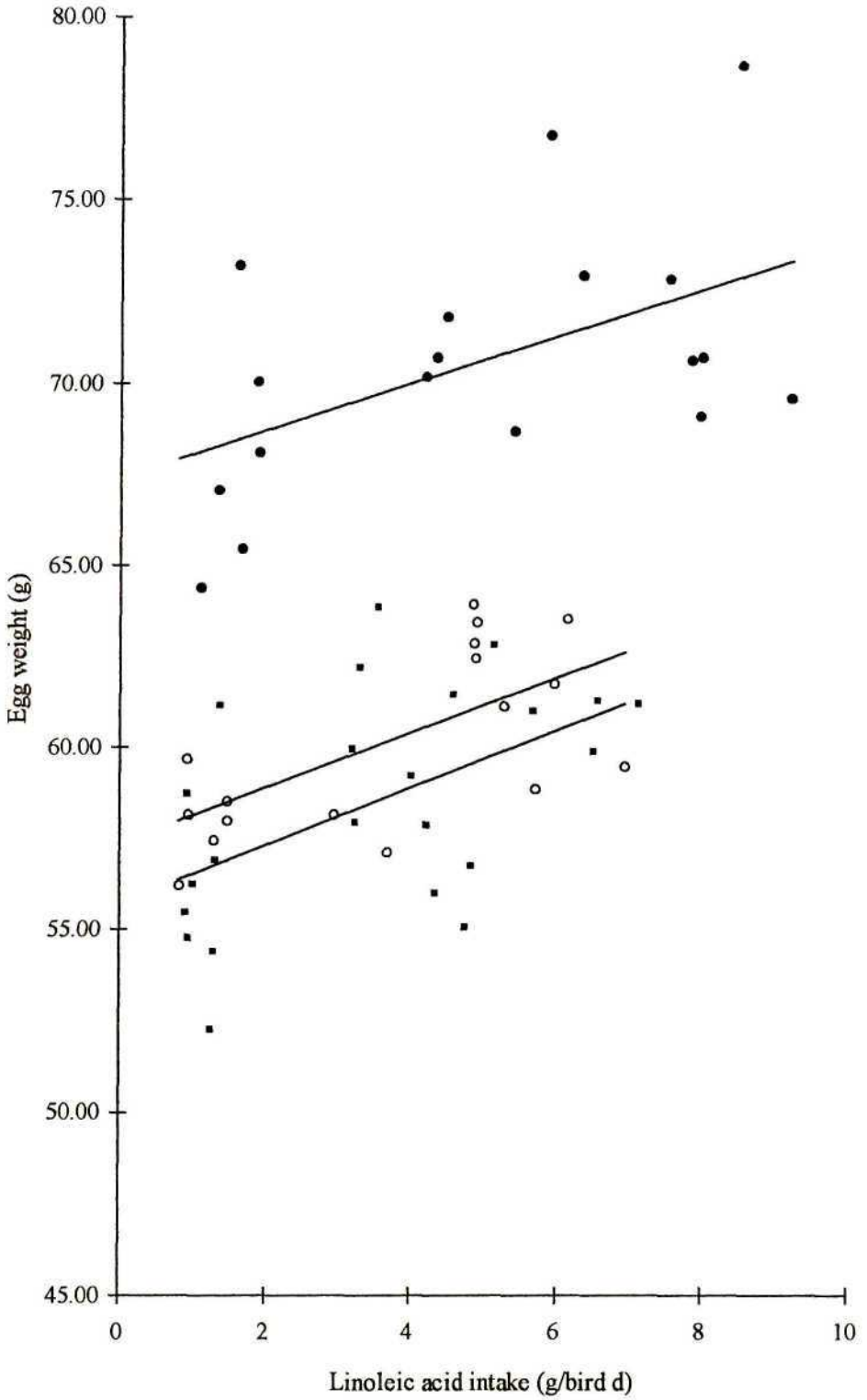

Fig. 2.2 —*Response in egg weight to linoleic acid intake among broiler breeders (•), Amberlink (■) and Hyline-Brown (○) laying hens*

Rate of laying

Mean rate of laying for the broiler breeders, Amberlink and Hyline-Brown laying hens, over the range of dietary linoleic acid content is shown in Table 2.5. Egg production of the two commercial laying strains was high, regardless of the level of linoleic acid in the diet. Whereas the slope of the responses in rate of lay of the two commercial strains did not differ significantly from zero, the number of eggs produced by the broiler breeders increased linearly ($P<0.05$) with linoleic acid intake.

The lack of response in rate of lay in the laying strains to linoleic acid intake is in accordance with the findings of the authors listed in Table 2.1. However, the increase in rate of laying in the broiler breeders, of an additional two eggs/ 100 hen d for a 1g increase in linoleic acid intake/d, has not been reported previously and is of considerable interest. This result could be interpreted in two different ways. Assuming all hens were to consume the same amount of each of the different feeds on offer (which was the case in this experiment, Table 2.7), rate of laying could be expected to increase by 3.2eggs/ 100 hen d for each 10g/ kg increase in dietary linoleic acid content. Alternatively, it could be argued that the broiler breeder has a requirement for linoleic acid for egg production, and that this variable cannot be maximised with an intake of less than about 6 to 7g linoleic acid/ bird d.

The difference in response between broiler breeders and laying hens cannot be explained on the basis of the different methods of allocation of feed to the different strains, or of differences in nutrient content, other than linoleic acid, in the different feeds on offer. Whereas broiler breeders were allocated a fixed amount of food each day (180g/ bird d), not all of this food was consumed, and in effect the birds consumed similar amounts of food on all treatments (Table 2.7). This was also the case with the laying strains, where food was available *ad libitum*. The two basal feeds differed only in oil content, i.e. all nutrients other than the constituents of the oil were the same in all feeds on offer. The Effective Energy (EE) contents (Emmans, 1994) of the two basal feeds differed slightly (10.85 and 10.89MJ EE/ Kg for the summit and the dilution diets respectively) because of the substitution of oil for sugar and starch. But this would have had an effect only if the environmental temperature during the experiment had been in excess of about 32C which was not the case. It would appear that broiler breeders respond positively in rate of lay to dietary linoleic acid, and this

observation warrants further investigation.

TABLE 2.5

Response in mean rate of laying (eggs/ 100 bird d) to dietary linoleic acid content among three groups of laying strains over the final three week period of the trial

Linoleic acid content (g/kg)	Broiler Breeders[1]	Amberlink[2]	Hyline- Brown[3]	Mean
8.10	50.25	92.90	87.01	76.72 ± 2.65
12.2	54.74	89.31	83.78	75.94 ± 2.55
28.1	57.10	88.71	89.66	78.49 ± 2.44
36.6	65.82	94.09	88.07	82.66 ± 2.32
45.7	62.49	95.28	88.51	82.09 ± 2.55
55.6	67.92	95.34	89.64	84.30 ± 2.75
Mean	59.72 ±1.87	92.61 ±1.60	87.78 ±1.90	
Regression coefficient[4]	1.83 ±0.60[a]	0.91 ± 0.74[a]	0.71 ± 0.85[a]	
P	0.004	0.221	0.404	R^2 0.82

Residual S.E. (40 df) 0.076
Strain x diet interaction NS (P<0.05)
[1]52-54 weeks of age, [2]30-32 weeks of age, [3]57-59 weeks of age
[4]Linear response to linoleic acid intake
[a-b]Linear regression coefficients with superscripts in common are not significantly different (P<0.05)

Egg output

Egg output, outlined in Table 2.6, for the final three week period, responded positively and linearly to dietary linoleic acid intake. Quadratic effects were not significant. The linear regression coefficients (Table 2.6) were not significantly different between strains. Since egg production of the two commercial laying strains was not significantly affected by linoleic acid intake, the linear response was solely due to the effect of dietary linoleic acid intake on egg weight. However, the influence of linoleic acid intake between 1.26 and 8.88g/ bird d on egg output of broiler breeders can be attributed to a concomitant effect on egg weight and rate of laying.

TABLE 2.6

Response in mean egg output (g/ bird d) to dietary linoleic acid content among three groups of laying strains over the final three week period of the trial

Linoleic acid content (g/kg)	Broiler Breeders[1]	Amberlink[2]	Hyline-Brown[3]	Mean
8.10	32.88	52.27	48.24	44.47 ± 1.34
12.2	37.70	50.09	49.89	45.89 ± 1.34
28.1	40.46	53.88	53.44	49.26 ± 1.34
36.6	47.66	56.22	55.62	52.81 ± 1.34
45.7	44.19	55.14	53.33	51.24 ± 1.27
55.6	50.16	56.22	55.67	54.20 ± 1.47
Mean	42.17 ± 1.04	54.06 ± 0.90	52.70 ± 0.93	
Regression coefficient[4]	1.74 ± 0.35[a]	1.20 ± 0.43[a]	1.33 ± 0.45[a]	
P	<0.001	0.007	0.004	R^2 0.71

Residual S.E. (48 df) 4.41
Strain x diet interaction NS (P<0.05)
[1]52-54 weeks of age, [2] 30-32 weeks of age, [3]57-59 weeks of age
[4]Linear response to linoleic acid intake
[a-b]Linear regression coefficients with superscripts in common are not significantly different (P<0.05)

Food intake

Daily food intakes by the three strains of hens and on each of the six dietary linoleic acid contents are given in Table 2.7. There was no significant interaction between the two factors. The consistently high food intake exhibited by each of the four Hyline-Brown hens, at a linoleic acid content of 36.6g/kg, is noted, but is not possible to explain. The broiler breeders were allocated 180g of food per day on the assumption that these hens require about 2000kJ of AME per day (Bowmaker and Gous, 1991). The feeds used in the experiment were designed to contain 11.3MJ/kg, this being the optimum nutrient density of a feed for commercial laying hens under the prevailing circumstances. The broiler breeders did not consume all of the food allocated to them, as evidenced by the mean intakes presented in Table 2.7, indicating that, for the level of performance of these birds, 2000kJ AME/ d is more than

required. The linoleic acid content of the feed had no influence on the amount of food consumed by the hens (Table 2.7), nor did the amount of food consumed relate to the weight or number of eggs produced by any of the birds on this experiment.

TABLE 2.7

Response in mean food intake(g/ bird d) to dietary linoleic acid content among three groups of laying strains over the final three week period of the trial

Linoleic acid content (g/kg)	Broiler Breeders[1]	Amberlink[2]	Hyline-Brown[3]	Mean
8.10	165.4	115.6	107.2	129.4 ± 2.70
12.2	149.9	106.9	116.9	124.5 ± 2.47
28.1	158.7	118.1	118.7	131.9 ± 2.34
36.6	160.4	117.3	133.6	137.1 ± 2.50
45.7	171.1	111.7	112.3	131.7 ± 2.34
55.6	159.8	116.5	111.7	129.3 ± 2.47
Mean	160.9 ± 1.91	114.4 ± 1.66	116.7 ± 1.66	
Regression coefficient[4]	0.19 ± 0.14[a]	0.08 ± 0.12[a]	0.07 ± 0.0.12[a]	
P	0.172	0.520	0.570	R^2 0.82

Residual S.E. (49df) 8.11
Strain x diet interaction NS (P<0.05)
[1]52-54 weeks of age, [2] 30-32 weeks of age, [3]57-59 weeks of age
[4]Linear response to linoleic acid content
[a-b]Linear regression coefficients with superscripts in common are not significantly different (P<0.05)

Body weight change

Body weight change, averaged over the last three weeks of the trial for strain and linoleic acid content, is given in Table 2.8. No significant interactions between strain and linoleic acid content were observed. Since the constant terms, when fitting the regression equation of body weight change against linoleic acid content, were not significantly different from zero, comparisons between linear coefficients only were

made (Table 2.8) A positive, linear trend was observed for the broiler breeders and the Amberlink hens, indicating that as the concentration of linoleic acid was increased in the diets, the birds tended to gain equivalent amounts of weight. Such findings have been reported by Brake (1990). Since the chemical composition of the birds was not determined, and the dietary treatments contained equivalent amounts of energy, it can be speculated that the increase in weight could have been due to an increase in oviducal protein synthesis. Contrary to these findings, the Hyline-Brown birds appeared neither to gain nor loose weight as the concentration of linoleic acid increased.

TABLE 2.8

Response in mean body weight change (g/ bird d) to dietary linoleic acid content among three groups of laying strains over the final three week period of the trial

Linoleic acid content (g/kg)	Broiler Breeders[1]	Amberlink[2]	Hyline-Brown[3]	Mean
8.10	0.66	1.08	-0.80	0.31 ± 1.49
12.2	-0.72	1.26	0.57	0.37 ± 1.57
28.1	4.66	3.67	2.44	3.59 ±1.93
36.6	3.21	3.50	1.49	2.73 ± 1.49
45.7	5.33	2.64	0.70	2.89 ± 1.57
55.6	6.11	6.20	1.36	4.56 ± 1.65
Mean	3.21 ± 1.17	3.06 ± 1.05	0.96 ± 1.22	
Regression coefficient[4]	0.13 ± 0.03^{a}	0.09 ± 0.03^{ab}	0.03 ± 0.03^{b}	
P	<0.001	0.008	0.292	R^2 0.81

Residual S.E. (46 df) 5.16
Strain x diet interaction NS(P<0.05)
[1]52-54 weeks of age, [2] 30-32 weeks of age, [3]57-59 weeks of age
[4]Linear response to linoleic acid content
[a-b]Linear regression coefficients with superscripts in common are not significantly different (P<0.05)

Egg components

The mean response in yolk, albumen and shell weight to dietary linoleic acid content among the three laying strains for the six week period are detailed in Tables 2.9-2.11. There were no statistically significant interactions between strain of hen and dietary linoleic acid content. Neither yolk weight nor shell weight exhibited a significant linear, or quadratic response, to dietary linoleic acid intake. However, in all three strains, albumen weight was the component which responded in a linear manner (Table 2.11) to dietary linoleic acid content. There was no significant evidence of curvature. The slope of the response was the same for the broiler breeders and Hyline-Brown birds while the response among the Amberlink hens was significantly more gradual. Since it is accepted that there is an allometric relationship between yolk and albumen weight, it is unusual that a significant yolk weight response was not apparent. The regression of the natural log (*ln*) of yolk weight against *ln* albumen weight for the pooled data (0.36 ± 0.05) indicated that as yolk weight increased, so albumen weight increased at a proportionally greater rate (R^2 0.80). However, this allometric relationship appeared to be strain- or age-related since the allometric relationships for each of the three strains were not significant. This conclusively illustrates that albumen weight was influenced by linoleic acid intake, but without a concomitant change in yolk weight.

TABLE 2.9

Response in mean yolk weight (g) to dietary linoleic acid content among three groups of laying strains over the final three week period of the trial

Linoleic acid content (g/kg)	Broiler Breeders[1]	Amberlink[2]	Hyline-Brown[3]	Mean
8.10	21.18	13.72	16.17	17.03 ± 0.30
12.2	20.30	13.20	17.49	17.00 ± 0.30
28.1	21.05	14.97	16.28	17.44 ± 0.30
36.6	20.40	14.09	17.77	17.42 ± 0.30
45.7	18.54	14.16	16.77	16.49 ± 0.32
55.6	21.62	14.34	16.42	17.46 ± 0.32
Mean	20.52 ± 0.23	14.08 ± 0.19	16.82 ± 0.19	
Regression coefficient[4]	-0.01 ±0.02[a]	0.02 ±0.02[a]	-0.00 ±0.02[a]	
P	0.685	0.431	0.970	R^2 0.90

Residual S.E. (49 df) 0.97
Strain x diet interaction NS($P<0.05$)
[1]52-54 weeks of age, [2] 30-32 weeks of age, [3]57-59 weeks of age
[4]Linear response to linoleic acid content
[a-b]Linear regression coefficients with superscripts in common are not significantly different ($P<0.05$)

TABLE 2.10

Response in mean shell weight (g) to dietary linoleic acid content among three groups of laying strains over the final three week period of the trial

Linoleic acid content (g/kg)	Broiler Breeders[1]	Amberlink[2]	Hyline-Brown[3]	Mean
8.10	6.30	5.18	5.03	5.50 ± 0.19
12.2	5.77	5.00	5.24	5.34 ± 0.17
28.1	6.27	5.66	5.21	5.71 ± 0.17
36.6	6.43	5.32	5.82	5.86 ± 0.17
45.7	6.08	5.46	4.77	5.44 ± 0.17
55.6	7.23	5.75	4.95	5.97 ± 0.19
Mean	6.35 ± 0.13	5.40 ± 0.12	5.17 ± 0.12	
Regression coefficient[4]	0.02 ±0.01[a]	0.01 ±0.01[a]	-0.00 ±0.01[a]	
P	0.590	0.178	0.693	R^2 0.70

Residual S.E. (49df) 0.57
Strain x diet interaction NS(P<0.05)
[1]52-54 weeks of age, [2] 30-32 weeks of age, [3]57-59 weeks of age
[4]Linear response to linoleic acid content
[a-b]Linear regression coefficients with superscripts in common are not significantly different (P<0.05)

TABLE 2.11

Response in mean albumen weight (g) to dietary linoleic acid content among three groups of laying strains over the final three week period of the trial

Linoleic acid content (g/kg)	Broiler Breeders[1]	Amberlink[2]	Hyline-Brown[3]	Mean
8.10	40.52	37.80	35.76	38.02 ± 1.00
12.2	42.05	37.17	37.89	39.04 ± 0.95
28.1	43.62	38.31	38.54	40.16 ± 0.95
36.6	44.34	38.96	39.55	40.95 ± 0.95
45.7	45.08	38.19	39.40	40.89 ± 0.95
55.6	45.47	39.89	39.07	41.48 ± 1.00
Mean	43.51 ± 0.70	38.39 ± 0.67	38.36 ± 1.22	
Regression coefficient[4]	0.10 ± 0.02[a]	0.04 ± 0.02[b]	0.06 ± 0.02[ab]	
P	<0.001	0.026	0.003	R^2 0.96

Residual S.E. (52 df) 3.30
Strain x diet interaction NS(P<0.05)
[1]52-54 weeks of age, [2] 30-32 weeks of age, [3]57-59 weeks of age
[4]Linear response to linoleic acid content
[a-b]Linear regression coefficients with superscripts in common are not significantly different (P<0.05)

These findings are in direct contrast to those of Jensen *et al.* (1958), Shutze and Jensen (1963) and Sell *et al.* (1987). These authors reported that yolk weight responded to dietary fat content. However, Whitehead (1991) determined, with young layers, that there was variation in the responses of the different components. Yolk was relatively more responsive to smaller inclusions of maize oil, and albumen to larger amounts (Whitehead, 1993). With ageing layers, the increase in egg weight was attributed soley to an increase in albumen weight (Whitehead, 1991). Since the inclusion rates in this study cover a far wider range of linoleic acid intakes than those used in any previous trial, and since the lowest intakes recorded previously were not obtainable without sacrificing daily protein or energy requirements, small responses in yolk weight at the lowest intakes of linoleic acid may have gone undetected, but over the range of linoleic acid intakes studied, there was no response in yolk weight.

Whitehead (1993) showed that feeding a diet containing a high concentration of maize oil, resulted in elevated oestradiol (pg/ml) concentrations. In fact, a linear response was measured. Since the rate of secretion of most oviduct proteins is under the control of oestrogen (Okulicz *et al.*, 1985; cited by Whitehead, 1993), this provides evidence for a mechanism to explain that fatty acids, specifically linoleic acid, have a stimulatory effect on oviducal protein secretion. However, the process by which dietary linoleic acid influences oestrogen metabolism is as yet unclear. The effect would appear to be attributable to the chemical composition of the fatty acid, namely, a chain length of 18 carbons and a moderate degree of unsaturation.

CHAPTER 3

THE INFLUENCE OF NUTRIENT INTAKE AND 20 WEEK BODY WEIGHT ON EARLY LAYING PERFORMANCE

3.1 INTRODUCTION

One of the greatest problems facing the broiler breeder industry is depressed laying performance throughout the laying cycle. Typically, peak production is lower than that predicted by the breeding companies, as is laying persistency post-peak. In an attempt to achieve the production goals set by breeding companies, broiler breeders are often fed in excess of the daily allowances recommended by the breeding companies. However, the production of settable eggs has not been improved concomitantly with this additional feed, leading to speculation that the poor performance may not be due to underfeeding but to overfeeding, either prior to, or during the laying period.

Broiler breeder hens are conventionally restricted throughout the growing and laying periods to prevent excessive body weight gains and to prevent the poor egg production, fertility and hatchability rates exhibited by full-fed birds (McDaniel *et al.*, 1981). Performance appears to be dependent on the degree of restriction imposed since severe forms of restriction have been found to depress egg output and the uniformity of body weights (Blair *et al.*, 1976). Recommendations by breeding companies of the way in which broiler breeders should be fed are contradictory in the extreme. Suggested feed intake patterns include features such as: (1) feeding ahead of the average requirement (lead feeding) prior to peak egg production. This method is used to ensure a strong development of maturity in the flock and to ensure maximum early egg size; (2) a maximum intake to provide for a strong peak production and continued development of egg size and bird weight; and (3) withdrawal of feed after peak to control body weight gains and to reduce the risk of over weight birds (Fisher, 1998a). Some companies suggest that the timing of feed changes should be linked to the attainment of a specific flock age (Ross Breeders Ltd., 1995; Lohman Indian

River, 1997) with adjustments made for the attainment of uniform body weights (Shaver Poultry Farms Ltd., 1997) or gain in body weight (Euribrid BV, 1997), whereas the Cobb Breeding Company Ltd. (1997) recommends altering feed intake according to hen-day production. Often, a pre-breeder feed is recommended up to 22 weeks of age. Thereafter, the producer has the choice of using either one or two breeding rations according to the age of the flock. Feeding recommendations are based on the assumption that the nutrient requirements of broiler breeders can be provided for by altering the daily food allowance of the flock. However, it is doubtful that the daily energy and amino acid requirements change concomitantly.

Very little nutritional research has been conducted during the early laying period and the results of this research are contradictory. Lilburn and Myers-Miller (1990) determined that early hen-day production was significantly improved by accelerating feed allowances during the pre-lay and early stages of production. These results concurred with other reports (McDaniel *et al.*, 1983; Robbins *et al.*, 1986) wherein *ad libitum* feeding, compared to various controlled feeding treatments, improved production. However, the advancement of sexual maturity of these birds would appear to be the cause of the additional production, and it is evident that there is a delicate balance between feeding for the onset of production and obtaining a maximal number of eggs per hen over the entire laying cycle (Lilburn and Myers-Miller, 1990). Recent findings (Yu *et al.*, 1992; Robinson *et al.*, 1995) suggest that restriction during the early laying period improves laying performance, thus indicating that broiler breeders are very sensitive to over feeding during the pre-lay and early laying periods. This observation is confirmed by Hocking (1996) who stated that careful control of food intake after photostimulation is necessary for optimising ovulation rate. In fact, Yu *et al.*(1992) determined that overfeeding resulted in significantly larger ovaries and in an increase in the incidence of erratic ovipositions, defective eggs and multiple ovulations.

Broiler breeder producers are of the opinion that body weight and uniformity of the flock at 20 weeks of age is a predictor of the subsequent laying performance observed in the flock. However, it has been determined that variability of 20 week body weight, often attributed to competition for a limited quantity of feed during rearing (Robinson and Robinson, 1991), had no influence on subsequent egg

production, egg weight, or body weight gain up to 34 weeks of age (Robinson *et al.*, 1995).

The experiment reported here was designed (1) to identify discrepancies, if any, between theoretical energy and amino acid requirements of broiler breeder hens from 20 to 40 weeks of age and those recommended by various breeding companies, and (2) to investigate the influence of 20 week body weight and nutrient intake, on performance and physiological parameters, during the early lay period.

3.2 MATERIALS AND METHODS

Literature review

The latest editions of all broiler breeder production manuals were consulted in order to calculate the recommended daily energy, lysine and methionine intakes of the various breeds between the ages of 22 and 40 weeks, and to compare these with calculated theoretical estimates. Production manuals were accepted for review if they met the following criteria:

- Potential egg weight and rate of lay were listed on a daily basis for each week in order to calculate egg output.
- Body weight and food intake per day were listed at least every second week.
- Recommended energy (MJ/kg), lysine (%) and methionine (%) concentrations were specified.

Only seven production manuals, the Ross 308 (Ross Breeders Ltd.,1995), Hybro N (Euribrid BV, 1997), Cobb 500 (Cobb Breeding Co. Ltd., 1997), Abor Acres (Arbor Acres Farm Inc., 1997), Shaver Starbro (Shaver Poultry Farms Ltd., 1997), Hubbard (Hubbard Farms Inc., 1997) and the Avian 24K (Avian Farms International, 1997) satisfied the requirements stipulated above. Recommended daily energy, lysine and methionine intakes of the various genotypes were calculated by multiplying the food intakes by the respective nutrient concentrations.

Theoretical calculations

The daily amounts of lysine and methionine required by the average individual in the flock for each week of the laying period, were calculated using the factorial approach, namely $I = aE + bW$, where the coefficients of response, a and b, were 16.88 and 11.22 for lysine and 7.03 and 1.52 for methionine respectively (Bowmaker and Gous, 1985). The values of E (egg output, g/ bird d) and W (body weight, kg) were those supplied by the breeding companies. The standard deviations of mean egg output and body weight were set at 0.2 and 0.12, respectively. The additional amount worth feeding the flock (Fisher *et al.*, 1973) was calculated by increasing the requirement of the average individual in the flock by an amount that would be equivalent to meeting the requirements of 0.965 of the population (the value of x in the Reading Model being 1.8).

The energy intakes were calculated according to Emmans (1974):

$$\text{ME intake (kJ/ bird d)} = \text{FLA} \times (\text{W} (586 - 8.4\text{T})) \times 8.4\text{E},$$

using values for feather loss score (FLA) of 1.3 and a dry bulb temperature (T) of 24C.

Experiment

Birds and management

A total of 3640 broiler breeder female chicks were housed at day old in eight control environment rooms of four pens each. The rooms were light tight and provided 23L:1D initially, decreasing to the minimum daylength (8L:16D) at ten days of age. This lighting pattern was maintained until the birds were 18 weeks of age. The feeding treatments imposed were designed to ensure that the birds followed one of three growth curves, namely, the Ross standard growth curve for pullets, 0.88 of the Ross standard and 1.11 of the Ross standard. Food allocation was therefore dependent on the body weights of the birds which were recorded twice weekly. Water was

supplied by means of nipple drinkers, and food, in chick feeders up to two weeks of age. Thereafter, feed was distributed by hand, uniformly over the litter.

At 20 weeks of age, 360 of these pullets were chosen at random from the population, subject of certain body weight constraints (see below), and placed in individual cages in a controlled environment house (10m x 7.7m x 3.5m). Cross ventilation was provided by six fans covered by baffels to ensure that the house was light tight. The wire cages (75cm x 48cm x 33cm) were arranged in six rows, back to back. Each row consisted of two tiers of 48 cages respectively. Nipple drinkers and drip - cups were provided at the junctions of every cage, thus giving each bird access to two drinkers. A feed trough (10cm x 30cm) and a wooden perch were supplied for each cage. The lighting pattern provided the birds with 12L:12D at 20 weeks. The number of hours of light was increased by an hour a week to a maximum of 16L:8D at 24 weeks of age. The trial ran for a period of 16 weeks.

Treatments and feeds

The experiment consisted of three treatments, namely, 20 week body weight (three levels), nutrient density (two levels), and food allocation (four levels).The broiler breeder hens were selected from the base population according to their body weight on an 'empty' basis. The three body weight categories of 120 birds respectively, were: 1900-2100g, 2100-2300g and 2300-2500g. Two feeds of a high (H) and low (L) nutrient density were formulated to provide all the hens' requirements at an intake of 150 or 180g/ bird d, respectively. Initially, L was obtained by diluting H with 170 g/kg sunflower husks. During the sixth week of the trial, a new L feed was formulated, since analysis revealed that the diluent did not effect a 0.17 difference in energy and protein content between the feeds. This feed was subsequently introduced from week nine of the trial. The feeds were mixed in a commercial feed mill and were analysed for AME, crude protein, total amino acid, calcium and phosphorus content (Table 3.1).

TABLE 3.1

Composition (g/kg) of the high (H) and low (L) nutrient density feeds

Ingredient	H	L_1	L_2
Maize meal	696.16	580.13	622.00
Wheat bran	13.89	11.58	-
Soybean oilcake meal 50	144.59	120.49	112.37
Fishmeal 65	14.13	11.78	-
Sunflower oil	50.00	41.67	-
DL Methionine	0.94	0.78	1.04
Monocalcium phosphate	7.55	6.29	7.06
Vitamin/mineral premix[1]	2.50	2.08	2.50
Limestone	67.05	55.88	56.19
Salt	3.19	2.66	4.85
Sunflower husks	-	167.77	193.99

Analysis	Calculated	Actual	Calculated	Actual	Calculated	Actual
AME (MJ/kg)	13.3	13.2	11.1	12.3	11.1	11.6
Protein	139.6	13.2	116.3	128.7	117.1	109.9
Lysine	6.8	7.3	5.7	7.7	5.4	6.2
Methionine	3.2	3.1	2.7	2.7	2.8	2.4
Threonine	5.2	5.1	4.3	5.2	4.3	4.5
Tyrosine	-	3.7	-	3.3	-	3.2
Arginine	8.5	9.4	7.1	8.1	6.8	8.5
Histidine	3.9	4.0	3.3	3.8	3.2	3.4
Isoleucine	6.0	5.9	5.0	6.0	5.1	5.2
Phe + Tyr	12.1	-	10.1	-	9.9	-
Valine	7.0	7.1	5.9	7.4	5.9	6.2
Calcium	26.7	30.0	22.4	29.1	22.2	22.5
Phosphorus	5.1	4.8	4.3	4.8	4.0	4.2

L_1 Feed used for 9 weeks
L_2 Feed used for remaining 7 weeks
[1] *Vitamin premix provided/kg diet*: 13000IU vitamin A, 3000IU vitamin D_3, 30mg ∝-tocopheryl acetate, 3mg menadione, 2mg thiamin, 6mg riboflavin, 5mg pyridoxine, 15 μg cyanocobalamin, 2mg folic acid, 100μg biotin, 15mg pantothenic acid, 60mg nicotinic acid. *Mineral premix provided/kg diet*: 20mg Cu, 3mg I, 40mg Fe, 70mg Mn, 100mg Zn, 0.2mg Se, 0.5mgCo, 0.5mg Mo

Both H and L were allocated to each of the three groups of pullets by means of a step-up feeding programme resulting in four levels of feed allowance at 30 weeks (Fig. 3.1). Up to 24 weeks of age, feed allocation was increased by 10g/d each week, while subsequent increments occurred every second week. The maximum feed allowances for each of the four categories were 150, 160, 170, and 180g/ bird d, respectively.

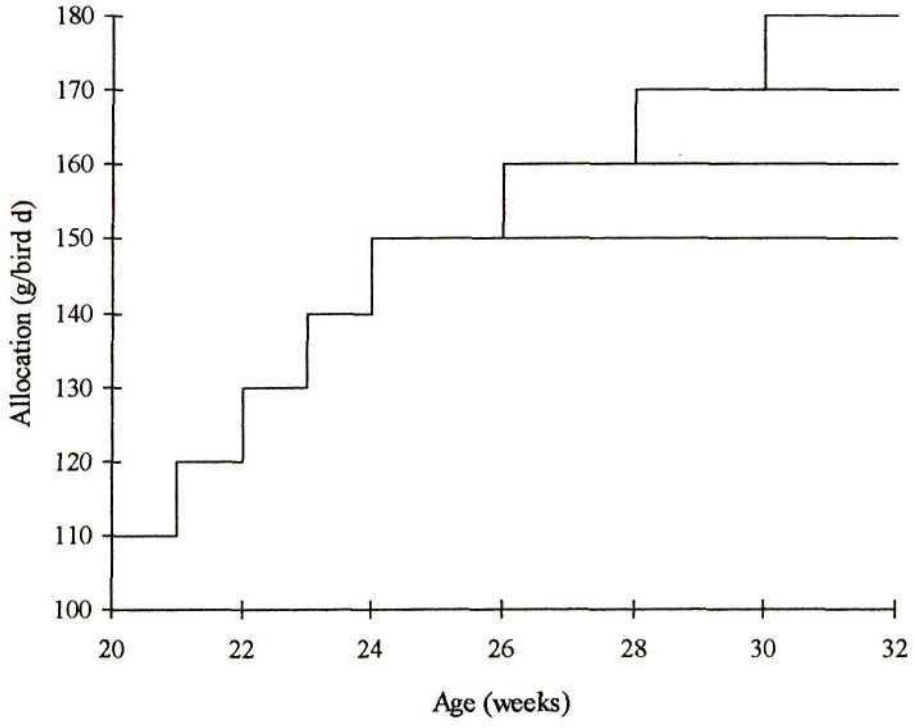

FIG. 3.1 —*Pattern of daily allocation of both the high and low nutrient density feeds over the course of the trial*

Apportionment of feeds

Daily feed allocations were weighed to within 1g of the required weight and stored in plastic-coated cardboard containers. Once weekly, seven containers per hen

were placed in a box which was divided by a cardboard partition, and placed on the top cages. This allowed for easy feeding management since birds on the top tier were fed from containers in the left partition, while birds on the lower tier were fed from the right partition. Feeding took place in the morning.

Measurements

Body weight was recorded initially to partition the birds into the three weight categories and every second week thereafter.

Food remaining in each feed trough at the end of each day was not removed but allowed to accumulate until the end of the week. Food intake was recorded weekly by subtracting the amount of food remaining from the amount fed.

Egg weight was recorded twice a day for three days of each week, and egg production was recorded daily.

Egg output was calculated for each week by multiplying average egg weight per hen with the mean rate of lay.

Maximum rate of lay, for each bird, was calculated over a modal two week period i.e. by calculating the highest mean number of eggs/ 100 bird d produced within any consecutive two week period.

Mortalities were recorded and each dead bird regarded as a missing plot. Birds were culled if they became lame.

At the beginning of the trial, three extra birds were selected from the parent population according the respective weight categories and killed by means of carbon dioxide poisoning. At the end of the trial three birds, a top, an average and a poor producer, from each of the 24 treatments were killed by means of cervical dislocation and their ovaries and oviducts dissected out and weighed. The feather-free carcasses were then minced individually to form an homogenous blend. A sample from each carcass was then subjected to chemical composition analysis. Body water content was determined by freeze drying the samples. Body protein was determined on a water-free basis in a LECO nitrogen analyser as N x 6.25. Body lipid of the 20 week old broiler breeders was analysed using the Soxhlett fat-extraction method. Gross energies (MJ/kg dry matter) were determined on the 36 week old hens and used to predict the lipid content of the birds using the following equation developed at the

University of Natal:

$$L = -0.8756 \text{ (s.e. } 0.0527) + 0.04754 \text{ (s.e. } 0.0020) \times GE$$

where, L=lipid (g/g dry matter) and GE = gross energy (MJ/kg dry matter).
All procedures conformed to those specified by the Association of Official Analytical Chemists (AOAC, 1975).

Statistical analyses

The design of the experiment was a 3 x 4 x 2 factorial, randomised block design. However, the design was unbalanced since a few birds died or became lame during the course of the trial. The mean responses and standard errors of the measured variables for the three factors, and the weight x nutrient density x food allocation interactions were determined using the general linear model of Minitab Rel. 10Xtra (1995). Since the allocation treatment is classified as a continuous variable, the means within the interaction tables, for each of the measured parameters, from week 30, were regressed against allocation, using 20 week body weight and nutrient density as dummy variables, respectively. T-tests for unequal samples were conducted on the main effect means (20 week body weight, nutrient density and feed allocation during the pre- and peak-production periods), in order to identify significant differences.

Regression analyses were applied to determine the response between particular variates (y) and predictors (x), using the three factors as dummy variables.

During the post-peak period, the variables, egg weight, rate of lay, egg output and body weight change, of birds laying in closed cycles, were regressed against both amino acid and energy intakes, using allocation, 20 week body weight and nutrient density as dummy variables, respectively. Data from birds laying above 50eggs/ 100 bird d were used to avoid confounding the effects with the efficiency of utilisation of that nutrient. Multiple regression analyses of both energy and amino acid intakes, on body weight, change in body weight and egg output at two time periods post-peak were conducted. The intercepts were dropped from the equations, since there was no theoretical basis for their inclusion.

3.2 RESULTS AND DISCUSSION

Literature review

Amino acids

The comparisons between daily recommended and calculated lysine and methionine intakes, respectively, between 22 to 40 weeks of age, are illustrated graphically (Fig's 3.2 and 3.3). It is evident that there are large discrepancies between calculated and recommended intakes throughout the period. These are especially marked during the production period before peak. As a result, the over-consumption of amino acids could result in heavier, particularly fatter birds. More importantly, the intake of amino acids in excess of requirement, could lead to an increased follicular hierarchy, resulting in an increased frequency of multiple ovulations and the production of defective eggs (Hocking, 1996). In addition, there is greater variation between genotypes when requirements are stipulated according to breeding company recommendations, compared to when calculated. Moreover, breeding company recommendations are that both methionine and lysine requirements should reach a maximum at approximately 26 weeks of age, while the theoretical maximum appears to be achieved only at about 31 weeks of age.

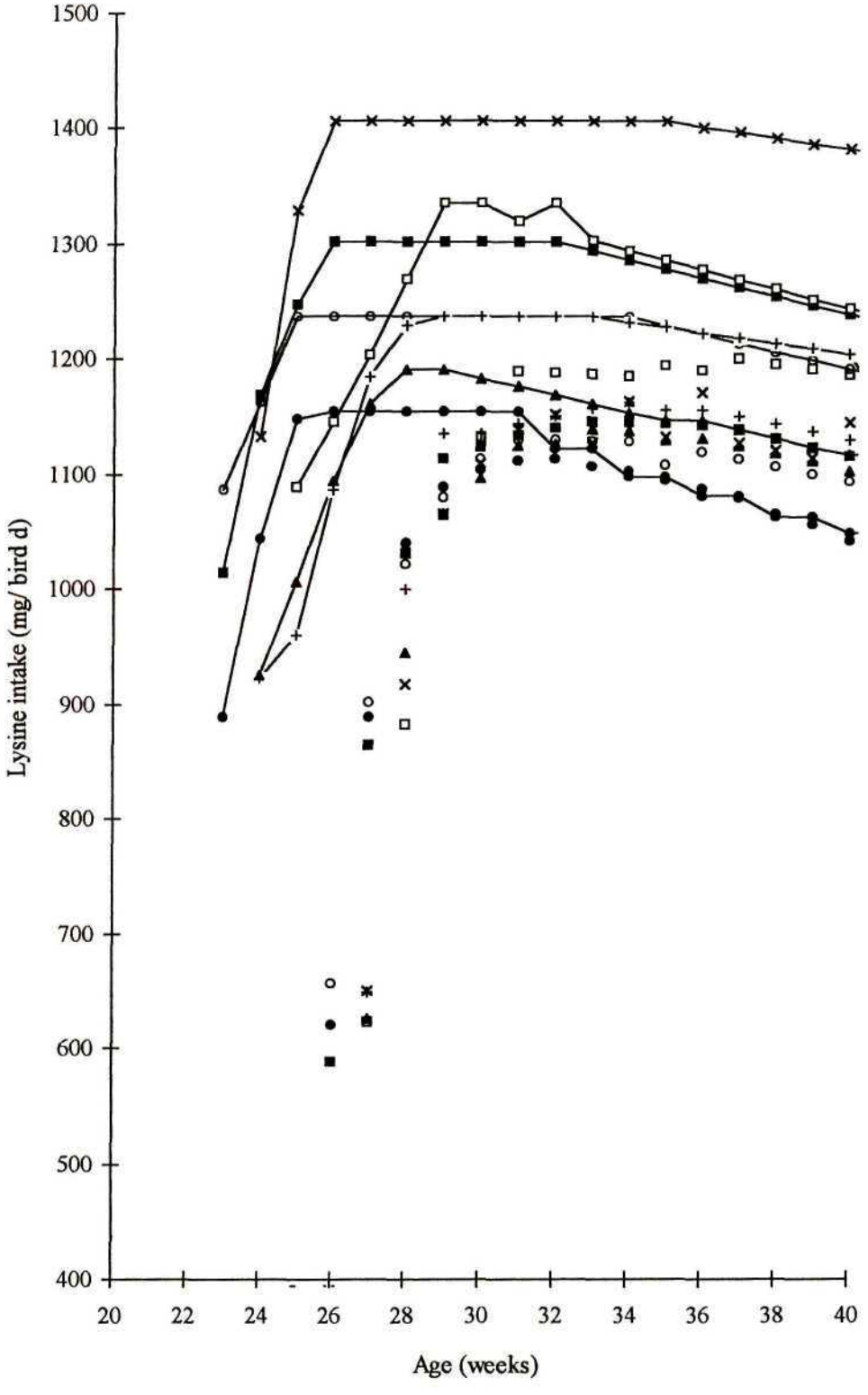

FIG. 3.2 —*Calculated lysine intakes (mg/ bird d) for Ross 308 (•), Hybro N (○), Cobb 500 (■), Arbor Acres (□), Shaver Starbro (+), Hubbard (×) and Avian 24K (▲) broiler breeders and lysine intakes recommended by the respective breeding companies (lines connecting the symbols) over the first 20 weeks of production*

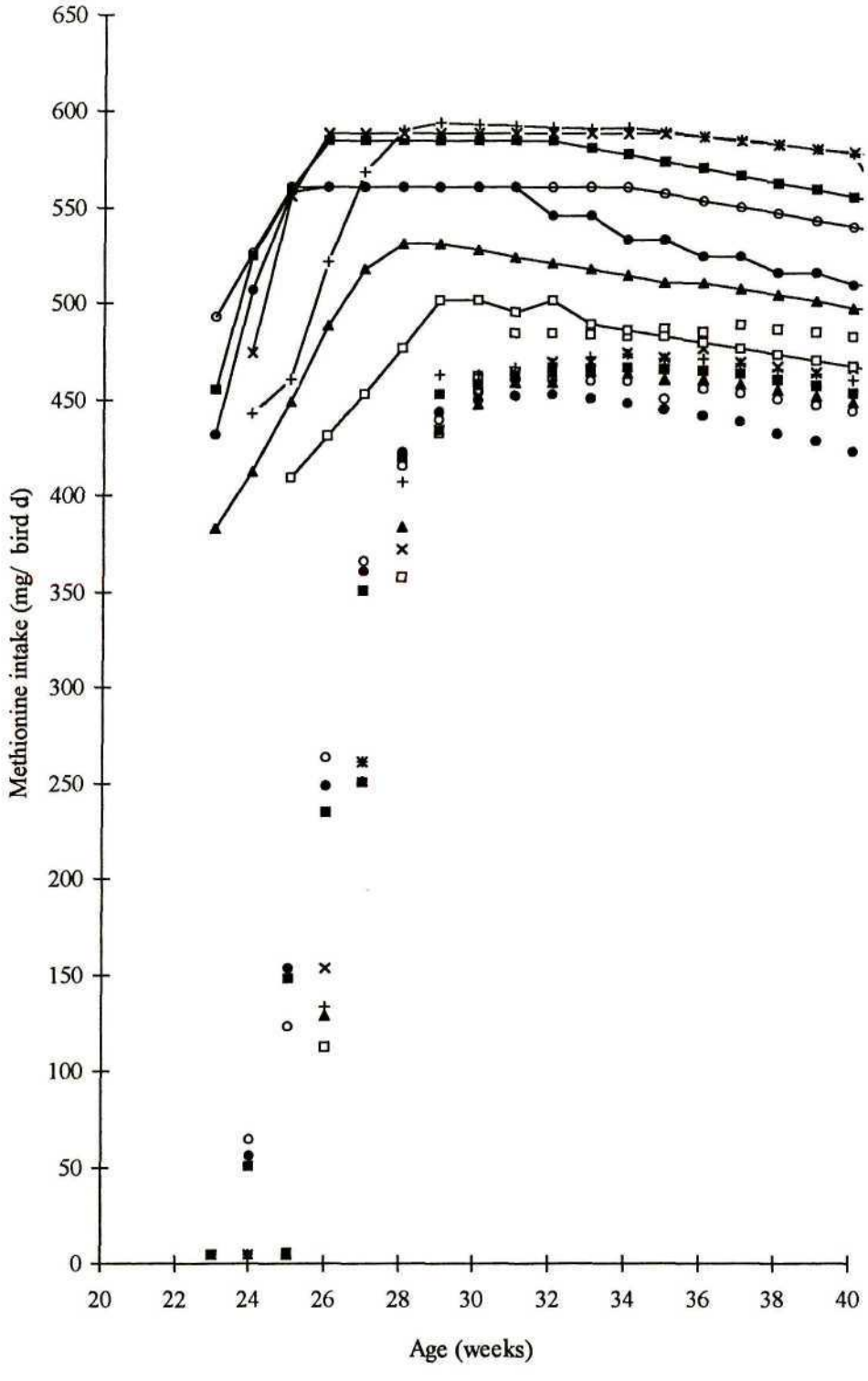

FIG. 3.3 —*Calculated methionine intakes (mg/ bird d) for Ross 308 (•), Hybro N (○), Cobb 500 (■), Arbor Acres (□), Shaver Starbro (+), Hubbard (X) and Avian 24K (▲) broiler breeders and methionine intakes recommended by the respective breeding companies (lines connecting the symbols) over the first 20 weeks of production*

Energy

Fig. 3.4 graphically illustrates the differences between the recommended and calculated daily energy requirements for the seven broiler breeder strains between 23 to 40 weeks of age. In the early stages of laying, the calculated requirements are considerably lower than those recommended by the breeding companies. From about 32 weeks of age, calculated intakes are on average, higher than recommended. However, the most notable difference is in the recommendation that daily energy intakes should decline after peak production whilst calculated energy requirements for egg production, maintenance and growth, continue rise. It is not certain if breeders need to grow during the laying period. The equation used to calculate energy intake assumed that the hens would not be gaining any weight when on a balanced feed. If weight gain is essential to optimising production in broiler breeders, the differences between recommended and calculated energy intakes early in the laying period would be negligible. However, the discrepancies during the post-peak period would escalate.

It is evident that the major differences between calculated and recommended amino acid and energy requirements, are in the relative amounts specified and in the patterns of change in amino acid and energy requirements with age. Adjusting food intake according to the level of production or age of the bird, as is stipulated in the production manuals, assumes that the requirements for energy and protein, specifically lysine and methionine, change in parallel. It has been illustrated that the requirements for amino acids and energy are theoretically lower than those recommended by the breeding companies during the pre-peak period of production. However, during the post-peak period, the calculated energy requirements increase, while the calculated amino acid intakes remain level with the respective requirements recommended by the breeding companies. Therefore, it is apparent that the energy and protein requirements of young broiler breeder hens demands further research.

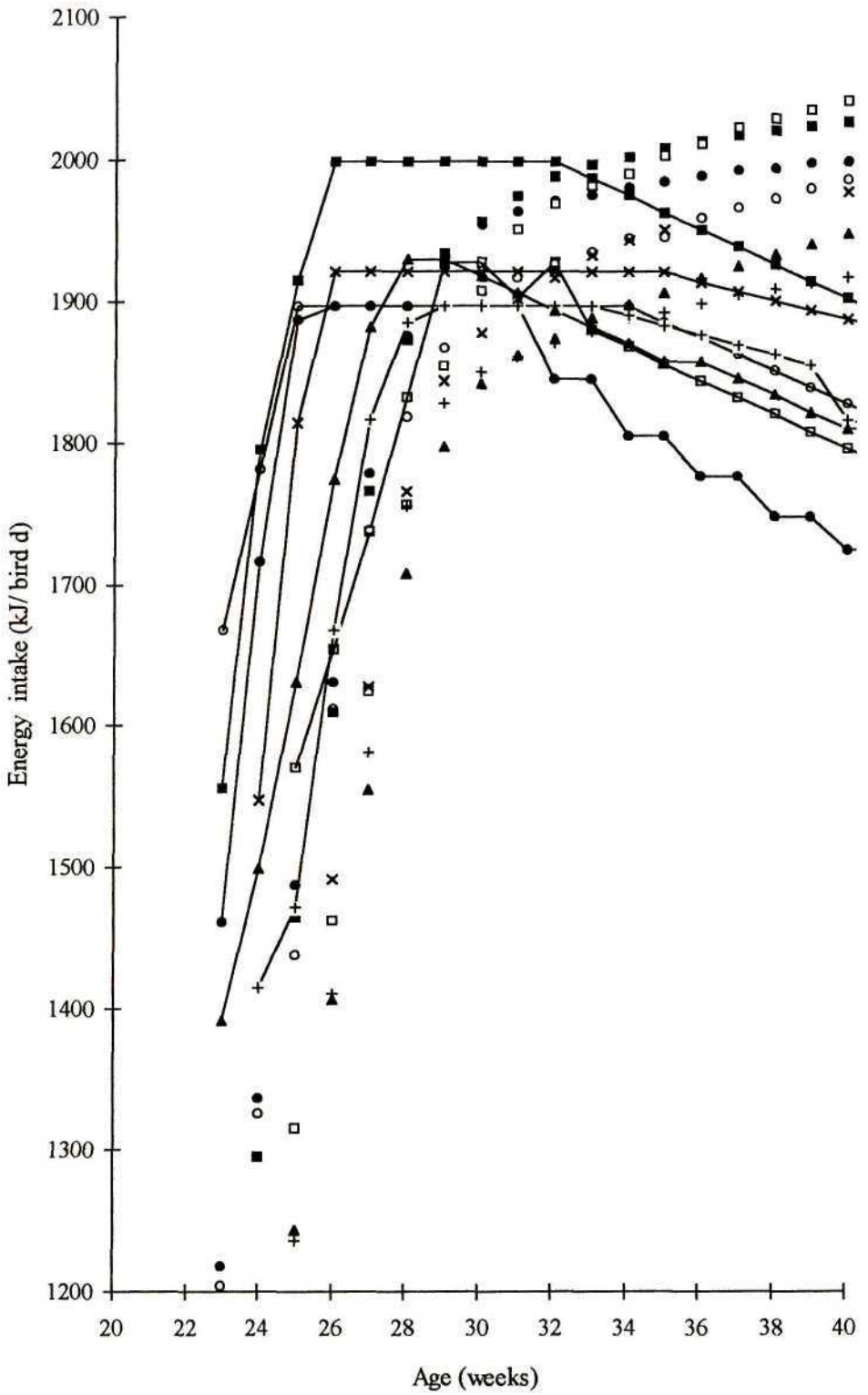

FIG. 3.4 —*Calculated energy intakes (kJ/ bird d) for Ross 308 (•), Hybro N (○), Cobb 500 (■), Arbor Acres (□), Shaver Starbro (+), Hubbard (X) and Avian 24K (▲) broiler breeders and energy intakes recommended by the respective breeding companies (lines connecting the symbols) over the first 20 weeks of production*

Experiment

Pre-peak production period (20-26 weeks)

Initial body weights. The mean body weights and standard errors of the birds selected for the three weight categories at 20 weeks of age are outlined in Table 3.2. In all three cases, the coefficient of variation was 0.3% indicating that the three weight ranges were discrete or non-overlapping. It is apparent that the chemical composition of the sexually immature broiler breeders was independent of body weight (Table 3.2).

TABLE 3.2

Body weight (g) and chemical composition (g/kg) of the 20 week old broiler breeders in the three weight categories

Weight[1]	Body weight[2]	Moisture[3]	Protein[4]	Fat[5]	Ash[6]
1	1985[a]	679.3[a]	170.7[a]	64.40[a]	85.66[a]
2	2211[b]	669.2[a]	169.2[a]	83.33[a]	78.33[a]
3	2441[c]	664.6[a]	174.5[a]	81.24[a]	79.72[a]

[2]Residual S.E. (336df) 0.07
[3]Residual S.E. (15df) 14.01
[4]Residual S.E. (15df) 5.28
[5]Residual S.E. (15df) 16.45
[6]Residual S.E. (15df) 7.29
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-c]Columns with superscripts in common are not significantly different ($P<0.05$)

Sexual maturity. The average age at which the birds laid their first egg was significantly affected by both 20 week body weight and nutrient density (Table 3.3). On average, the heaviest birds came into lay five days before the lightest birds, while a lapse of only three days existed between those on H, compared to those birds on L. On average, all birds were allocated 150g/ bird d at this time, hence the lack of significance of the food allocation treatment. The interaction between nutrient density and food allocation was significant ($P<0.05$). This reiterates the fact that birds on the

H feed reached point of lay at a faster rate than those on the L feed.

TABLE 3.3

Mean age at sexual maturity(d)

	Allocation (g)									
	150		160		170		180			
Nutrient Density	H	L	H	L	H	L	H	L	H	169.9[a]
									L	173.3[b]
Weight[1]									Weight	
1	174.7	179.5	173.4	171.7	174.1	175.1	169.5	176.4	174.3[a]	
2	168.8	173.8	169.7	175.1	172.5	172.4	168.1	170.9	171.4[b]	
3	165.9	173.0	168.0	173.5	168.6	167.0	166.1	171.1	169.1[c]	
Allocation	172.6[a]		171.9[a]		171.6[a]		170.3[a]			

Residual S.E. (331df) 9.5
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-c]Main effects with superscripts in common are not significantly different (P<0.05)

The average body weight at which the birds came into lay (Table 3.4) was significantly correlated with nutrient density. The broiler breeders fed H were statistically heavier at first egg compared to those fed L. A direct, positive relationship appeared to exist between the weight groups selected at 20 weeks and mean body weight at first egg. As was expected, allocation had no effect on body weight at point of lay, and the four factor interactions were not statistically significant.

TABLE 3.4

Mean body weight (g) at first egg

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	3 075[a]
Density									L	3 012[b]
Weight[1]									Weight	
1	3 098	3 000	3 005	2 807	3 042	2 891	2 985	2 916	2 968[a]	
2	3 050	3 060	3 047	3 074	3 129	2 958	2 993	2 969	3 035[b]	
3	3 131	3 182	3 124	3 223	3 170	2 987	3 130	3 075	3 128[c]	
Allocation	3 087[a]		3 047[a]		3 030[a]		3 012[a]			

Residual S.E. (321df) 7.87
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-c]Main effects with superscripts in common are not significantly different (P<0.05)

The regression of age at sexual maturity on body weight at first egg was linear and positive. However, the slopes of the responses (and the intercepts), derived by including the three factors as dummy variables, respectively, were not significantly different from one another. Hence, a common linear model was accepted to describe the response in age at sexual maturity to body weight at first egg for birds in the various weight groups and on the two nutritional treatments (Table 3.5). The model indicates that the longer the birds took to start laying, the heavier they became before the onset of production. If age at sexual maturity was dependent upon a particular body weight at point of lay, the regression of the two variables would not be possible (Bornstein *et al.*, 1984). However, the linear model implies the attainment of a particular body weight at sexual maturity is not the main stimulus for bringing the birds into production. These findings are supported by Pearson and Herron (1982).

TABLE 3.5

Regression coefficients relating age at sexual maturity to body weight at first egg (g) and initial egg weight to age at sexual maturity (g)

	Constant (b_0)	Linear coefficient (b_1)	R^2
Body weight at first egg VS age at sexual maturity	108.07 ± 3.80	0.02 ± 0.001	0.45
Age at sexual maturity VS initial egg weight	-5.35 ± 5.10^{NS}	0.33 ± 0.30	0.27

Regression coefficients $P<0.05$
^{NS}Non-significantly different from zero ($P<0.05$)

Research by Robinson and Robinson (1991) confirm the findings that low weight birds at 20 weeks come into lay later, and that heavy weight birds at 20 weeks have a heavier body weight at sexual maturity. However, Robinson *et al.* (1995) determined that 20 week body weight had no residual effect on age at sexual maturity, possibly because of the narrow body weight ranges used. Moreover, a higher plane of nutrition, prior to the onset of lay, appears to advance ovarian function or, specifically, influence follicular growth (Hocking, 1993).

The average weight of eggs produced within the first week of production was not significantly influenced by 20 week body weight or by either of the nutritional treatments (Table 3.6). Age at first egg maintained a positive, linear relationship with initial egg weight (Table 3.5) that was similar for birds in the three weight groups, two nutrient densities and four allocations. These findings support the fact that egg weight is solely a function of bird age.

TABLE 3.6

Average egg weight (g) during the first week of production

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	51.11[a]
Density									L	51.86[a]
Weight[1]									Weight	
1	50.62	54.26	51.58	49.37	51.34	51.51	51.26	52.00	51.49[a]	
2	49.85	51.97	52.67	53.18	50.39	52.86	49.86	50.85	51.55[a]	
3	49.60	52.51	50.66	52.44	52.81	50.35	52.72	51.04	51.69[a]	
Allocation	51.47[a]		51.65[a]		51.54[a]		51.29[a]			

Residual S.E. (326df) 5.22
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-b]Main effects with superscripts in common are not significantly different ($P<0.05$)

Peak production period (26-31 weeks)

Mean peak performance of all the treatments, calculated over the modal two week period, was 89.8eggs/ 100 bird d (Table 3.7). All broiler breeders in this trial reached maximum egg production at approximately 28 weeks of age, which was independent of the treatments imposed on the birds after 20 weeks of age (Table 3.8). Factor interactions were not statistically significant. The only factor influencing maximum rate of laying, calculated over the modal two week period, was nutrient concentration (Table 3.7). Birds were able to lay equally well regardless of their weight at 20 weeks. In addition, it was of no benefit to feed broiler breeders in excess of 150g/ bird d to achieve an excellent peak production since the responses to all three food allocations at 28 weeks were not significantly different from one another. Maximum rate of laying and the age at which the birds reached peak were not significantly related.

TABLE 3.7

Maximum rate of laying (eggs/ 100 bird d) calculated over the modal two week period

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	90.27[a]
Density									L	89.30[b]
Weight[1]									Weight	
1	88.25	87.07	89.01	90.16	90.16	92.86	91.43	88.10	89.63[a]	
2	91.75	90.14	91.84	86.98	87.76	88.89	92.06	89.12	89.82[a]	
3	90.79	89.21	88.10	89.52	89.38	91.11	92.70	88.78	89.95[a]	
Allocation	89.54[a]		89.27[a]		90.02[a]		90.36[a]			

Residual S.E. (320df) 7.49
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-b]Main effects with superscripts in common are not significantly different (P<0.05)

TABLE 3.8

Age (weeks) at which maximum rate of laying was reached

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	27.86[a]
Density									L	27.66[a]
Weight[1]									Weight	
1	27.60	28.14	28.08	27.13	28.79	27.36	28.27	27.43	27.85[a]	
2	27.13	27.93	27.79	28.20	28.50	27.33	27.87	28.29	27.88[a]	
3	27.20	27.53	28.29	27.33	27.62	27.47	27.20	27.79	27.55[a]	
Allocation	27.59[a]		27.80[a]		27.84[a]		27.81[a]			

Residual S.E. (319df) 2.16
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-b]Main effects with superscripts in common are not significantly different (P<0.05)

Post-peak production period (31-36 weeks)

Performance

Egg weight. At 30 weeks of age, all four of the food allocations were imposed on the broiler breeders. From 31 weeks, it was assumed that the birds had adjusted to the final food increment. Table 3.9 details the mean weight of the eggs of the hens on the 24 treatments over the final six week period of the trial. Birds on the H feed produced eggs which were approximately one gram heavier than those produced on the L feed. 20 week body weight had no significant residual effect on mean egg weight. Regressing mean egg weight against allocation, a continuous variable, yielded non significant linear, and quadratic trends which were not improved by adding weight and nutrient density as dummy variates. This was expected since none of the factor interactions were significant.

TABLE 3.9

Mean egg weight (g) between 31 and 36 weeks of age

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	64.00[a]
Density									L	63.17[b]
Weight[1]									Weight	
1	62.97	61.66	64.92	61.73	64.07	63.13	63.93	64.68	63.39[a]	
2	62.40	63.22	64.76	64.31	64.88	62.79	62.86	63.31	63.57[a]	
3	63.82	63.62	63.04	63.90	65.19	63.69	65.19	62.07	63.81[a]	
Allocation	62.95		63.78		63.96		63.67			

Residual S.E. (319df) 3.60

[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)

[a-b]Main effects with superscripts in common are not significantly different (P<0.05)

Rate of laying. Egg production over the final six week period (Table 3.10) was consistently high (on average 81.75eggs/ 100 bird d) over all the treatments. However, birds on the H feed laid on average significantly more eggs (1.88eggs/ bird d) compared to the birds on L. 20 week body weight did not appear to influence rate of laying, and the factor interactions were significant ($P<0.05$). Rate of lay displayed a linear trend ($P<0.05$) with the amount of food allocated, and the slopes of the response for the three weight categories and the nutrient densities, respectively, were not significantly different from one another (Table 3.11). Therefore, regardless of body weight at 20 weeks of age, and the concentration of nutrients in the feed, rate of lay increased by 0.20 ± 0.04eggs/100 bird d for every ten gram increment in daily food allocation.

TABLE 3.10

Mean rate of laying (eggs/ 100 bird d) between 31 and 36 weeks of age

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	82.69[a]
Density									L	80.81[b]
Weight[1]									Weight	
1	75.68	78.91	79.49	80.32	86.98	80.95	85.71	83.50	81.44[a]	
2	80.61	80.78	84.18	80.32	82.23	80.36	88.28	82.78	82.13[a]	
3	81.03	76.19	81.38	80.79	81.50	82.38	86.39	82.48	81.52[a]	
Allocation	78.87		81.08		82.40		84.44			

Residual S.E. (314df) 8.15
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-b]Main effects with superscripts in common are not significantly different ($P<0.05$)

TABLE 3.11

Linear regression coefficients relating rate of lay (eggs/ 100 bird d), egg output (g/ bird d), body weight (g), body weight change (g/ bird d) and food intake (g/ bird d) to allocation for the three weight categories and two nutrient densities

Variable		Rate of lay (eggs/ 100 bird d)	Egg output (g/ bird d)	Body weight (g)	Body weight gain (g/ bird d)	Food intake (g/ bird d)
		0.20 ±0.04	0.15 ±0.03	8.34 ±4.73^{NS}	0.18 ±0.05	0.89 ±0.04
	R^2	0.54	0.50	0.12	0.38	0.96
Weight[1]	1	0.26 ±0.07^{a}	0.22 ±0.06^{a}	6.89 ±8.55^{aNS}	0.15 ±0.09^{aNS}	0.87 ±0.06^{a}
	2	0.14 ±0.07^{a}	0.09 ±0.06^{aNS}	10.02 ±8.55^{aNS}	0.10 ±0.09^{aNS}	0.91 ±0.06^{a}
	3	0.18 ±0.07^{a}	0.13 ±0.06^{a}	8.13 ±8.55^{aNS}	0.20 ±0.09^{aNS}	0.89 ±0.06^{a}
	R^2	0.60	0.57	0.21	0.38	0.98
Nutrient	H	0.14 ±0.05^{a}	0.19 ±0.03^{a}	7.27 ±2.57^{a}	0.21 ±0.02^{a}	0.93 ±0.05^{a}
Density	L	0.26 ±0.04^{a}	0.10 ±0.03^{a}	9.41 ±2.57^{a}	0.15 ±0.02^{a}	0.85 ±0.05^{a}
	R^2	0.71	0.72	0.88	0.94	0.97

[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
$^{a-b}$Regression coefficients within columns, for each factor, with superscripts in common are not significantly different ($P<0.05$)
^{NS}Not significantly different from zero ($P<0.05$)

Egg output. Mean egg output between 31 and 36 weeks of age for the various weight categories, feed allocations and nutrient densities are outlined in Table 3.12. The factor interactions were not significant. On average, egg output on H was 2.07g/ bird d greater than that on L. Body weight at 20 weeks had no significant influence on mean egg output. Table 3.11 details the linear response in mean egg output to allocation which was independent of weight or nutrient density. However, it should be noted that even though the slopes of the three weight groups were not significantly different from one another, the response slope of the second weight group did not differ significantly from zero due to the large variation in egg production of these birds within each of the four feed allocations.

TABLE 3.12

Mean egg output (g/ bird day) between 31 and 36 weeks of age

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	53.03[a]
Density									L	50.96[b]
[1]Weight									Weight	
1	47.57	48.56	51.43	49.54	55.71	51.04	54.71	53.97	51.57[a]	
2	50.26	51.03	54.53	51.57	54.20	50.02	55.84	52.28	52.47[a]	
3	51.67	48.41	51.14	51.49	52.99	52.42	56.27	51.22	51.95[a]	
Allocation	49.58		51.62		52.56		53.76			

Residual S.E. (313) 5.06
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-b]Main effects with superscripts in common are not significantly different (P<0.05)

Compared to current findings, variation in the effect of degree of restriction on production parameters such as egg weight and egg production as reported by some researchers such as Blair *et al.* (1976), McDaniel (1983), McDaniel *et al.* (1981), Robbins *et al.* (1986), Lilburn *et al.* (1990) and Robinson *et al.* (1995) highlights the fact that production responses are dependent on the degree of restriction, rearing treatment and strain differences.

Body weight. Mean body weight over the six week period was influenced by nutrient intake (Table 3.13). The interaction between allocation and nutrient density was significant (P<0.05) indicating that, over the range of food allocated, birds tended to be significantly heavier on the H feed than on the L feed. The body weights of the birds in the three distinct weight groups selected at 20 weeks, were still significantly different (P<0.05) from one another 16 weeks later. However,

the mean differences between the three groups had decreased. Body weight maintained a positive, linear relationship with allocation ($P<0.01$) for the two nutrient densities, the slopes of which were not significantly different (Table 3.11). The fit of the linear model was not improved by including 20 week body weight as a dummy variable. Within each weight group, the broiler breeders maintained their body weights to a large extent, regardless of the change in food allocation.

TABLE 3.13

Mean body weight (g) between 31 and 36 weeks of age

	Allocation (g)									
	150		160		170		180			
Nutrient Density	H	L	H	L	H	L	H	L	H	3813[a]
									L	3343[b]
Weight[1]									Weight	
1	3530	3209	3721	3130	3798	3280	3766	3357	3474[a]	
2	3638	3219	3738	3272	3938	3371	3935	3490	3575[b]	
3	3743	3348	3836	3427	3997	3442	4005	3569	3671[c]	
Allocation	3448		3521		3638		3687			

Residual S.E. (320df) 176.07
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-d]Main effects with superscripts in common are not significantly different ($P<0.05$)

Body weight change. Body weight change, averaged over the last six weeks of the trial, was not significantly different within the three weight groups (Table 3.14). On average, birds consuming H tended to gain significantly more weight than those on L. As the amount of food allocated to the hens increased, so the birds tended to gain similar amounts of weight regardless of the concentration of the nutrients in the feed (Table 3.11). However, the interaction between allocation and nutrient density was statistically significant ($P<0.05$) indicating that the birds gained, on

average, more weight on the H feed compared to the L feed over the range of food allocations. Mean body weight gain of the birds in the three weight categories was not significantly influenced by allocation (Table 3.11).

TABLE 3.14

Mean body weight change (g/ bird d) between 31 and 36 weeks of age

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	9.75[a]
Density									L	4.82[b]
[1]Weight									Weight	
1	7.67	3.02	9.54	3.40	9.19	5.99	11.96	8.06	7.35[a]	
2	7.79	1.93	9.86	2.55	10.02	6.10	12.00	9.27	7.44[a]	
3	6.34	2.58	9.09	1.62	10.91	5.72	12.62	7.63	7.06[a]	
Allocation	4.89		6.01		7.99		10.26			

Residual S.E. (319df) 2.76
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-d]Main effects with superscripts in common are not significantly different (P<0.05)

Food intake. Significantly more (1.8g/ bird d) of L was consumed over the six week period than H (Table 3.15). Food intake was influenced by 20 week body weight. The heaviest birds at 20 weeks of age tended to consume more food on a daily basis than birds in either of the other two categories, possibly because of their greater maintenance requirements. The interactions between nutrient density, and body weight and food allocation, respectively, were highly significant. This indicates that as 20 week body weight and the amount of food allocated increased, birds tended to consume more of L in relation to H, in an attempt to consume sufficient of a limiting nutrient in order to meet the daily requirement for that nutrient (Emmans, 1981). Food intake responded positively and linearly to food allocation, which was expected (Table 3.14). The linear regression coefficients were not significantly

different between weight groups or nutrient densities.

TABLE 3.15

Mean food intake (g/ bird d) between 31 and 36 weeks of age

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	160.6[a]
Density									L	162.5[b]
Weight									Weight	
1	144.4	147.8	154.1	158.1	166.2	166.7	168.3	175.1	160.1[a]	
2	148.2	148.9	156.7	158.2	165.6	167.1	174.7	176.9	162.0[b]	
3	149.7	148.6	158.0	157.8	167.4	167.0	174.3	177.4	162.6[c]	
Allocation	147.9		157.2		166.7		174.4			

Residual S.E. (311df) 3.77
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-c]Main effects with superscripts in common are not significantly different (P<0.05)

Chemical composition. Mean protein content (Table 3.16) of the feather-free carcasses at the end of the trial was not influenced by 20 week body weight nor by food allocation. The interactions between the three factors were not significant. The linear and quadratic trends between protein content and allocation were not significant. However, birds on the L feed had a higher protein content per kg body weight (3.7g/kg) than birds on the H feed (P<0.05). It can be speculated that delay in sexual maturity of the birds on L, of approximately three days, allowed the birds to gain slightly more protein weight (approximately 1g/ bird d) than those on H, if it is assumed that protein growth ceases after the onset of sexual maturity.

TABLE 3.16

Mean body protein concentration (g/kg) of the feather-free carcass at 36 weeks of age

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	147.4[a]
Density									L	151.1[b]
[1]Weight									Weight	
1	141.2	152.4	152.8	159.3	155.7	151.6	147.9	145.7	150.8[a]	
2	147.5	152.3	142.4	151.3	140.2	153.7	149.7	149.0	148.3[a]	
3	144.7	153.2	151.0	149.6	152.0	150.3	143.6	144.6	148.6[a]	
Allocation	148.6		151.1		150.6		146.7			

Residual S.E. (48df) 7.20
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-b]Main effects with superscripts in common are not significantly different (P<0.05)

The mean lipid concentration of the feather-free carcasses (Table 3.17) was significantly affected by nutrient density. Birds maintained on the H feed for 16 weeks were approximately 13 percent fatter than those on the L feed. Linear and quadratic trends for lipid content and allocation were not statistically significant and 20 week body weight did not appear to influence lipid content. The factor interactions were not significant.

The lack of an effect of 20 week body weight on carcass characteristics is supported by Robinson *et al.* (1995), who stated that carcass composition is not significantly affected by early lay period treatment, but by the level of feed allocation and egg production rate during the latter part of the production period.

TABLE 3.17

Mean body lipid concentration (g/kg) of the feather-free carcass at 36 weeks of age

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	258.5[a]
Density									L	229.4[b]
[1]Weight									Weight	
1	277.6	206.9	232.5	203.1	246.7	228.2	252.2	256.5	238.0[a]	
2	253.0	228.5	279.0	217.0	297.1	216.7	227.6	236.0	244.4[a]	
3	256.9	212.2	242.4	243.9	257.1	232.6	279.8	270.9	249.5[a]	
Allocation	239.2		236.3		246.4		253.8			

Residual S.E. (48df) 31.72
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-b]Main effects with superscripts in common are not significantly different ($P<0.05$)

Physiology. The mean ovary weights of the 36 week old broiler breeders are detailed in Table 3.18. Ovary weight was not influenced by 20 week body weight. However, birds on H had significantly ($P<0.05$) heavier ovaries than those on L. The interactions between the three factors were not statistically significant. Ovary weight maintained a significant linear relationship ($P<0.05$) with allocation. However the statistical fit (R^2 0.18) of the model was not significant. Including 20 week body weight and nutrient density as dummy variables, respectively, revealed trends that were not significantly different from zero. The fit was not significantly improved by the inclusion of quadratic terms. There were only two observed cases of internal laying which were not specific to any particular treatment. In general, the birds were extremely oily and had eggs in their oviducts.

TABLE 3.18

Mean ovary weight (g) at 36 weeks of age

	Allocation (g)									
	150		160		170		180			
Nutrient Density	H	L	H	L	H	L	H	L	H	75.05[a]
									L	61.69[b]
[1]Weight									Weight	
1	73.43	50.67	85.85	68.50	86.67	61.03	73.83	76.53	72.06[a]	
2	65.73	57.60	91.43	57.57	72.40	61.90	76.03	65.23	68.49[a]	
3	56.40	55.73	69.50	63.80	65.67	60.77	83.70	60.90	64.56[a]	
Allocation	59.93		72.78		68.07		72.71			

Residual S.E. (46df) 15.28
[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[a-b]Main effects with superscripts in common are not significantly different ($P<0.05$)

To identify factors influencing ovary weight other than the treatments imposed on the birds, ovary weight was regressed against body weight, protein concentration and lipid concentration, using 20 week body weight, allocation and nutrient density as dummy variables, respectively. Ovary weight maintained a positive, linear relationship (R^2 0.23) with body weight (Fig 3.5). The constant term was 1913.50 ± 401.4 ($P<0.05$) and the slope was 26.22 ± 5.87 ($P<0.05$).The coefficient of determination (R^2) increased to 0.74 when 20 week body weight was included in the model. However, the constant and slope terms of the three categories were not significantly different from one another indicating they may be described by one equation. Including allocation and nutrient density as dummy variables, respectively, resulted in non-significant trends.

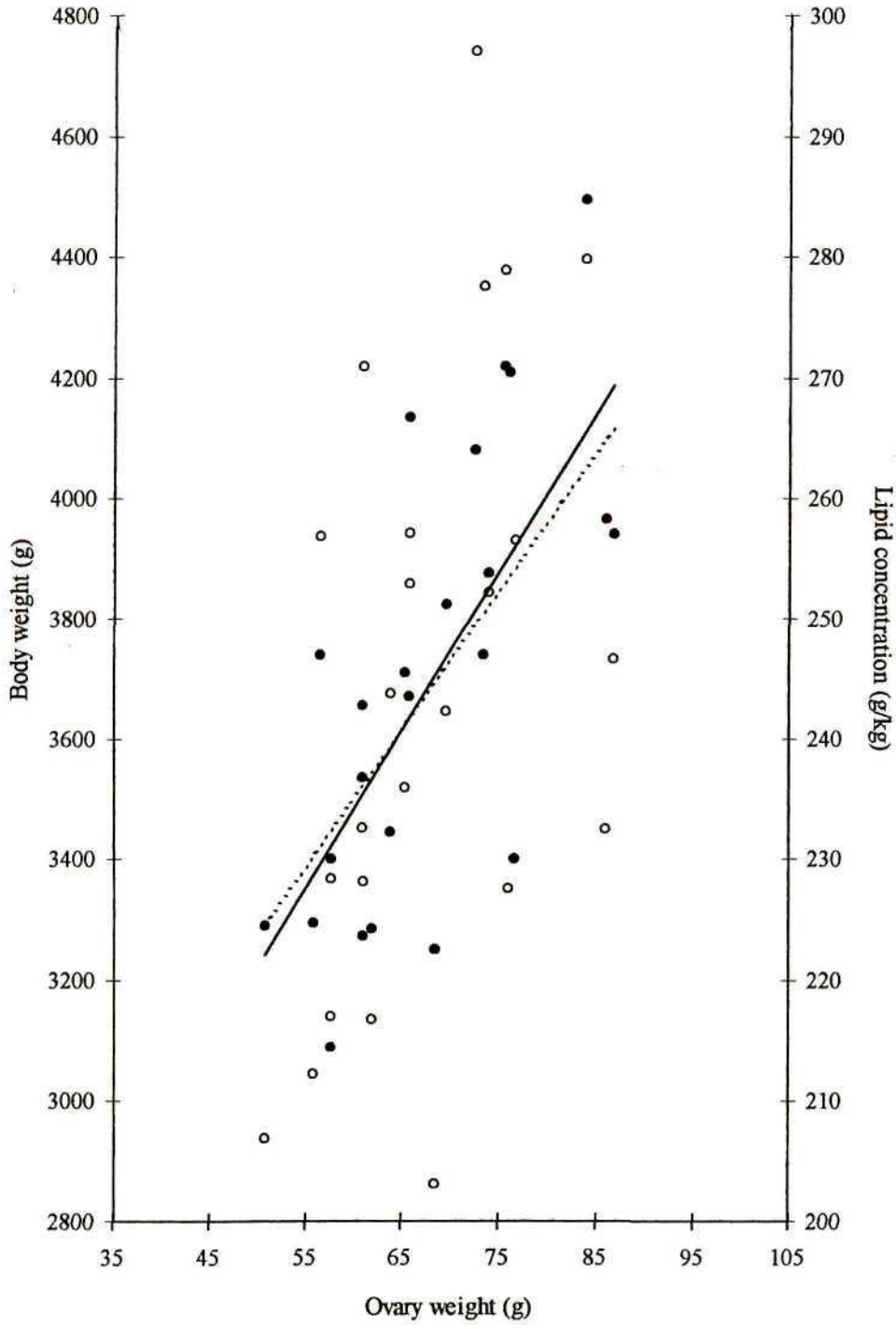

FIG. 3.5 —*Response in body weight (•) represented by the solid line, and body lipid concentration (∘) represented by the dotted line, to ovary weight*

The regression of ovary weight against lipid concentration was significant ($P<0.05$). The constant term was 24.64 ± 18.28 ($P<0.05$) while the slope was 0.18 ± 0.07 ($P=0.027$). However, the R^2 value of 0.23 was not significant indicating that the variation in lipid concentration cannot be completely explained by the fitted model. There was no significant improvement with the addition of quadratic terms. Fitting the model using the respective dummy variables resulted in non-significant trends. The relationship between ovary weight and protein concentration was random.

The relationship between ovary weight, in particular follicular number, and body weight at point of lay is linear (Hocking, 1993). It is interesting that the trend is still observed later in the laying cycle. Yu *et al.* (1992) also noted that the ovaries of full fed broiler breeders at 33 weeks, were heavier than ovaries of birds maintained on restriction but that an increase in ovary weight was negatively correlated with egg production. Since internal laying, follicular atresia, the production of broken, soft-shelled or shell-less eggs are related to the number of yellow follicles (Hocking *et al.*, 1987; Hocking, 1993), it is unusual that a decline in production of birds maintained on H was not observed.

To assess to what extent post-peak rate of lay, egg output and egg weight can be explained by ovarian function, these variables were regressed against ovary weight using the three factors as dummy variables. Rate of laying of birds in the second and third weight categories maintained a positive, linear relationship ($P<0.05$) with ovary weight as did the birds allocated 170 and 180g of food per day (Table 3.19). The lightest birds at 20 weeks of age and those maintained on the lowest allocations exhibited non-significant trends. Egg production of the birds on H and L was unrelated to ovary weight. No significant linear or quadratic trends between egg weight and ovary weight were exhibited by the birds in the three weight categories, two nutrient densities or four allocations. Egg output of the birds in the three weight groups, as well as the birds allocated 170 and 180g of food a day, was linearly, and positively related to ovary weight (Table 3.19). Nutrient density had no significant effect on the linear relationship between egg output and ovary weight. These results show that the variation in reproductive performance cannot be adequately explained by ovary weight.

TABLE 3.19

Regression coefficients relating rate of lay (eggs/ 100 bird d) and egg output (g/ bird d) to ovary weight (g)

		Rate of lay (eggs/ 100 bird d)		Egg output (g/ bird d)	
		Constant (b_0)	Linear coefficient (b_1)	Constant (b_0)	Linear coefficient (b_1)
Allocation	150	84.94± 6.11[a]	-0.10±0.10[a NS]	53.07±4.63[a]	-0.06±0.08[a NS]
	160	80.48±6.01[a]	0.01±0.09[ab NS]	49.00±4.56[a]	0.04±0.06[ab NS]
	170	67.12±5.64[a]	0.23±0.08[b]	40.48±4.28[a]	0.18±0.06[b]
	180	70.53±7.33[a]	0.20±0.10[b]	37.64±5.56[a]	0.23±0.08[b]
	R^2	0.74		0.76	
Weight[1]	1	72.51±3.80[a]	0.12±0.07[a NS]	41.20±4.21[a]	0.14±0.06[a]
	2	61.39±5.84[a]	032±0.13[a]	36.24±6.24[a]	0.24±0.09[a]
	3	65.82±8.67[a]	0.24±0.11[a]	38.85±5.05[a]	0.20±0.08[a]
	R^2	0.45		0.54	

[1]Weight 1 (1900-2100g); 2 (2100-2300g); 3 (2300-2500g)
[NS]Non-significantly different from zero (P<0.05).
[a-b]Regression coefficients within columns, for each factor, with superscripts in common are not significantly different (P<0.05)

Mean oviduct weights of the 36 week old broiler breeders were statistically similar over the weight groups, allocations and nutrient densities. The factor interactions were not significant. No statistically significant relationships existed between oviduct weight and body weight, protein concentration or lipid concentration, respectively.

Nutritional requirements

Amino acids. The regression coefficients relating the production variables, egg weight, rate of lay, egg output and body weight change (Appendix Tables 1-3) to lysine and methionine intake, respectively, are given in Tables 3.20 and

3.21. Methionine intake was calculated to range between 350 and 529g/ bird day. Lysine intakes varied between 918 and 1250g/ bird d. There was no significant evidence of a quadratic effect with any of the variables measured.

Although egg weight was linearly related ($P<0.05$) to lysine and methionine intake, the fit of the model to the data was not statistically significant. The inclusion of the three factors in the experiment as dummy variables did not result in a significant improvement of the fit. Interestingly, the response of the lightest birds at 20 weeks in egg weight to amino acid intake reflected the response in egg output, namely that the lightest birds displayed the lowest intercept (lowest maintenance requirement) and the steepest slope (greater efficiency of utilisation) compared to the larger birds at 20 weeks. The linear trend in egg weight with increased lysine intake, exhibited by the birds allocated 170g/ bird d, has no biological explanation, but is probably due to variation within the group, since the responses were not significantly different from each other.

TABLE 3.20

Regression coefficients relating egg weight (g), rate of laying (eggs/ 100 bird d), egg output (g/ bird d) and body weight change(g/ bird d) to lysine intake (mg/ bird d)

		b_o	b_1	R^2
Egg weight (g)		57.92±2.13	0.005±0.002	0.25
Allocation	150	60.54±6.01[a]	0.002±0.006[aNS]	0.41
	160	55.24±6.19[a]	0.008±0.006[aNS]	
	170	50.90±5.79[a]	0.012±0.005[a]	
	180	58.02±6.84[a]	0.005±0.006[aNS]	
Weight	1	52.89±3.98[a]	0.010±0.004[a]	0.36
	2	61.42±3.78[a]	0.002±0.003[aNS]	
	3	58.96±3.59[a]	0.005±0.003[aNS]	
Nutrient density	H	61.10±4.21[a]	0.002±0.004[aNS]	0.28
	L	57.81±4.43[a]	0.006±0.004[aNS]	
Rate of laying (g/100 bird d)		58.76±3.76	0.022±0.004	0.64
Allocation	150	51.80±11.13[a]	0.022±0.011[a]	0.69
	160	64.07±11.45[a]	0.017±0.011[aNS]	
	170	66.88±10.73[a]	0.014±0.010[aNS]	
	180	50.60±12.65[a]	0.029±0.011[a]	
Weight	1	52.25±6.86[a]	0.028±0.006[a]	0.71
	2	64.09±6.52[a]	0.017±0.006[a]	
	3	58.40±6.20[a]	0.021±0.006[a]	
Nutrient density	H	59.71±7.58[a]	0.021±0.008[a]	0.64
	L	57.67±7.97[a]	0.023±0.007[a]	
Egg output (g/ bird d)		32.26±2.70	0.019±0.003	0.71
Allocation	150	31.12±8.34[a]	0.020±0.008[a]	0.73
	160	34.05±8.58[a]	0.017±0.008[a]	
	170	31.78±8.04[a]	0.019±0.007[a]	
	180	27.03±9.48[a]	0.023±0.008[a]	
Weight	1	24.00±4.75[a]	0.026±0.004[a]	0.78
	2	38.43±4.51[b]	0.013±0.004[b]	
	3	33.01±4.29[ab]	0.018±0.004[ab]	
Nutrient density	H	35.58±5.37[a]	0.015±0.005[0]	0.73
	L	30.26±5.64[a]	0.006±0.005[a]	
Body weight change (g/ bird d)		-32.99±3.002	0.040±0.003	0.90
Allocation	150	-41.41±6.66[a]	0.048±0.007[a]	0.97
	160	-56.29±5.99[a]	0.061±0.006[a]	
	170	-27.37±5.61[b]	0.033±0.005[b]	
	180	-25.66±6.62[b]	0.033±0.006[b]	
Weight	1	-30.14±5.69[a]	0.037±0.005[a]	0.92
	2	-34.09±5.41[a]	0.040±0.005[a]	
	3	-35.62±5.17[a]	0.041±0.005[a]	
Nutrient density	H	-36.75±4.86[a]	0.042±0.005[a]	0.95
	L	-17.10±5.50[b]	0.025±0.005[b]	

[NS] Non-significantly different from zero ($P<0.05$); [a-b] Regression coefficients for each variable and factor with superscripts in common are not significantly different ($P<0.05$)

TABLE 3.21

Regression coefficients relating egg weight (g), rate of laying (eggs/ 100 bird d), egg output (g/ bird d) and body weight change (g/ bird d) to methionine intake (mg/ bird d)

		b_o	b_1	R^2
Egg weight (g)		59.67±1.44	0.009±0.003	0.26
Allocation	150	61.62±3.46^{a}	0.003±0.009^{aNS}	0.39
	160	59.76±3.42^{a}	0.010±0.008^{aNS}	
	170	56.51±3.38^{a}	0.016±0.008^{aNS}	
	180	60.91±3.65^{a}	0.006±0.008^{aNS}	
Weight	1	56.86±2.72^{a}	0.015±0.006^{a}	0.36
	2	61.88±2.54^{a}	0.004±0.006^{aNS}	
	3	59.98±2.51^{a}	0.009±0.006^{aNS}	
Nutrient density	H	61.10±4.25^{a}	0.005±0.010^{aNS}	0.26
	L	58.50±4.43^{a}	0.011±0.009^{aNS}	
Rate of laying (g/100 bird d)		66.99±2.74	0.035±0.006	0.59
Allocation	150	64.90±6.34^{a}	0.039±0.016^{a}	0.69
	160	72.66±6.27^{a}	0.021±0.015^{aNS}	
	170	73.14±6.20^{a}	0.021±0.014^{aNS}	
	180	66.45±6.69^{a}	0.038±0.014^{a}	
Weight	1	64.06±5.18^{a}	0.042±0.012^{a}	0.64
	2	68.42±4.85^{a}	0.033±0.011^{a}	
	3	67.68±4.78^{a}	0.032±0.011^{a}	
Nutrient density	H	59.80±7.61^{a}	0.054±0.020^{a}	0.64
	L	58.12±7.92^{a}	0.053±0.016^{a}	
Egg output (g/ bird d)		39.17±1.99	0.030±0.005	0.66
Allocation	150	40.01±4.84^{a}	0.026±0.012^{a}	0.72
	160	43.18±4.79^{a}	0.021±0.011^{aNS}	
	170	40.46±4.73^{a}	0.027±0.010^{a}	
	180	39.78±5.11^{a}	0.030±0.011^{a}	
Weight	1	35.02±3.78^{a}	0.040±0.009^{a}	0.70
	2	41.72±3.53^{a}	0.028±0.005^{a}	
	3	40.03±3.48^{a}	0.027±0.008^{a}	
Nutrient density	H	35.58±5.50^{a}	0.040±0.014^{0}	0.71
	L	31.21±5.73^{a}	0.046±0.012^{a}	
Body weight change (g/ bird d)		-19.25±2.18	0.064±0.005	0.88
Allocation	150	-20.46±3.19^{a}	0.067±0.008^{a}	0.97
	160	-27.46±2.83^{a}	0.082±0.007 ^{a}	
	170	-11.41±2.80^{b}	0.047±0.006^{ab}	
	180	-7.831±3.02^{b}	0.043±0.006^{ab}	
Weight	1	-18.67±4.00^{a}	0.063±0.009^{a}	0.90
	2	-17.54±4.28^{a}	0.061±0.010^{a}	
	3	-21.69±3.95^{a}	0.069±0.009^{a}	
Nutrient density	H	-36.76±4.87^{a}	0.111±0.013^{a}	0.94
	L	-16.83±5.48^{b}	0.059±0.011^{b}	

^{NS}Non-significantly different from zero (P<0.05); $^{a-b}$Regression coefficients for each variable and factor with superscripts in common are not significantly different (P<0.05)

Rate of lay was linearly related to both lysine and methionine intake over the aforementioned ranges. Including allocation as a dummy variable revealed that the production of birds consuming 150 and 180g/bird d was influenced positively by an increase in amino acid intake. The amount of lysine and methionine available to the hens on the lowest allocation did not appear to be sufficient to satisfy the requirements for maximum production. The trend exhibited by the birds on the highest allocation can be attributed to a few hens within the group laying at an extremely high rate. The response of the birds within the three weight groups and two nutrient densities, respectively, could be described by the general equation (Tables 3.20 and 3.21).

The relationship between egg output and lysine and methionine intake, respectively, over the allocations, weight groups and nutrient densities, was linear and positive without any indication of a plateau having been reached. The non-significant ($P<0.05$) response in egg output to lysine intake, exhibited by the birds allocated 160g/bird d appeared to be coincidental, rather than a true effect. Interestingly, the response to lysine intake differed between the hens in the weight categories. Numerically, the lightest birds displayed the lowest intercept (lowest maintenance requirement) and the steepest slope (greatest efficiency of utilisation).

The broiler breeder hens tended to gain significantly more weight as the daily lysine and methionine intakes increased. The response was dependent on the amount, and the nutrient density of the feed allocated. Lower daily allocations resulted in a significantly ($P<0.05$) greater weight gain. However, this does not appear to be a real effect of the allocation treatment, but a reflection of the nutrient density treatment imposed on the birds, within the four allocations. Mean body weight gain of the birds fed lower allocations of L, was relatively lower than gains achieved by the birds fed higher allocations of L. In fact there were eight cases where the birds actually lost weight on the lower allocations of L. Since the slopes of the response in weight gain of hens fed different allocations are reliant on nutrient density, it appears that the relatively lower gains at the lower allocations of L, reduced the intercepts, and hence, increased the slopes of the response at the lower allocations. The hens gained significantly more weight on the H feed. This is because, at the lower intakes (L) there was less lysine and methionine available, above that required to satisfy egg output, that could be deposited as fat.

Energy. The regression coefficients relating the production variables, egg weight, rate of lay, egg output and body weight change, to energy intake, which ranged from 1643 to 2323kJ/ bird d, are given in Table 3.22. In general egg weight was linearly related to energy intake. However, when the dummy variables, allocation and nutrient density were included, only the birds allocated 170g/ bird d and those on the L feed displayed linear trends. It is apparent that low variation within these groups is the cause of the observed effect. Again the egg weight of the lightest birds at 20 weeks increased with increased energy intake.

Rate of laying of birds on the lowest food allocation increased significantly ($P<0.05$) with increasing energy intake. The lack of a response by birds on the remaining allocations indicate that energy was not a factor limiting production above an allocation of 160g/bird d. Production was linearly related to energy intake over the two nutrient densities and body weight categories with the exception of the middle category, which is not possible to explain.

Egg output was influenced linearly, and positively by energy intake over the three weight groups and two nutrient densities. The trend exhibited by the birds allocated 170g/bird day can be ascribed to the low variation within that group. A non-significant relationship between egg output and energy intake was observed over the remaining food allocations, indicating that, regardless of the amount of food allocated, daily energy requirements needed to support egg output were satisfied.

A linear trend in body weight gain with increasing energy intake was observed. Again, the gain was greater when the birds were allocated either 150 or 160g/bird d and when they were on the H feed.

TABLE 3.22

Regression coefficients relating egg weight (g), rate of laying (eggs/ 100 bird d), egg output (g/ bird d)and body weight change (g/ bird d) to energy intake (kJ/ bird d)

		bo	b1	R^2
Egg weight (g)		57.43±1.70	0.003±0.001	0.41
Allocation	150	60.93±4.14[a]	0.001±0.002[aNS]	0.51
	160	58.35±4.36[a]	0.003±0.002[aNS]	
	170	53.72±4.14[a]	0.005±0.002[a]	
	180	59.29±5.83[a]	0.002±0.003[aNS]	
Weight	1	54.46±3.08[a]	0.005±0.002[a]	0.50
	2	58.45±3.49[a]	0.003±0.002[aNS]	
	3	59.71±2.74[a]	0.002±0.001[aNS]	
Nutrient density	H	58.48±4.08[a]	0.003±0.002[aNS]	0.43
	L	53.79±4.33[a]	0.005±0.002[a]	
Egg production (g/100 bird d)		63.34±3.64	0.010±0.002	0.57
Allocation	150	59.60±9.13[a]	0.011±0.005[a]	0.62
	160	68.80±9.89[a]	0.007±0.005[aNS]	
	170	69.96±9.12[a]	0.006±0.005[aNS]	
	180	60.68±12.84[a]	0.011±0.006[aNS]	
Weight	1	56.66±6.28[a]	0.013±0.032[a]	0.67
	2	70.57±7.13[a]	0.006±0.004[aNS]	
	3	62.46±5.60[a]	0.010±0.003[a]	
Nutrient density	H	59.03±8.81[a]	0.012±0.005[a]	0.56
	L	60.73±9.35[a]	0.011±0.004[a]	
Egg output (g/ bird d)		35.06±2.65	0.009±0.001	0.68
Allocation	150	36.19±6.68[a]	0.008±0.004[aNS]	0.71
	160	39.72±7.02[a]	0.006±0.004[aNS]	
	170	36.10±6.68[a]	0.008±0.003[a]	
	180	34.85±9.40[a]	0.009±0.004[aNS]	
Weight	1	28.25±4.67[a]	0.012±0.009[a]	0.74
	2	40.64±5.30[a]	0.006±0.008[a]	
	3	36.38±4.16[a]	0.008±0.008[a]	
Nutrient density	H	33.20±6.29[a]	0.010±0.004[a]	0.70
	L	29.23±6.68[a]	0.011±0.003[a]	
Body weight change (g/ bird d)		-28.42±2.66	0.019±0.001	0.91
Allocation	150	-30.89±3.91[a]	0.021±0.002[a]	0.98
	160	-42.53±3.52[b]	0.026±0.002 [a]	
	170	-19.35±3.35[c]	0.014±0.001 [b]	
	180	-16.62±4.71[c]	0.014±0.002 [b]	
Weight	1	-25.68±4.89[a]	0.018±0.003[a]	0.92
	2	-30.80±5.54[a]	0.020±0.003[a]	
	3	-30.04±4.36[a]	0.019±0.002[a]	
Nutrient density	H	-38.56±5.65[a]	0.025±0.003[a]	0.94
	L	-18.32±6.34[b]	0.014±0.003[b]	

[NS]Non-significantly different from zero (P<0.05); [a-b]Regression coefficients for each variable and factor with superscripts in common are not significantly different (P<0.05)

It is apparent that broiler breeder hens are unable to maximise egg output over the range of lysine, methionine and energy intakes used in this experiment, without concomitantly influencing body weight gain. In fact, the correlation between egg output and body weight change was 0.754 ($P<0.05$). However, there were individual response differences that are worth mentioning. At least one bird performed poorly when body weight gain exceeded 20g/bird d and at least two birds maintained an egg output of above 55g/bird d while losing weight. There were nine observed cases where egg output was greater than 55g/bird d with a body weight gain between zero and five grams per day. However, if the flock average is considered, it is evident that broiler breeder hens are incapable of partitioning their nutrient intakes in such a way that they can maximise performance on a lower intake of either lysine, methionine or energy. It is also unlikely that any other nutrient could have limited performance.

The mean egg output of birds laying at a rate above 0.5 between 31 and 36 weeks of age was 52.1g/ bird d and was higher than that presented as the potential for broiler breeders by the seven breeding companies. Clearly, broiler breeder hens have a greater potential for egg production than is currently perceived. The high egg output was attained on an energy intake of 1898kJ/ bird d, a lysine intake of 1047mg/ bird d and a methionine intake of 434mg/ bird d. In comparison to the recommendations made by Ross Breeders Ltd. (1995), the difference in energy intake was negligible. In the experiment, the equivalent of 5g/ bird d more feed was offered than would be recommended, whereas, an additional 33g/ bird d and an additional 10g/ bird d of the given feed would have been necessary in order to meet the recommended methionine and lysine requirements, respectively.

However, Ross Breeders Ltd. (1995) recommend an energy intake of 1898kJ/ bird d at 31 weeks of age and 1777kJ/ bird d at 36 weeks of age. The later intake is lower than the energy value (1898kJ/ bird d), determined experimentally to give higher egg outputs than those presented by the breeding company. Therefore, is apparent that the energy intakes of broiler breeders should not drop off (if at all) in the post-peak period, as is recommended, in order to exploit laying potential.

When the experimental energy and methionine intakes were compared to the theoretical values over the post-peak period, the differences were negligible. The equivalent of 6g/ bird d, and approximately 2g/ bird d, less feed was offered than was

calculated, to meet the energy, and methionine requirements, respectively. In contrast, egg output was improved by feeding 17g/ bird d less than what was calculated to meet the requirement for lysine. Therefore, it is evident that the theoretical values estimating both energy and methionine intakes, are closer to those determined experimentally, compared to those recommended by Ross Breeders Ltd. (1995) However, the disparities between the experimental, theoretical and recommended lysine intakes, indicates that further research is essential.

It should be emphasised that the observed responses in egg output, egg weight, egg production and body weight change were attributable to both energy and amino acid intakes. Pearson and Herron (1982) reported linear responses in egg output and body weight gain to increasing energy concentrations, which were not influenced by protein intake. However, the lysine, methionine and energy intakes in this experiment are highly correlated due to the nature of the nutritional treatments imposed on the birds. As a result, there is ambiguity as to whether the effects can be confidently attributed to either energy or protein intake. Therefore, an experiment in which energy and protein were made to vary independently during the latter part of the laying period, would be of particular value.

It is interesting to note how the hens partitioned nutrient intake between body weight, change in body weight and egg output. Table 3.23 details the multiple regression equations during two time periods after peak production. The coefficient for body weight gain probably represents a combination of an increase in body lipid content and ovary weight, and would appear to be a factor worth considering when attempting to estimate the requirements of broiler breeder hens after peak production. However, an increase in ovary weight is generally associated with multiple ovulations and consequently, internal laying and double-yolked eggs (Hocking, 1983).

TABLE 3.23

Multiple regression analyses of energy intake (kJ/ bird day) and methionine and lysine intake (mg/ bird day) on body weight (g), change in body weight (g/ bird day) and egg output (g/ bird day) at 30-32 weeks and 34-36 weeks of age, respectively

Age (weeks)	Response variate	Regression coefficients		
		Body weight (b_1)	Change in body weight (b_2)	Egg output (b_3)
30-32	Energy intake (kJ/ bird d)	451.38 ± 12.77	8.02 ± 1.11	7.31 ± 0.81
	Methionine intake (mg/ bird d)	96.70 ± 3.52	3.32 ± 0.31	1.38 ± 0.22
	Lysine intake (mg/ bird d)	240.13 ± 6.73	3.64 ± 0.59	3.96 ± 0.43
34-36	Energy intake (kJ/ bird d)	406.61 ± 10.16	3.89 ± 1.10	8.24 ± 0.68
	Methionine intake (mg/ bird d)	97.08 ± 2.72	1.87 ± 0.30	1.09 ± 0.18
	Lysine intake (mg/ bird d)	224.40 ± 5.32	2.59 ± 5.77	4.14 ± 0.35

Multiple regression equations $P < 0.05$

CHAPTER 4

THE INFLUENCE OF BROILER BREEDER AGE, OR EGG SIZE, ON PRE- AND POST-NATAL GROWTH OF BROILERS

4.1 INTRODUCTION

In rearing broilers to market age, the main objective lies in attempting to exploit their genetically pre-determined potential growth rates. However, the achievement of potential growth is hampered by environmental, nutritional and social factors. High temperatures, limited macronutrients or deficiencies in micronutrients and competition for food, often result in diminished growth rates. However, restriction of potential growth begins even before the broilers are hatched, during pre-natal development. The degree of restriction depends on several factors including egg nutrient levels, egg environment, and egg size, all of which are influenced by parental flock age.

Wiley (1950) reported that embryonic development during the later stages of incubation may be restricted within small eggs laid by young broiler breeders, by the limited space within the eggshell. Bray and Iton (1962) termed the effect of initial egg weight on embryo weight, 'a temporary environmental influence which begins after 11 days of incubation', while correlations of near zero have been reported between egg weight and embryo weight up to 14 days of incubation (Hassan and Nordskog, 1971; Al-Murrani, 1978). However, there was no indication in the literature of an attempt to fit growth models to the embryo weights throughout the incubation period in order to identify differences in growth patterns between embryos from differently sized eggs. Moreover, rapid genetic selection for broiler growth over the past two decades has occurred without a concomitant alteration in egg size with age of the parent stock. Therefore, it is expected that egg environment and egg size will have a far greater influence on embryonic growth in the present day.

Growth restriction of embryos within small eggs has been attributed to the protein content of the egg (Al-Murrani, 1978), the decreased oxygen availability (Tullet and Deeming, 1982), the inefficient use of egg nutrients (Shanawany, 1984),

disturbances in the mechanisms of embryonic metabolism (Noble and Connor, 1987), and the degree of yolk-sac utilisation (Wilson, 1991). Tullet and Burton (1982) claimed that the variation in chick weight at hatch was not only accounted for by the size of the egg, but was explained by water loss during incubation. Thus, when chicks are incubated in a moist, rather than a dry environment, the chicks are heavier at hatch.

Since pre-incubation egg weight has a profound effect on the weight of the hatchling, the potential effect of egg weight on subsequent growth becomes a practical and economically important concern. Previous research has shown that rearing small chicks could be profitable if grown separately (Morris *et al.*, 1968; Hearn, 1986). However, there was no evidence in the literature of an attempt to impose different nutritional treatments on chicks of different weight in order to assess the growth responses. It is possible that chicks of different sizes have disparate nutritional requirements for maintenance and growth, and that these needs are unable to be met by using a single compounded ration (Emmans, 1978). Belyavin (1993) has suggested that each chick can theoretically choose a blend between two feeds which, in some proportion, meet its unique requirements for protein, and possibly other nutrients. Indeed, research has shown that when offered a choice of low and high protein feeds, broilers were able to grow at a rate not significantly different to broilers given the optimum diets, by making an appropriate choice from the two feeds (Shariatmadari and Forbes, 1990a; b). Further restriction to growth after hatching has been attributed to the yolk reservoir (Daly and Peterson, 1990). A heavier yolk residual at hatch results in heavier chicks at older ages (Kulka and Duskin, 1964).

The objectives of the present study were (1) to investigate the effect of initial egg weight (parental flock age) on embryonic weight, chemical composition, water loss and yolk absorption throughout the incubation period, and (2) to investigate the effect of initial egg weight on post-hatch growth of broilers offered one of two diets differing in protein, or amino acid content, or a choice between the two.

4.2 MATERIALS AND METHODS

Experiment 1

Experiment 1 was conducted to determine the effect of flock age (egg size) on water loss, embryonic growth and chemical composition. The eggs were obtained from three Ross broiler breeder flocks which were reared on a restricted diet. The flocks, of ages 30, 35 and 52 weeks respectively, were kept under the normal restriction programmes for broiler breeders. Two trays per age group were randomly allocated to the mid-section of a trolley and set at a dry bulb temperature of 37.8C and a wet bulb temperature of 30C for 18 days. The trays rotated 90° about a vertical axis every hour. On the 18th day of incubation, the eggs were transferred to the hatcher (dry-bulb temperature 37.5C, relative humidity 65%)

Embryo weights

Three trays, each containing 96 eggs were assigned to the three flock ages respectively. The eggs were numbered and set under the above mentioned incubator conditions. On the fourth and fifth days of incubation, four and six eggs respectively, from each flock age, were randomly selected and the embryos killed by means of carbon dioxide gassing of the eggs in a closed chamber. From the sixth day of incubation, ten eggs from each of the three flock ages were selected and gassed. The eggs were cracked open and the embryos dissected out by removing the yolk-sacs and associated embryonic membranes. Embryos were blotted dry and weighed to four decimal places.

Water loss

One tray, containing 96 eggs, was assigned to each of the three flock ages. The eggs where numbered, then weighed initially to obtain the mean egg weights before setting. The eggs were weighed individually every second day using a two-place balance until the 20th day, once the eggs had been transferred to the hatcher. Weight loss from the eggs over the incubation period was assumed to be water loss.

Laboratory analysis

After weighing, the embryos were subjected to carcass analysis to determine the changes in chemical composition during embryonic development. Because of the small size of the embryos, all embryos from each flock age, at the stipulated time periods, were grouped together for analysis. Composition analysis commenced from the tenth day of incubation. Body water content was determined by freeze drying the samples. Lipid content of the dry matter was subsequently analysed using the Soxhlett fat-extraction method. Body protein was determined on a water- and fat-free basis in a LECO Nitrogen analyser as N x 6.25. The body ash content was determined using fat-free, water-free samples by ashing in a furnace at 500C. All procedures conformed to those specified by the Association of Official Analytical Chemists (AOAC, 1975).

Statistical Analyses

Analysis of variance (GLM) was performed on the embryo weight data to determine whether significant differences ($P<0.05$) existed between the flock ages over the incubation period (Minitab Rel. 10.5Xtra, 1995). T-tests for unequal samples were then carried out on embryo weights at each weighing at the one percent level of significance to detect specific parental flock age differences. The embryo weights were transformed (log (weight + 1)) in order to stabilise the variation in weight over the incubation period, before fitting the growth trends. By means of the fitcurve procedure of Genstat 5 Rel. 3.2 (Genstat 5, 1995), the log transformed data was fitted to the exponential model:

$$Y = A + BR^x$$

where A = constant, B = linear coefficient (rate of increase), R = coefficient. BR^x = combined rate of growth. X = time of incubation and Y = embryo weight (g) using the three flock ages as factors. To establish whether the curves differed between the treatments, the A, the B and then the R parameters were determined for each of the treatments in a stepwise procedure. The change in variance was accepted as the statistical indicator at each step.

Linear regression analysis was applied to the egg weight data for each flock

age over time to determine whether parental flock age had a significant effect on weight loss.

The chemical components of the carcasses were regressed over time to detect significant age x time differences and significant ($P< 0.05$) linear and curvilinear component correlations. Allometric relationships between components were determined by regression using the general equation :

$$\ln C = a + b \ln BP$$

where lnC = natural logarithm (log) of the component weight (g), a = natural log of the constant term, b = regression coefficient (slope), lnBP = natural log of protein weight (g). Body protein was used as the independent variable and body water, body ash and body lipid, respectively, as the dependent variables.

Experiment 2

Experiment 2 was conducted in order to determine the effect of initial egg weight (flock age) on embryonic growth, water loss from the egg and yolk absorption during the pre-natal period, and on subsequent performance during the post-natal period.

Pre-natal phase

The eggs obtained from three Ross 788 flocks aged 24, 31 and 54 weeks were graded, and then weighed individually, to sort the eggs into four discrete and non-overlapping weight categories. The categories, each consisting of 396 eggs or rather, three trays of 132 eggs, respectively, were as follows: small (40.5-49.5g); medium (50.5-59.5g); large (60.5-69.5g) and jumbo (70.5-79.5g). Large to jumbo sized eggs laid by the young flock (double-yolked eggs), misshapen eggs and eggs with hairline cracks were rejected. The 12 trays were randomly allocated to the mid-section of a

trolley. The eggs were pre-heated until the egg core-temperature was 35C and then placed in a multistage Buckeye setter. Dry-bulb temperature ranged between 37.8C in the first zone and 37.5C in the fourth zone. Wet-bulb temperature was maintained at 28.5C. The trays rotated 90^0 about a vertical axis every hour. The eggs were transferred to the hatcher (dry-bulb temperature 36.7C, relative humidity 72.1%) on the 19^{th} day of incubation.

Embryo weights. One tray of eggs per weight category was numbered and the eggs in each tray were weighed individually. From the third to the 20^{th} day of incubation, six eggs per treatment were randomly selected, weighed, and the embryos killed by placing the eggs in an environment too cold to sustain embryonic metabolism. The eggs were cracked open and the embryos dissected out by removing the yolk-sacs and associated embryonic membranes. Embryos were blotted dry and weighed to four decimal places. From the 15^{th} day of incubation, it was possible to dissect out the yolk-sacs, blot them dry and weigh them to two decimal places, since the embryonic blood supply had completely surrounded the sac, thereby providing strength to the usually weak yolk-sac walls.

Water loss. Two trays per treatment were used to estimate mean water loss from the eggs during incubation. Initially, the trays were weighed empty and full in order to calculate mean egg weight for a particular tray. On a daily basis, and up to the 20^{th} day of incubation, the full trays were weighed to four decimal places and egg weight calculated.

Statistical analyses. Embryo weight and embryo weight as a proportion of initial egg size, were analysed using a general linear model to determine whether significant differences ($P<0.05$) existed between egg weight categories from the third to 21^{st} day of incubation (Minitab Rel. 10.5Xtra, 1995). T-tests for unequal samples were then conducted on the embryo weight data to identify the times during incubation when the treatment means became significantly different from one another. Exponential models were fitted to the log transformed (log (y+1)) embryo weights and proportions over the incubation period as in experiment 1.

In order to simultaneously establish the differences in growth over time and the points during the incubation period where the treatments became significantly different from one another, an attempt was made to fit an exponential broken stick model to the log transformed embryo weight data. However, the model assumed that responses between the treatments up to the estimated break point were similar. It was also not possible to establish more than one break point within the model. In order to do this, the data for each of the four treatments would have to have been fitted separately. A loss in information would have resulted and more importantly, it would not have been possible to compare break points between treatments, which was the original objective.

In addition, an attempt was made to fit the log transformed embryo proportion data to the exponential model, using pre-incubation egg weight as a covariant. However, it was not possible to include the covariant in the model since the fitcurve directive accepted the inclusion of one variate, in this case time, only. Therefore, the model had to be abandoned.

Relative growth rate was calculated as the change in embryo weight per day over the geometric mean ($e^{0.5 \times \ln wt(t1) + 0.5 \times \ln wt(t2)}$). An exponential model was then fitted to the data using ln embryo weight as the x-variate. The relative growth rates during the first three days were excluded from the model due to their high influence and variance. To establish whether the curves differed between the treatments, the A, the B and then the R parameters were determined for each of the treatments in a stepwise procedure. The change in variance was accepted as the statistical indicator at each step.

Linear regression was applied to the egg weight data using the initial egg weight categories as dummy variables to detect whether the rates of water loss over the incubation period differed significantly one from the other.

The general linear model was applied to the yolk-sac weight and the proportion of yolk-sac weight to body weight data, to identify significant differences ($P<0.05$) between the egg category means for each day of incubation. The two variables were also regressed over time to identify statistically different rates of resorption between the four egg weight categories.

Post-natal phase

Birds and management. After hatching, 180 viable chicks per weight category were placed in four electrically heated five-tier battery brooders. Broilers were housed in the middle three tiers of the brooders in groups of 15. Each tier consisted of four pens that were provided with both water and feed troughs attached to the exterior of the pen. Two smaller troughs, instead of one, were used when the chicks were offered a choice of feeds. The position of the troughs used for choice feeding were randomised over the egg weight category treatments to prevent selection according to the relation of the trough with the heater or water supply. Temperature was maintained at a comfortable level and ventilation was provided by a fan. The lighting pattern provided the chicks with 23L:1D over the experimental period of three weeks.

Treatments and feeds. Chicks hatched from the eggs in the four weight categories were offered one of two feeds differing in protein level, or a choice between the two. Therefore, the trial consisted of 4 x 3 treatments replicated four times and randomised within each brooder or block. A high (H) and a low (L) protein feed were formulated (Table 4.1) to be iso-energetic. The amino acid content of H was 1.6 times that of L. The two diets contained equal concentrations of canola seed meal to prevent selection for, or against, this protein source. The feeds were analysed for AME, crude protein, amino acid, calcium and phosphorus content.

TABLE 4.1

Composition (g/kg) of the high protein (H) and low protein (L) feeds

Ingredient	H	L
Maize meal	184.45	304.87
Soyabean oilcake 48	361.29	184.20
Canola seed meal	170.00	170.00
Fish meal 65	58.00	29.63
Sunflower husks	116.34	179.66
Sand	29.75	45.96
L-lysine HCL	1.33	0.68
DL methionine	1.56	1.14
Vitamin and mineral premix[1]	2.50	2.50
Limestone	10.62	13.40
Monocalcium phosphate	11.60	14.59
Salt	2.55	3.36
Sunflower oil	50.00	50.00

Analysis	Calculated	Actual	Calculated	Actual
AME (MJ/kg)	13.00	13.46	13.00	12.70
Protein	280.0	316.0	190.0	192.7
Lysine	18.0	23.1	11.4	13.4
Methionine	6.0	5.8	4.1	3.5
Methionine + cystine	10.3	-	7.2	-
Threonine	10.9	11.0	7.2	7.2
Tryptophan	3.5	-	2.2	-
Arginine	18.3	20.3	11.6	10.9
Histidine	7.9	8.1	5.3	5.2
Isoleucine	12.3	14.8	8.1	8.6
Leucine	20.9	23.3	14.6	15.5
Phe + Tyr	21.9	22.7	14.4	13.5
Valine	13.8	17.4	9.4	10.4
Calcium	10.0	12.8	10.0	12.2
Phosphorus	5.0	5.3	5.0	5.2
Sodium	1.8	-	1.8	-

[1]*Vitamin premix provided/kg diet*: 35mg thiamin, 23mg riboflavin, 8mg pyridoxine, 0.8mg biotin, 5.7mg pteroylmonoglutamic acid, 7mg menaphthone, 30μg cyanocobalamin, 213mg nicotinic acid, 354 ascorbic acid, 212.7μg retinol, 10.2mg cholecalciferol (2000μg/g), 6mg $\propto$-tocopherol (250mg/g), 125mg maize starch.
Mineral premix provided/kg diet: 479 mg KH_2PO_4, 365mg NaCl, 23mg ferric citrate, 9mg $MgSO_4.H_2O$, 0.46mg KI, 0.58mg $CuSO_4$, 9mg $ZnCo_3$, 0.46mg $Na_2MoO_4.H_2O$

Allocation of feeds. The chicks were allowed *ad libitum* access to the feeds.

Measurements. Body weight was recorded at the beginning of the trial, and at the end of each week, on a pen basis.

Relative growth rate was calculated by dividing the change in body weight by the geometric mean body weight for each week.

Food intake was recorded by subtracting the weight of the bucket containing the feed at the end of the week from the weight of full bucket at the beginning of the week. When chicks were removed for carcass analysis within the week, a record was made of food intake according to the number of existing chicks on that day.

Food conversion efficiency (FCE) was calculated by dividing the change in body weight by the amount of food consumed for each week of the post-natal phase.

After hatching, four chicks from each weight category, and on eight other occasions, four chicks from each treatment were randomly selected, killed by cervical dislocation and their yolk-sacs dissected out and weighed. All four chicks per treatment were then minced together to form an homogenous blend. A sample was then subjected to chemical analysis. Body water content was determined by freeze drying the samples. The procedure conformed to that specified by the Association of Official Analytical Chemists (AOAC, 1975). The body protein and body lipid contents of the chicks were not analysed due to a backlog at the University laboratory.

Statistical analyses. The design of the experiment was a 4x3 randomised block design. Variables were analysed using the general linear model to identify significant differences ($P<0.05$) within the two treatments and to identify significant interactions between chick size (egg weight category) and dietary treatment (Minitab Rel. 10.5Xtra, 1995). T-tests for unequal samples were used to detect significant differences between means within a particular main effect.

Body water contents were regressed against time using the egg size categories and feed treatments as dummy variables, respectively.

Relative growth rates were regressed against ln body weight using the egg weight categories as dummy variables.

Yolk-sac weight data were regressed over time, using dummy variables to

identify different rates of resorption.

One-sample t-test computations were performed on the mean proportion of pre-starter selected in relation to total food intake for each week of the trial and for each weight category. The hypothesis tested was that the mean proportion chosen differed significantly ($P<0.05$) from 0.5.

4.3 RESULTS AND DISCUSSION

Experiment 1

The mean initial egg weights within each category were as follows: 30 week old flock 59.87g ± 0.38g; 35 week old flock 65.12g ± 0.41g; 52 week old flock 69.87 ± 0.40. The mean egg weights within each category differed significantly from one another ($P<0.05$).

Embryo weights

The mean weights of the embryos, together with the standard errors, at the stipulated time periods, for the three parent flocks are given in Table 4.2 . Embryos from eggs laid by the 30 week old flock were significantly lighter than those laid by both the 35 and the 52 week old flocks from day five. Inconsistent differences were obtained between the 35 and 52 week old parent flocks. However, the embryos from the 52 week old flock were statistically heavier than those from the 35 week old flock during the final two days of incubation.

TABLE 4.2

Mean embryo weights (g) and standard errors of three flock ages during incubation

	Age of parent flock (weeks)		
Time (days)	30	35	52
4	0.02±0.003[a]	0.03±0.003[a]	0.04±0.004[a]
5	0.17±0.02[a]	0.22± 0.02[b]	0.20±0.02[b]
6	0.46±0.03[a]	0.52±0.03[b]	0.56±0.03[c]
8	1.34±0.05[a]	1.49±0.05[b]	1.45±0.03[b]
10	2.95±0.10[a]	3.27±0.08[b]	3.29±0.09[b]
12	6.68±0.18[a]	7.45±0.17[b]	7.04±0.18[c]
14	13.36±0.40[a]	14.78±0.36[b]	14.39±0.36[b]
16	19.32±0.40[a]	21.87±0.40[b]	22.23±0.50[b]
18	28.18±0.80[a]	31.88±0.74[b]	32.97±0.74[c]
20	45.84±1.32[a]	50.14±1.17[b]	52.09±1.17[c]

[a-c]Rows with superscripts in common are not significantly different (P<0.05)

The exponential parameter estimates of the log transformed embryo weights over the incubation period are given in Table 4.3. It is evident that the embryos from the younger flock maintained a slower rate of growth than the embryos from the older flocks. The exponential parameters of both the 35 and the 52 week old flocks were similar.

TABLE 4.3

Exponential parameter estimates of the log transformed embryo weights (g) for the three flock ages over the incubation period (days)

	Exponential parameters		
Age (weeks)	A	B	R
30	14.61±2.58	-16.01±2.50	0.980±0.004
35	17.92±4.74	-19.24±4.66	0.984±0.005
52	18.09±4.38	-19.44±4.31	0.984±0.004
R^2		0.99	

The highly significant restriction of embryonic growth in the 30 week old flock (Table 4.2) from the fifth day of incubation may be due to the physical constraint imposed by the smaller egg size. This is in accordance with the findings of Wiley (1950). It appears that the restriction of growth occurs earlier than previously estimated by Hassan and Nordskog (1971) and Bray and Iton (1962) who determined that egg weight exerts an effect on embryo weight from day 14 and day 11, respectively. The earlier restriction observed, may be explained by the fact that modern broilers grow significantly faster due to the intense selection for rapid growth rate that has taken place.

The considerable increase in growth rate in the period between the last two weighings (between 18 and 20 days of age) could be attributed to the weight of the yolk-sac, since it was not removed from the embryos prior to weighing once resorption was initiated.

It has been shown that growth restriction may be more closely associated with egg composition, rather than egg size *per se*. Eggs from younger flocks have a thicker shell and a lower pore concentration, as reflected in a lower porosity of the eggshell (Peebles and Brake, 1986; Peebles *et al.*, 1987) and a more viscous albumen (Meur and Bauman, 1988, cited by Vick *et al.*, 1993). These factors would lead to impaired

gaseous exchange between the interior of the egg and the external environment. In fact, Tullet and Deeming (1982) have demonstrated that embryonic oxygen consumption is proportionately related to eggshell porosity in the range of 8 to 18mg H_2O/mm Hg d. Consequently, the diminished supply of oxygen would lead to a decreased metabolic rate (Tullet and Deeming, 1982; Shanawany, 1984) that would restrict embryonic growth especially during the later stages of pre-natal development.

The embryo derives nutrition for growth and development throughout the incubation period via the yolk and the albumen (Romanoff, 1960). However, the relative distributions of several egg components have been shown to be related to the age of the parent bird. As hens age, the percent of yolk per egg increases as does the volume of albumen, while the percent of total solids (protein), and the concentrations of phosphorus and chlorine in the albumen decline (Cunningham 1959a; 1959b). It has been suggested (Shanawany, 1984; Noble *et al.*, 1986; Noble and Connor, 1987) that as birds age, they become more efficient in depositing all the nutrients essential for embryonic growth and that embryos from older flocks may utilise the nutrients in the egg more efficiently than embryos from younger flocks. Al-Murrani (1978) determined that the protein content of eggs was the main factor influencing later embryonic weight. Therefore, since the volume of yolk proteins increase with increasing egg size or flock age (Cunningham, 1959a), embryos from smaller eggs laid by younger flocks are expected to maintain limited weight gains commensurate with the available protein supply.

Water loss

The mean egg weights at the different stages of incubation, for the three flock ages are presented in Table 4.4. Significant, linear differences in weight loss from the eggs were observed with time and between the flock ages. The eggs from the young flock lost significantly ($P<0.05$) less weight on a daily basis, and as a proportion of initial egg weight, than the eggs from the other two groups, while weight loss exhibited by the 35 week old flock was greatest. The parameters estimating egg weight and hence weight loss are given in Table 4.5.

TABLE 4.4

Mean egg weight (g) and standard errors for three flock ages during incubation

	Age of parent flock (weeks)		
Time (days)	30	35	52
0	59.87 ± 0.38	65.12 ± 0.41	69.87 ± 0.40
2	59.08 ± 0.38	63.97 ± 0.41	68.78 ± 0.40
4	58.46 ± 0.37	63.18 ± 0.41	67.98 ± 0.40
6	57.81 ± 0.37	62.48 ± 0.42	67.15 ± 0.40
8	57.16 ± 0.37	61.56 ± 0.42	66.23 ± 0.41
10	56.54 ± 0.37	60.55 ± 0.45	65.54 ± 0.43
12	55.75 ± 0.37	59.73 ± 0.45	64.76 ± 0.44
14	55.08 ± 0.37	58.81 ± 0.47	63.98 ± 0.44
16	54.42 ± 0.37	57.99 ± 0.48	63.28 ± 0.43
18	53.67 ± 0.37	57.04 ± 0.49	62.24 ± 0.44
	Total water loss over the 18 day incubation period (g/g)		
	0.10	0.12	0.11

TABLE 4.5

Parameter estimates of the decrease in egg weight (g) with time(days) for three flock ages

Age of parent flock (weeks)	Constant (b_0)	Linear coefficient (b_1)	R^2
30	60.521 ± 0.278^a	-0.6793 ± 0.0449^a	
35	65.897 ± 0.279^b	-0.8824 ± 0.0453^b	0.55
52	70.480 ± 0.279^c	-0.8180 ± 0.451^c	

[a-c]Regression coefficients within columns, with superscripts in common are not significantly different ($P<0.05$)

The total weight loss of the egg is equivalent to the loss of water from the egg, since the respiratory exchange of the embryo (respiratory quotient = 0.727) involves no change in mass (Drent, 1975; Rahn and Paganelli, 1991). The relationship between dry and wet bulb incubation temperatures determines the external humidity; and because the relative humidity within the egg is essentially 100 percent, the water-vapour pressure gradient has a great influence on water loss (Swann and Brake, 1990), as does the conductance of the shell to water vapour (Tullet, 1990; Vick *et al.*, 1993).

Tullet and Burton (1982) claimed that the variation in chick weight at hatch was not only accounted for by the size of the egg, but was explained by the water loss during incubation. Thus when eggs were incubated in a moist rather than a dry environment, the chicks were heavier at hatch and had a higher water : dry matter ratio. The results of the present experiment showed that even when eggs were incubated in identical conditions, differences in weight or water loss arose as a result of the age of the parent flock. Therefore, it is apparent that the authors did not consider age effects on eggshell, and egg composition, on conductance of the egg to water vapour.

The lower porosity of the eggs of young flocks (Peebles and Brake, 1986) as well as the thicker shell membrane (Britton, 1977), cuticle (Peebles *et al.*, 1987) and more viscous albumen (Meur and Bauman, 1988, cited by Vick *et al.*, 1993) pose as significant barriers to water vapour diffusion. As a result of the low water vapour conductance, growth of the embryo is limited, and its biochemical pathways are slowed down (Burton and Tullet, 1983; 1985). It is thought that the wetting of the shell membranes blocks embryonic oxygen uptake (Ar and Rahn, 1980) and restricts metabolism. Orlov (1961), cited by Rol'nik (1970) speculated that growth could be negatively affected if the majority of the water in the allantois was not eliminated after closure of the allantois at the small end of the egg after 11 days of incubation. This is because the excess water could hold up the flow of water that carries the supply of nutrients from the yolk and albumen to the embryo. However, the theory is not born out of experimental data.

Eggshell membranes and cuticles within large eggs are thinner at the end of the laying cycle (Britton, 1977; Peebles and Brake, 1987) and there is an increase in the thin outer layer of the albumen with a corresponding decrease in the thick outer layer

as a flock ages (Romanoff and Romanoff, 1949). These facts help to explain the greater loss of water from the large, compared to the small eggs laid by the young flock. The eggs laid by the 52 week old flock lost a slightly lower proportion of initial egg weight compared to the proportion of water lost by the eggs laid by the 35 week old flock. This is because large eggs produced by ageing broiler breeders have a lower eggshell water-vapour conductance, or porosity, compared to eggs produced in the mid-lay period, since egg production relative to daily calcium intake is lower (Peebles and Brake, 1986; 1987). However, the water loss of the eggs laid by the 35 week old flock was closest to the value of 0.12, recommended by Lundy (1969), to ensure optimal embryonic development and survivability.

Since the embryo *per se* appears to have a limited ability to compensate for low water loss (Swann and Brake, 1990), cuticle removal, manipulation of the incubator humidity, or both, may be practical methods to promote embryonic development within small eggs from young flocks. Removal of the thick cuticle using sodium hypochlorite (Peebles and Brake, 1987) increases water loss. However, incubator hygiene would need to be improved when adopting this procedure to prevent bacterial infection (Tullet, 1990). In addition, it has been shown (Vick *et al.*, 1993) that a lower wet-bulb temperature is able to overcome the resistance of the thicker cuticle-eggshell-membrane complex, combined with the thicker albumen of young flocks, to water loss (Peebles and Brake, 1987).

However, embryos in eggs larger than 65g do not benefit from, and could possibly be damaged by lower incubation wet-bulb temperatures because of their lower initial albumen and eggshell quality (Vick *et al.*, 1993). Therefore, constant incubation conditions throughout the laying cycle are not appropriate. It would appear that until eggs from different flock ages are incubated separately according to their specific wet-bulb temperature needs, to exact optimum water loss, water loss as a factor influencing embryonic growth should not be ignored.

Chemical composition

The change in the proportion of body lipid (BL), body protein (BP), body water (BW) and body ash (BA) over time (Appendix Table 4), reveals that chemical growth does not follow the same growth pattern exhibited by the embryo as a whole

(Fig. 4.1). The chemical composition of the embryo has a temporal sequence, with the amount of water in the developing embryo changing reciprocally with the changes in the amount of solids (Romanoff, 1967).

No significant ($P<0.05$) differences in mean water content were observed between the three flocks over the incubation period. Mean body water maintained a significant ($P<0.05$), negative, linear relationship with age of the embryo.

The mean protein concentrations of the embryos over the incubation period were not significantly influenced by flock age. Body proteins, which comprise the largest proportion of the embryo solids, increased linearly to 143 g/kg at the 20th day of incubation (Fig. 4.1). This is in contrast to findings of Romanoff (1967) who noted a final body protein weight of 100g/kg. However, with current intense selection for rapid protein growth in broilers, this difference is to be expected.

The concentration of lipid increased in a quadratic ($P<0.05$) manner with the growth of the embryo (Fig. 4.1). It has been suggested that the rapid gain in lipid is required for the maintenance of energy during the highly active period just before the emergence of the chick from the shell (Romanoff, 1967). Although the proportion of body lipid in the embryos from the 30 week old flock was continuously lower than that of the two older flocks, the differences over time were not statistically significant. Since the yolk-sacs of the embryos were not removed prior to chemical analyses, once resorption was initiated, non-significant differences in lipid concentration during the later part of the incubation period were expected. This is because Noble *et al.* (1986) determined that a lower proportion of total yolk lipids were associated with the embryos from a young flock, while a greater proportion of the lipid was associated with the yolk content, compared to the distribution of lipid between embryos and yolk sacs in ageing flocks.

Body ash, and hence embryonic skeletal growth (Romanoff, 1967), increased in a linear manner ($P<0.05$) over time, and was not influenced by parental flock age.

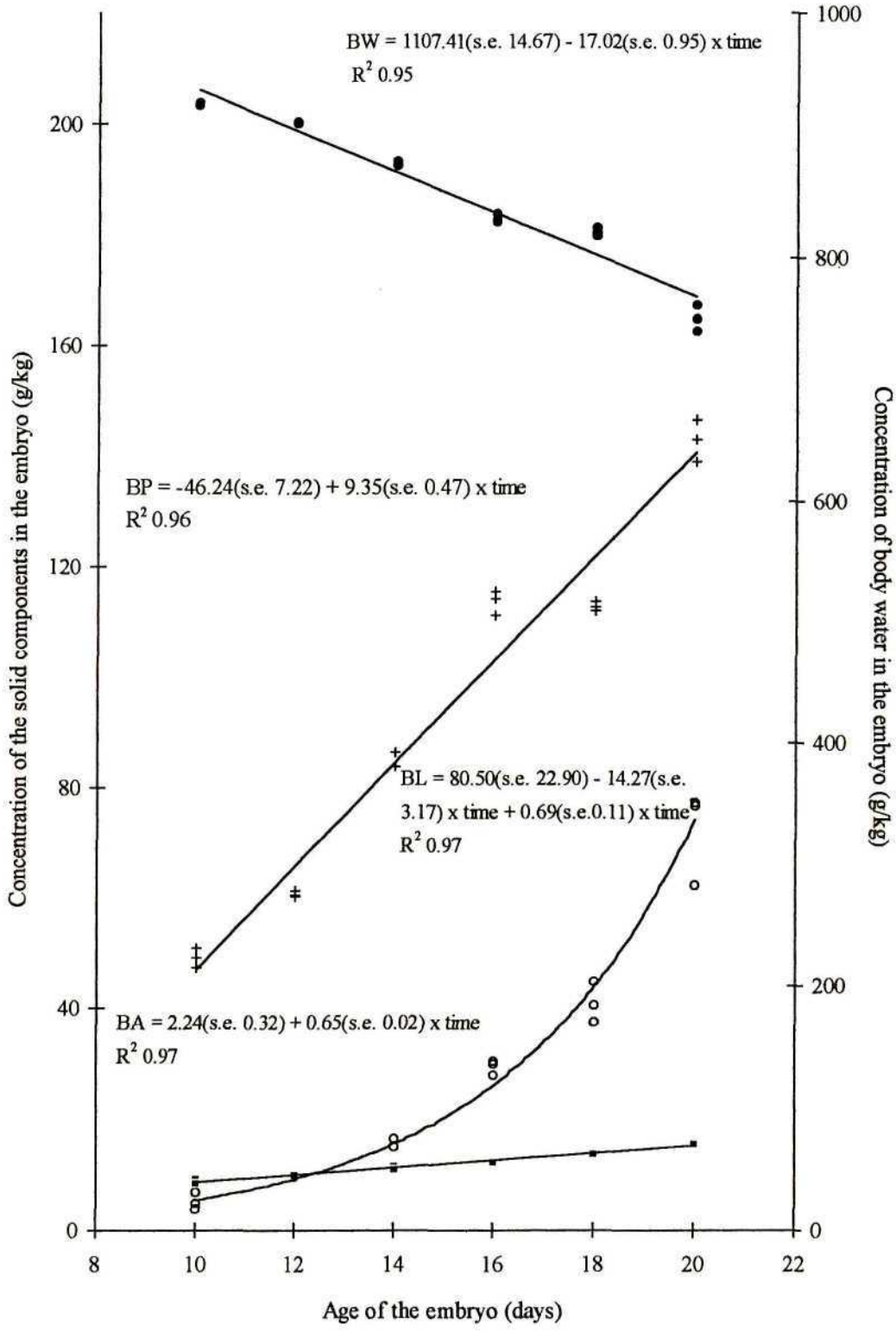

FIG. 4.1 —*The change in the concentration of body water (•), body protein (+), body lipid (○) and body ash (▪) with age of the embryo*

The reciprocal relationships between water and solids are demonstrated by the significant ($P<0.05$), negative correlations between the solids (BL and BA) and body water content (Table 4.6).

The increase in the proportion of solids is reflected in the significant ($P<0.05$), positive, linear correlation between body protein and body ash concentration. The positive, curvilinear correlations between body protein and body lipid content, and between body ash and body lipid content, emphasises the fact that protein and ash growth occurs at a proportionally slower rate than lipid growth.

TABLE 4.6

Regression analyses defining the relationships between body lipid (BL), body water (BW), body ash (BA) and body protein (BP) contents (g/kg)

	Constant (b_0)	Linear coefficient (b_1)	Quadratic coefficient (b_2)	R^2
BL vs BW	1149.60 ± 146.40	-2.29 ± 0.35	0.001 ± 0.00	0.99
BA vs BW	43.17 ± 1.65	- 0.04 ± 0.002	-	0.96
BP vs BW	-515.70 ± 129.30	2.02 ± 0.31	-0.002 ± 0.00	1.00
BP vs BA	-75.73 ± 10.42	14.19 ± 0.86	-	0.95
BP vs BL	37.66 ± 4.12	2.90 ± 0.28	-0.02 ± 0.00	0.96
BA vs BL	8.28 ± 0.24	0.18 ± 0.02	-0.001 ± 0.00	0.97

The allometric relationships between ln body protein, the independent variable, and ln body lipid, ln body ash and ln body water respectively, were significant (Table 4.7). As ln body protein increased, ln body lipid increased at a proportionately greater rate ($b = 1.4033 \pm 0.031$). ln body water and ln body ash increased relatively more slowly. From these relationships, it is possible to estimate the body weight of an embryo given the amount of body protein (g) at any stage of development. The relationship between ln body protein and ln body lipid is likely to be influenced by breed and parental flock nutrition, whereas those between ln body protein and either ln

body water or ln body ash would be likely to remain constant over breeds and other factors.

TABLE 4.7

Allometric relationships of body composition (g)

	Constant (b_0)	Linear coefficient (b_1)	R^2
lnBA vs lnBP	- 1.98 ± 0.02	0.86 ± 0.01	0.99
lnBL vs lnBP	- 1.58 ± 0.04	1.40 ± 0.03	0.99
lnBW vs lnBP	2.36 ± 0.02	0.66 ± 0.01	0.99

Experiment 2

Pre-natal phase

The mean initial egg weights within each category were as follows: small 45.96g ± 1.72g; medium 56.39g ± 1.88g; large 65.86g ± 2.99g; jumbo 74.32g ± 2.03g. The mean egg weights within each category differed significantly from one another ($P<0.05$).

Embryo weight. The trend in embryonic growth over the incubation period is presented graphically (Fig. 4.2) and in Appendix Table 5. The vertical lines (Fig. 4.2) represent the points at which embryos from the different egg size categories became significantly ($P<0.05$) different from one another. The embryos dissected out from the small eggs were significantly lighter than those from the other egg size categories from the eighth day of incubation. Embryos in the large and jumbo categories, were significantly heavier than those in the medium egg weight category from the 10^{th} and 12^{th} day of development, respectively. After 16 days in the setter, a significant separation in embryo weight was observed between the two largest categories. The

differences between the four groups persisted until hatching. The 21 day embryo weights were as follows: small 31.69g ± 0.15g; medium 38.94g ± 0.15g; large 45.38g ± 0.15g; jumbo 51.77g ± 0.15g.

The parameter estimates of the maximal exponential model predicting log (embryo weight +1) for the four egg size categories over time are given in Table 4.8. The constant term A, and the R coefficient, increased with egg weight, while the B coefficient became increasingly negative with mean initial egg weight. The complete, significantly different ($P<0.05$) models for each of the treatments indicate that growth rate of embryos increased as the initial mean egg size increased.

TABLE 4.8

Exponential parameter estimates of the log transformed embryo weights (g) for the four egg size categories over the incubation period (days)

	Exponential parameters		
Egg weight	A	B	R
Small[1]	10.80 ± 1.90	-11.92 ± 1.83	0.976 ± 0.005
Medium[2]	13.22 ± 2.52	-14.29 ± 2.46	0.980 ± 0.004
Large[3]	15.45 ± 3.41	-16.54 ± 3.35	0.983 ± 0.004
Jumbo[4]	20.23 ± 5.92	-21.25 ± 5.86	0.987 ± 0.004
R^2		0.99	

[1]40.5-49.5g; [2]50.5-59.5g; [3]60.5-69.5g; [4]70.5-79.5g

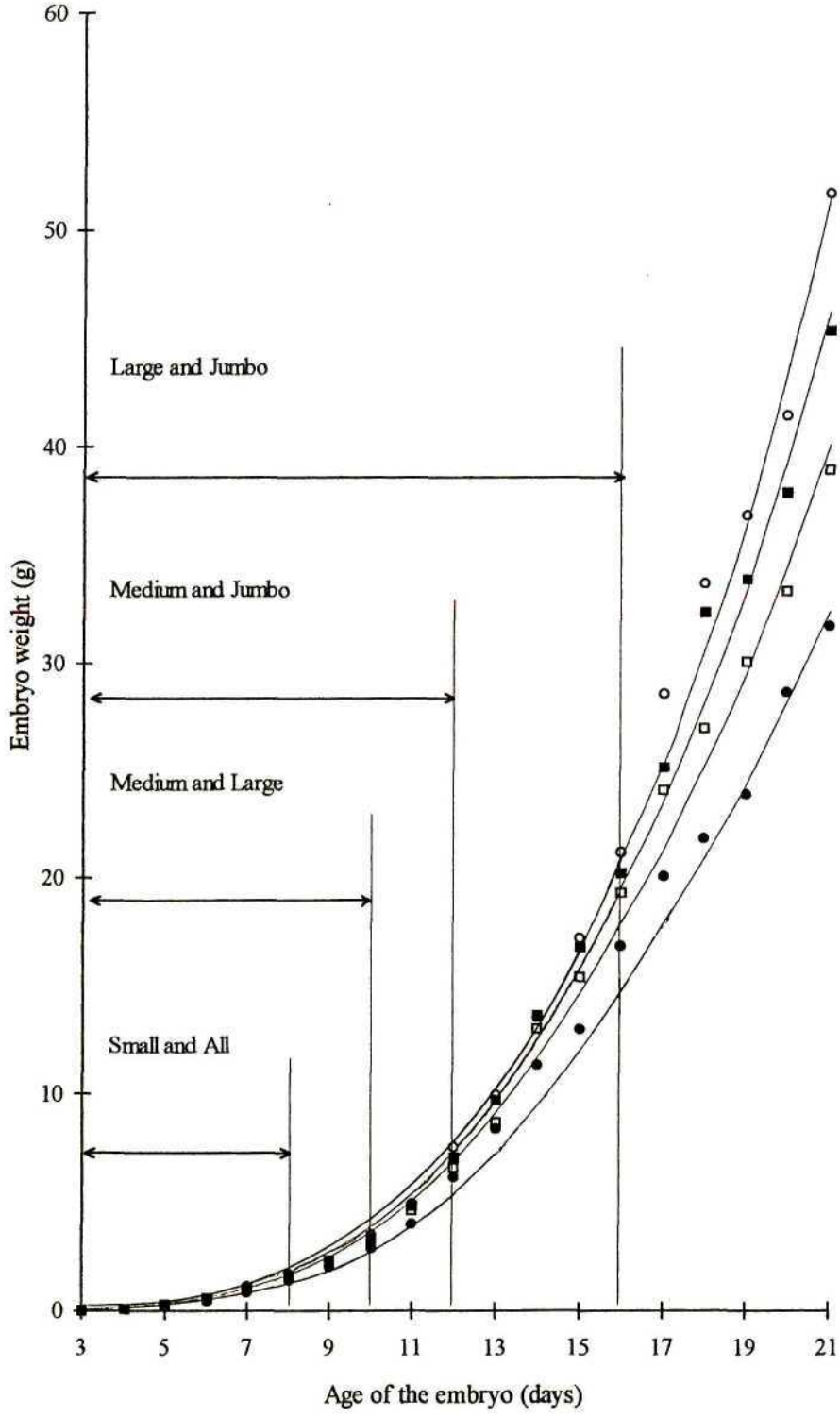

FIG. 4.2 —*Weights of embryos dissected out from small (•), medium (□), large (▲) and jumbo (○) sized eggs throughout the 21 day incubation period*

Embryo weight expressed as a proportion of initial egg weight maintained an exponential relationship with age of the embryo (Fig. 4.3). The means and standard errors are detailed in Appendix Table 6. In general, the embryos in the small category exhibited numerically higher body weights in relation to egg size compared to the embryos in the large and jumbo categories from day five. As egg size increased, so the proportion of embryo weight to egg weight decreased. However, this is a generalisation since there were occasions during incubation where the significant ($P<0.05$) differences in the embryo weight proportions were erratic between the egg weight categories.

Table 4.9 outlines the significant ($P<0.05$) exponential parameters describing the relationship between the log (Proportion of embryo weight to egg weight + 1) and time for the four egg weight categories. The A and R parameters increased significantly ($P<0.05$) with increasing mean egg size, while the B parameter decreased significantly ($P<0.05$) with egg weight category. The four statistically distinct models indicate that as initial egg weight increased, so the proportion of embryo weight (log + 1) to pre-incubation egg weight decreased in an exponential manner.

These findings seem to imply that embryonic growth is not simply influenced by the limited space within the eggshell, but that chick embryos grow at a rate commensurate with the available nutrient supply as was suggested by Shanawany (1984). If growth were dependent on egg size alone, the ratio of embryonic weight to initial egg weight would be equivalent over the range of egg sizes throughout the incubation period.

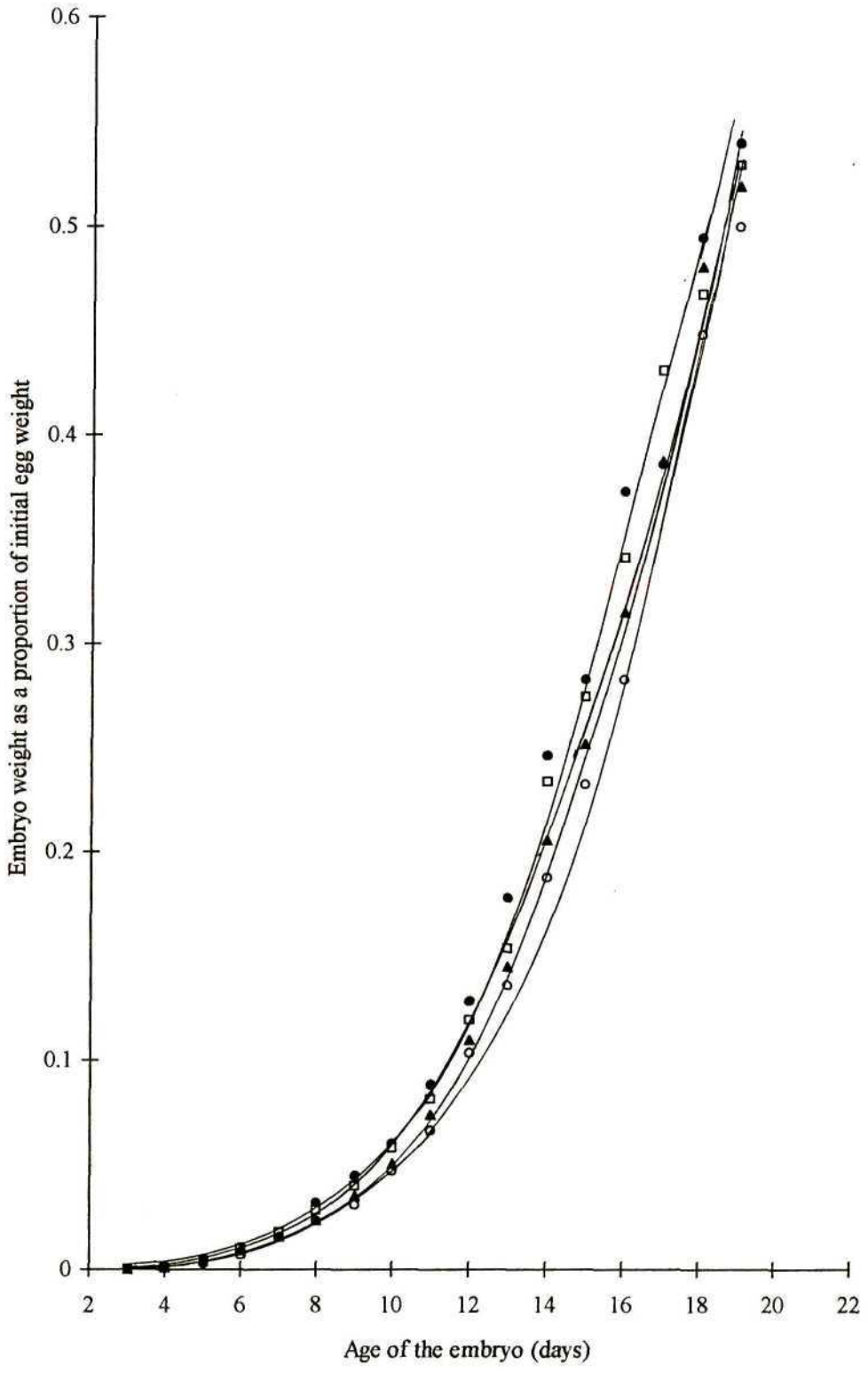

FIG. 4.3 —*Embryo weight expressed as a proportion of initial egg weight for the small (•), medium (□), large (▲) and jumbo (○) egg size categories over time*

TABLE 4.9

Exponential parameter estimates of the log transformed embryo weights (g) as a proportion of initial egg weight (g) for the four egg size categories over the incubation period (days)

	Exponential parameters		
Egg weight	A	B	R
Small[1]	-0.11 ± 0.02	0.06 ± 0.01	1.12 ± 0.008
Medium[2]	-0.11 ± 0.01	0.06 ± 0.01	1.12 ± 0.007
Large[3]	-0.09 ± 0.01	0.04 ± 0.01	1.14 ± 0.007
Jumbo[4]	-0.07 ± 0.01	0.03 ± 0.01	1.15 ± 0.008
R^2		0.98	

[1]40.5-49.5g; [2]50.5-59.5g; [3]60.5-69.5g; [4]70.5-79.5g

The exponential relationship between relative growth rate and ln embryo weight for the four egg weight categories is presented graphically (Fig. 4.4) and in Appendix Table 7. The constant terms differed significantly between egg weight categories. However, both the B and the R parameters were similar across the treatments (Table 4.10). Theoretically, the relationship between relative growth rate and ln body weight of broilers is negative and linear. During the initial stages of incubation, up to a weight (ln) of approximately 10g, relative growth rate does appear to decrease linearly as the embryos developed. However, towards the end of the incubation period, it is apparent that the embryos developed at an approximately constant rate relative to their body weights. This provides evidence to suggests that embryonic growth may be limited by the available nutrient or oxygen supply, or by the limited space within the egg shell. The increase in the constant term with an increase in pre-incubation egg weight, resulted in a concomitant horizontal shift in the individual response curves. As a result, the relative growth rates of the embryos within the smaller eggs were lower than those maintained by the embryos within the larger eggs for a given weight (ln).

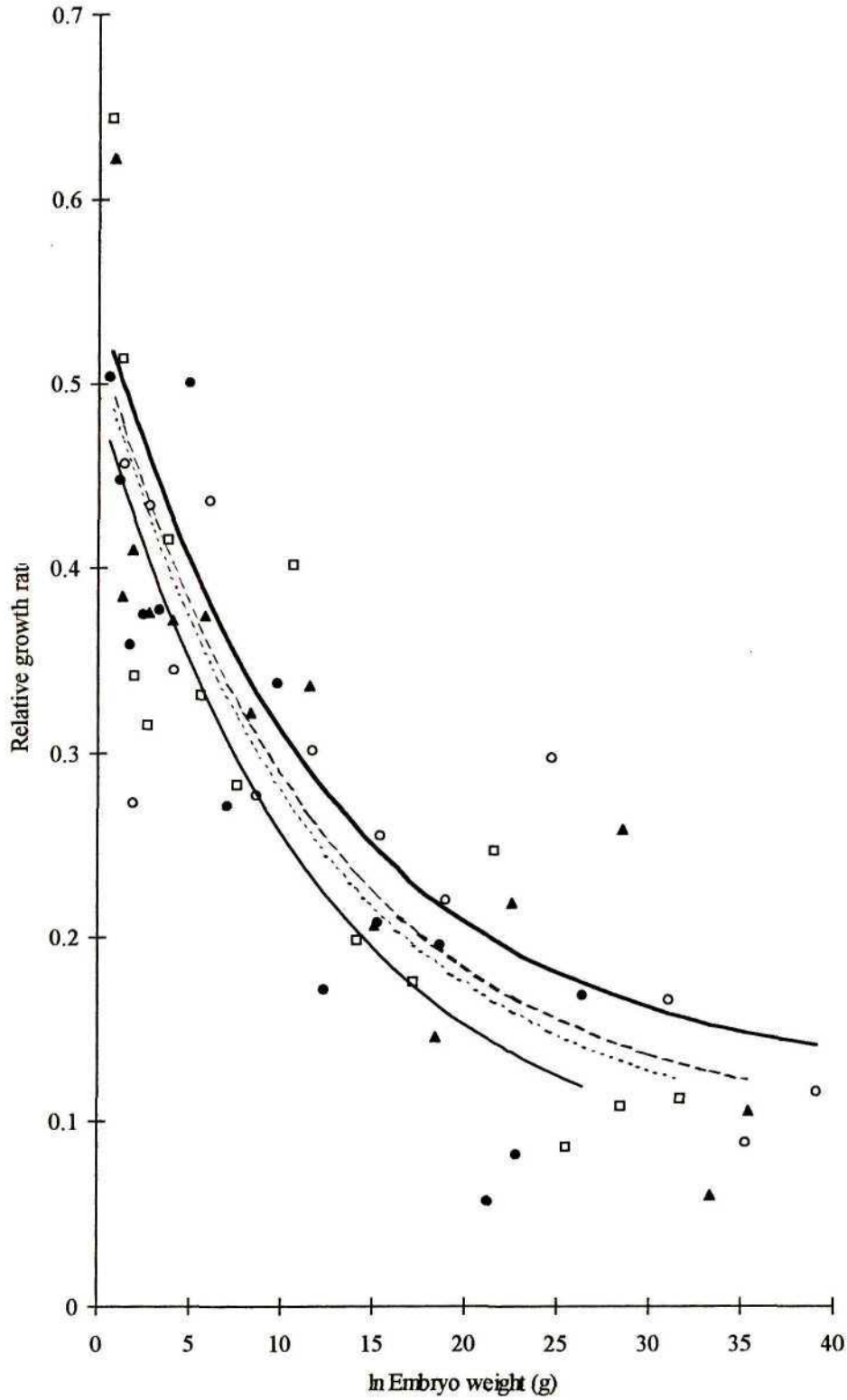

FIG. 4.4 —*Response in relative growth rate of the embryos within the small (—•—), medium (·····□·····), large (– –▲– –) and jumbo (—○—) egg size categories to ln embryo weight*

TABLE 4.10

Exponential parameters describing the relationship between relative growth rate and ln embryo weight (g) for the four egg size categories over the incubation period (days)

	Exponential parameters		
Egg weight	A	B	R
Small[1]	0.067 ± 0.04		
Medium[2]	0.089 ± 0.04	0.4205 ± 0.04	0.923 ± 0.02
Large[3]	0.097 ± 0.04		
Jumbo[4]	0.123 ± 0.04		
R^2		0.70	

[1]40.5-49.5g; [2]50.5-59.5g; [3]60.5-69.5g; [4]70.5-79.5g

Water loss. The linear decline in mean egg weight over time is illustrated in Fig. 4.5 and Appendix Table 8. The negative slopes (Fig. 4.5) were significantly ($P<0.05$) different from each other, indicating that as the initial egg weights increased, so the rate of decline in egg weight, specifically, water loss, increased. The observation that the small eggs lost significantly less weight over time than eggs within the other categories is consistent with the findings of experiment 1. However, the trend of increasing water loss with increasing egg size is contrary to the results obtained when eggs were segregated according to flock age. This can be attributed to the differences in incubation conditions. Although the eggs within the four egg weight categories lost significantly different amounts of water over time, the eggs lost similar volumes of water in relation to pre-incubation egg weight. However, Lundy (1969) has shown that optimum water loss is 0.12 of initial egg weight. Therefore, it appears that the embryos within the eggs of different sizes were water stressed, but were able to compensate for the high water loss conditions within the incubator to a similar extent, by osmoregulating the available water supply. It has been determined that embryos subjected to conditions of higher water loss, are able to conserve water by actively absorbing water from the albumen during the early stages of incubation (Simkiss, 1980), and from the allantois during the middle and later stages of embryonation (Hoyt, 1979) without affecting development. Therefore, of greater concern is the inhibition of growth of embryos from small eggs incubated in conditions of low water loss.

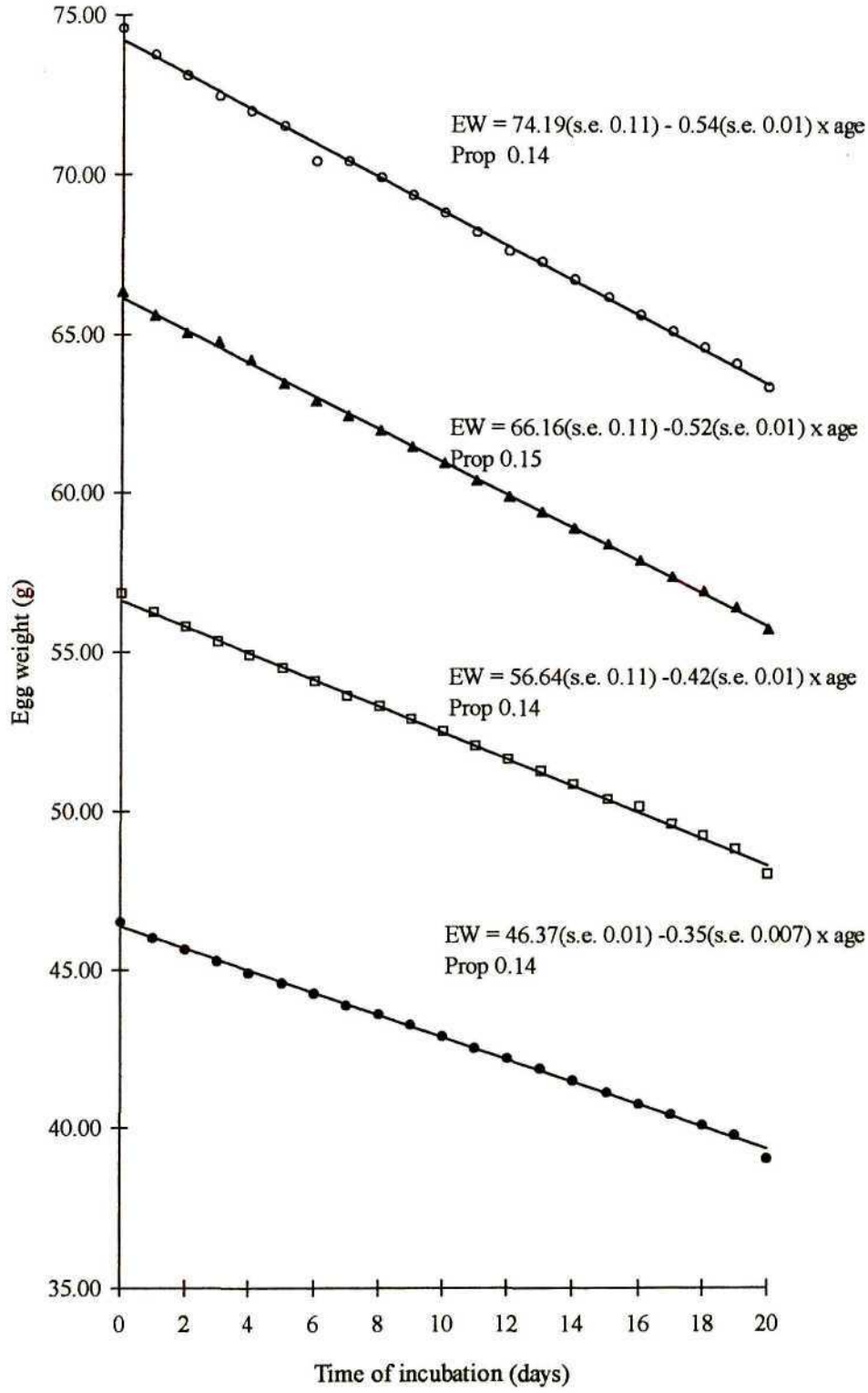

FIG. 4.5 —*Weight of the eggs within the small (•), medium (□), large (▲) and jumbo (○) egg size categories over the 21 day incubation period. Constants and linear regression coefficients are significantly different from one another (P<0.01)*
Prop: Proportion of weight loss in relation to pre-incubation egg weight; R^2 0.99

Yolk-sac weight. The weight of the yolk-sac from the 15th day of incubation until the third week after hatching is presented in Fig 4.6 and Appendix Table 9. The yolk-sac weights of the groups at day 15 and at hatching were as follows: small 9.43 ± 0.71 and 2.95 ± 0.50g; medium 13.80 ± 0.71 and 3.67 ± 0.50g; large 17.14 ± 0.71 and 5.36 ± 0.50g; jumbo 19.97 ± 0.64 and 6.42 ± 0.50g. Significant differences ($P<0.05$) were observed between the four groups throughout the six day period. During development, the rate at which the yolk-sac was absorbed appeared to be quadratic (Table 4.11) and dependent on initial egg size. Although the quadratic regression coefficients were not statistically different between the four groups, significant differences ($P<0.05$) in both the constant and linear coefficients were observed. Numerically, the constants increased, while the linear coefficients declined with increased initial egg weight. This indicates that, the larger the initial egg size, the greater the initial yolk-sac size, and the more rapid the resorption rate prior to hatching.

TABLE 4.11

Regression coefficients relating yolk-sac weight (g) to age of embryo (days) for the four weight categories from the 15th day of incubation to hatching

Egg weight	Constant (b_0)	Linear coefficient (b_1)	Quadratic coefficient (b_2)
Small[1]	-39.43 ± 11.19[a]	6.30 ± 1.25[a]	-0.20 ± 0.03[a]
Medium[2]	-30.61 ± 11.21[b]	6.17 ± 1.25[b]	-0.22 ± 0.03[a]
Large[3]	-23.66 ± 11.19[c]	5.97 ± 1.25[bc]	-0.22 ± 0.03[a]
Jumbo[4]	-18.13 ± 11.15[dNS]	5.80 ± 1.25[c]	-0.22 ± 0.03[a]
R^2		0.92	

[1]40.5-49.5g; [2]50.5-59.5g; [3]60.5-69.5g; [4]70.5-79.5g

[a-d]Regression coefficients within columns, with superscripts in common are not significantly different ($P<0.05$)

[NS]Not significantly different from zero

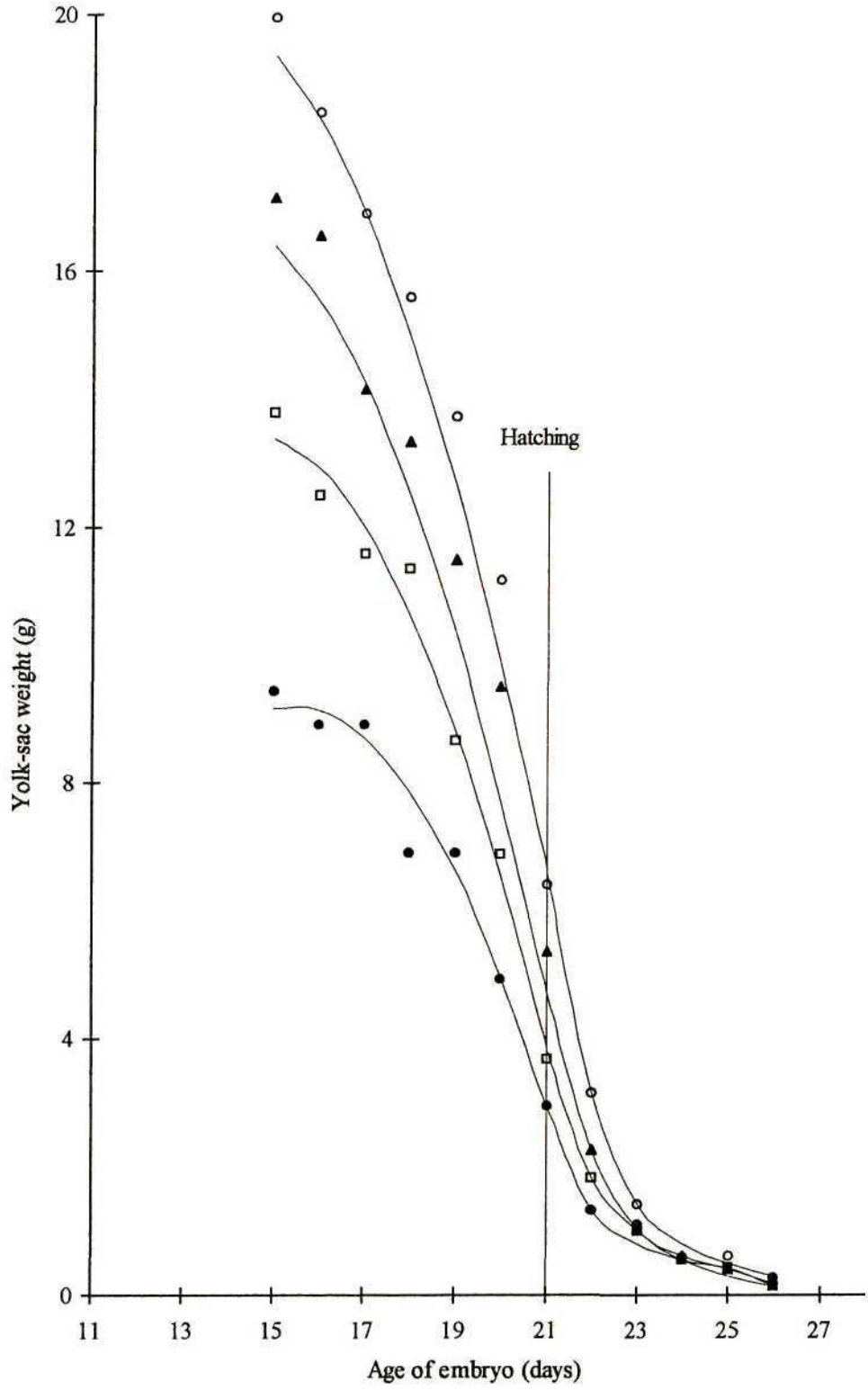

FIG. 4.6 —*Yolk-sac weight of embryos within the small (•), medium (□), large (■) and jumbo (○) egg size categories during the pre-natal period up to hatching (represented by the vertical line) and during the post-hatch period*

Yolk-sac weight was expressed as a proportion of body weight and graphed over time to hatching (Fig. 4.7). Throughout the five day period, there were significant differences ($P<0.05$) between the four groups. As the initial egg size increased, so the proportion of yolk-sac to body weight increased. These differences were no longer detectable from hatching. This is in accordance with findings of Latour *et al.* (1996).The trend over time was exponential. Therefore, the ln of the proportion was regressed over the final incubation period, using the four initial egg weight groups as dummy variables. The linear regression coefficients (Table 4.12) indicated that the rate of decline in the proportion of yolk-sac weight to body weight of the small embryos was less than that exhibited by the other three categories. The proportion was also significantly smaller at 15 days than the proportions exhibited by the other three egg weight categories.

TABLE 4.12

Regression coefficients relating ln yolk-sac weight (g) as a proportion of embryo weight (g) to age of embryo (days) for the four weight categories from the 15th day of incubation to hatching

Egg weight	Constant (b_0)	Linear coefficient (b_1)
Small[1]	2.22 ± 0.26^{a}	-0.23 ± 0.01^{a}
Medium[2]	3.49 ± 0.24^{b}	-0.28 ± 0.01^{b}
Large[3]	4.02 ± 0.23^{bc}	-0.29 ± 0.01^{b}
Jumbo[4]	4.56 ± 0.22^{c}	-0.31 ± 0.01^{b}
R^2	0.96	

[1]40.5-49.5g; [2]50.5-59.5g; [3]60.5-69.5g; [4]70.5-79.5g

[a-c]Regression coefficients within columns, with superscripts in common are not significantly different ($P<0.05$)

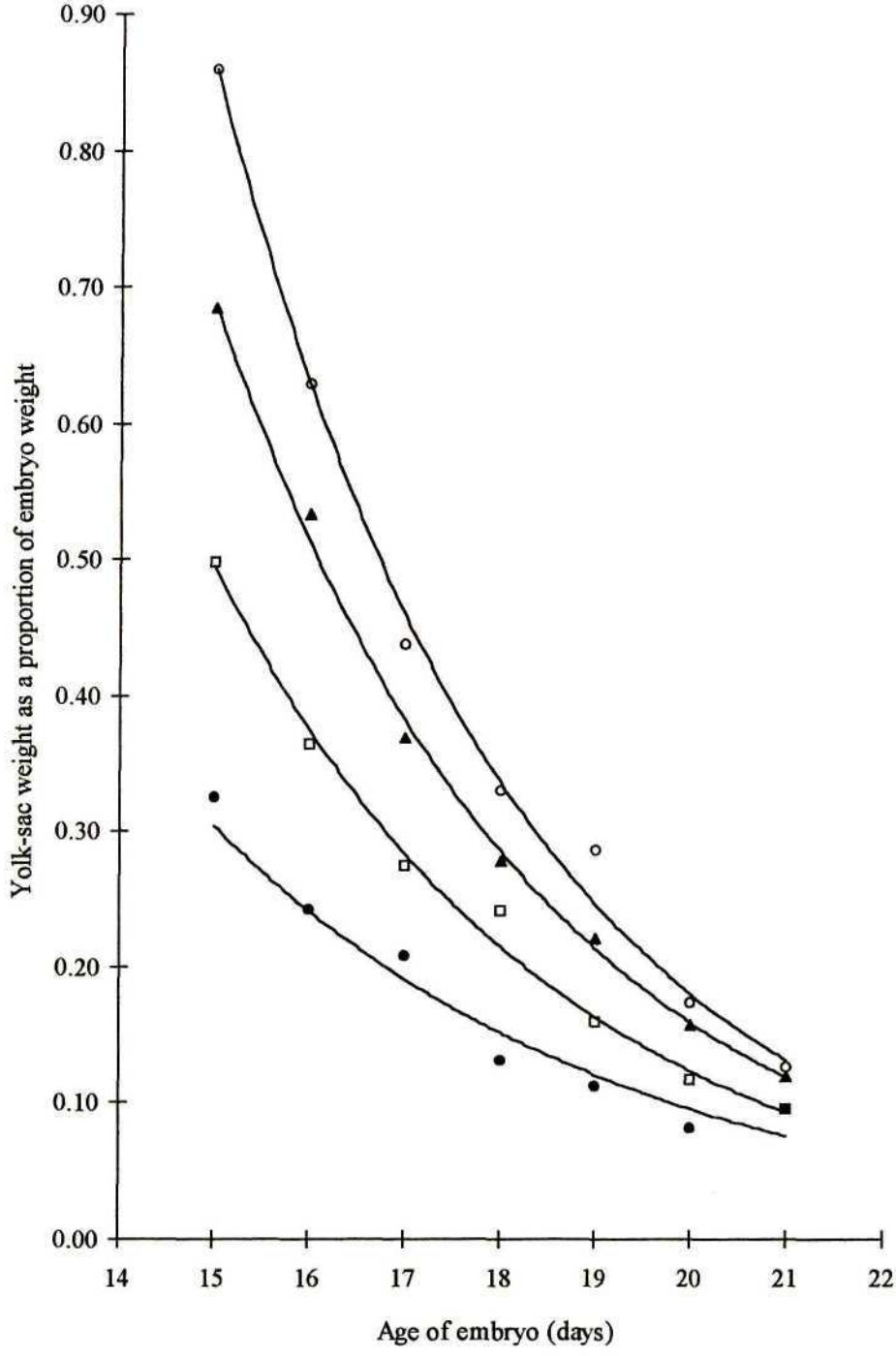

FIG. 4.7 —*Yolk-sac weight expressed as a proportion of embryonic weight within the small (•), medium (□), large (▲) and jumbo (○) egg size categories over the 21 day incubation period*

The last week of the 21 day developmental period of the chick embryo is notable as an intense period of lipid metabolism (Romanoff, 1960). During the rapid embryonic growth that occurs over this time, virtually the entire lipid content of the yolk, consisting in the main of triglycerides, free cholesterol and phospholipids, are mobilised and absorbed into the embryonic tissues (Romanoff, 1960). The yolk-sac membrane is a major site of conversion of free cholesterol in the yolk to cholesteryl esters, particularly cholesteryl oleate (Noble and Connnor, 1987), which is involved in lipoprotein synthesis and lipid assembly (Noble *et al.*, 1983) required for lipid transport. Since the embryo derives more than 90% of its total energy requirements from fatty acid oxidation (Romanoff, 1967), lipid metabolism is of vital importance to embryonic growth and development during the last week of incubation.

The differences in the rate at which the yolk-sacs were absorbed can be attributed to the effect of parental flock age on embryonic lipid metabolism and assimilation (Noble *et al.*, 1986; Noble and Connor, 1987; O'Sullivan *et al.*, 1991). Noble and Connor (1987) determined at the 15th day of incubation, that the concentration of cholesteryl esters in the yolk-sac membranes, of eggs laid by young flocks, was double the amount contained in the yolk-sac membranes of eggs from ageing birds. At the 19th day of incubation, the difference was four-fold and there was a concomitant decrease in the proportion of triglycerides present. Since the uptake of triglyceride is dependent on an association with cholesteryl esters, it appears that a malfunction may be present in the process of lipid assimilation within the eggs produced by younger hens, thus reducing access by the embryo to the major nutrient associated with its development during this time (Noble et al., 1986; Yafei and Noble, 1988). It has been suggested that the young hen is unable to provide adequate amounts of the necessary yolk apoproteins known to be required for lipoprotein assembly (Chapman, 1980). However, recent findings (Latour *et al.*, 1998) have suggested that the enzymes Δ6- and Δ9-desaturase or the hormonal levels of adrenocorticotropin and corticosterone may influence the enzymatic activities of yolk membranes within eggs from younger breeders differently. However, since it is possible to influence yolk fatty acids through dietary manipulation (Latour *et al.*, 1994) of broiler breeders, it may be possible to influence yolk fatty acid metabolism in embryos and newly hatched chicks (Latour, *et al.*, 1998) from smaller eggs. Further

research on the effect, if any, on subsequent growth and hatchability would need to be conducted.

Hatchability. The hatchability of eggs within the initial egg weight categories were not significantly different ($P<0.05$) from one another and were as follows: small 78.79 ± 3.27; medium 79.86 ± 3.27; large 80.42 ± 3.27; jumbo 78.11 ± 3.27. This is consistent with the findings of Proudfoot and Hulan (1981).

Post-natal phase

Body weight change. Mean body weight change was significantly ($P<0.05$) influenced by initial egg weight (Table 4.13). It appears that the ability to gain weight during the three week period after hatching was improved with an increase in initial egg weight. As a result, the significant differences ($P<0.05$) in body weight between the chicks in the egg weight categories persisted until the third week. This is in accordance with earlier findings (Gardiner, 1973; Merritt and Gowe, 1965; Morris *et al.*, 1968; Proudfoot and Hulan, 1981; Whiting and Pesti, 1984; Wyatt *et al.*, 1985; Hearn, 1986). The chicks gained significantly ($P<0.05$) less weight when maintained on L throughout the post-natal phase. During the second week, chicks offered a choice between H and L, gained significantly ($P<0.05$) more weight than chicks within the other two groups. However, during the first and third weeks, the difference in gain between the chicks on H, and the chicks offered a choice between H and L, were not statistically significant.

TABLE 4.13

Mean body weight change (g/bird d) of chicks produced from eggs of four weight categories, on three feed treatments, for each week of the trial

Week 1[1]	Feed H	Feed L	Choice of H and L	Mean
Small[4]	6.51	6.08	6.89	6.49±0.27[a]
Medium[5]	8.95	8.40	8.62	8.66±0.27[b]
Large[6]	9.68	9.10	9.73	9.50±0.28[c]
Jumbo[7]	10.19	8.91	10.76	9.95±0.27[d]
Mean	8.83±0.23[a]	8.12±0.23[b]	9.00±0.24[a]	
Week 2[2]				
Small	17.09	16.48	18.72	17.43±0.55[a]
Medium	21.28	20.44	21.64	21.12±0.55[b]
Large	22.85	21.97	23.05	22.62±0.58[c]
Jumbo	23.83	21.64	25.82	23.77±0.55[d]
Mean	21.26±0.47[a]	20.13±0.47[b]	22.31±0.49[c]	
Week 3[3]				
Small	34.98	28.29	33.34	32.20±0.81[a]
Medium	37.98	36.67	36.88	37.18±0.77[b]
Large	39.47	37.96	38.25	38.56±0.81[c]
Jumbo	39.60	37.60	40.57	39.26±0.77[c]
Mean	38.01±0.67[a]	35.13±0.69[b]	37.26±0.69[c]	

[1]Residual S.E. (35df) 0.92
[2]Residual S.E. (35df) 1.90
[3]Residual S.E. (35df) 3.45
[4]40.5-49.5g; [5]50.5-59.5g; [6]60.5-69.5g; [7]70.5-79.5g
[a-d]Main effects with superscripts in common are not significantly different (P<0.05)
Day-old body weights: small (31.39 ± 0.15); medium (38.94 ± 0.15); large (45.38 ± 0.15); jumbo (51.77 ± 0.15)
Size x diet interactions NS (P<0.05)

Body water content. The change in body water content of the chicks in the four egg weight categories (Fig. 4.8a) and three feed treatments (Fig. 4.8b) over time was negative and linear. The means and standard errors are presented in Appendix Table 10. The regression coefficients given in Table 4.14 illustrate that the chicks hatched out from small and medium sized eggs, had a significantly ($P<0.05$) higher initial body water content than chicks hatched out from large and jumbo sized eggs. Since body water and body lipid are negatively correlated (Velu *et al.*, 1972), it can be assumed that the chicks hatched out of the smaller eggs had a proportionately lower body lipid content than chicks from larger eggs. The change in body water, and hence body lipid, over time was independent of initial egg size and feed treatment.

TABLE 4.14

Regression coefficients relating body water content (g/ kg) to time for the four egg weight categories and three feed treatments

	Constant (b_0)		Linear coefficient (b_1)
Egg weight			
Small[1]	810.63 ± 4.19[a]		-3.35 ± 0.45[a]
Medium[2]	803.90 ± 4.19[a]		-3.72 ± 0.45[a]
Large[3]	788.67 ± 4.19[b]		-2.76 ± 0.45[a]
Jumbo[4]	789.23 ± 4.19[b]		-3.09 ± 0.45[a]
R^2		0.90	
Feed treatments			
H	801.88 ± 3.90[a]		-2.81 ± 0.42[a]
L	792.87 ± 3.90[a]		-3.85 ± 0.42[a]
Choice of H and L	798.75 ± 3.90[a]		-3.01 ± 0.42[a]
R^2		0.91	

[1]40.5-49.5g; [2]50.5-59.5g; [3]60.5-69.5g; [4]70.5-79.5g

[a-b]Regression coefficients within columns, for each factor, with superscripts in common are not significantly different ($P<0.05$)

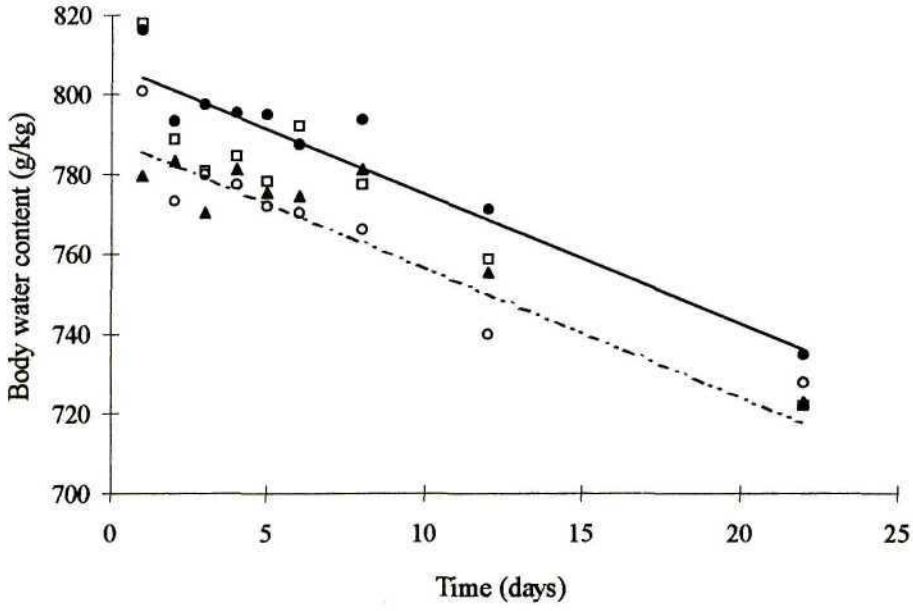

FIG. 4.8a —*The change in the concentration of body water over time of the chicks within the small (— •—), medium (— □—), large (···▲···) and jumbo (···○···) egg size categories*

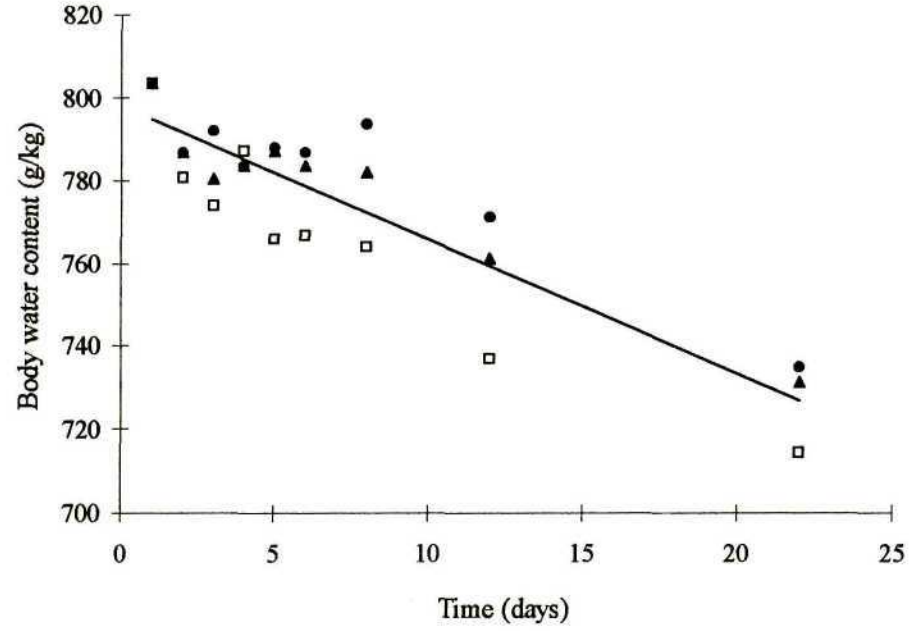

FIG. 4.8b —*The change in the concentration of body water over time of the chicks on the H (— •—), L (— □—), and choice (—▲—) feed treatments*

Relative growth rates. The quadratic relationships between the relative growth rates of the chicks within the four egg weight categories and ln Body weight over the three week period are presented graphically (Fig. 4.9) and in Appendix 11. Table 4.15 details the respective regression coefficients. Theoretically, the trend between relative growth rate and ln body weight of commercial broilers is linear. However, the observed quadratic trends indicate that growth of the chicks was limited during the first week post-hatch. The relative growth rates of the chicks in the small egg weight category were significantly lower than those achieved by the chicks in the other size categories during the first week. It is possible that performance of the smaller chicks may have been restricted by the lower residual yolk supply at hatching. In addition, it can be speculated that the smaller chicks may have had a higher temperature requirement during the first seven days. Therefore, in comparison to the chicks from the larger egg weight categories, a greater proportion of nutrients may have been utilised in maintaining core temperature. A lower proportion of the nutrients would then have been available for growth. Within the second and third weeks, the relative growth rate of the chicks decreased with ln body weight as initial egg weight increased. The trend also appeared to be linear over the final two week period.

TABLE 4.15

Regression coefficients relating relative growth rate to ln body weight (g) for the four weight categories over the three week period after hatching

Egg weight	Constant (b_0)	Linear coefficient (b_1)	Quadratic coefficient (b_2)
Small[1]	-0.315 ± 0.05[a]	0.201 ± 0.02[a]	-0.022 ± 0.002[a]
Medium[2]	-0.316 ± 0.05[a]	0.201 ± 0.02[a]	-0.022 ± 0.002[a]
Large[3]	-0.335 ± 0.05[b]	0.202 ± 0.02[ab]	-0.022 ± 0.002[a]
Jumbo[4]	-0.360 ± 0.06[c]	0.204 ± 0.02[b]	-0.021 ± 0.002[b]
R^2		0.94	

[1]40.5-49.5g; [2]50.5-59.5g; [3]60.5-69.5g; [4]70.5-79.5g

[a-d]Regression coefficients within columns, with superscripts in common are not significantly different (P<0.05)

[NS]Not significantly different from zero (P<0.05)

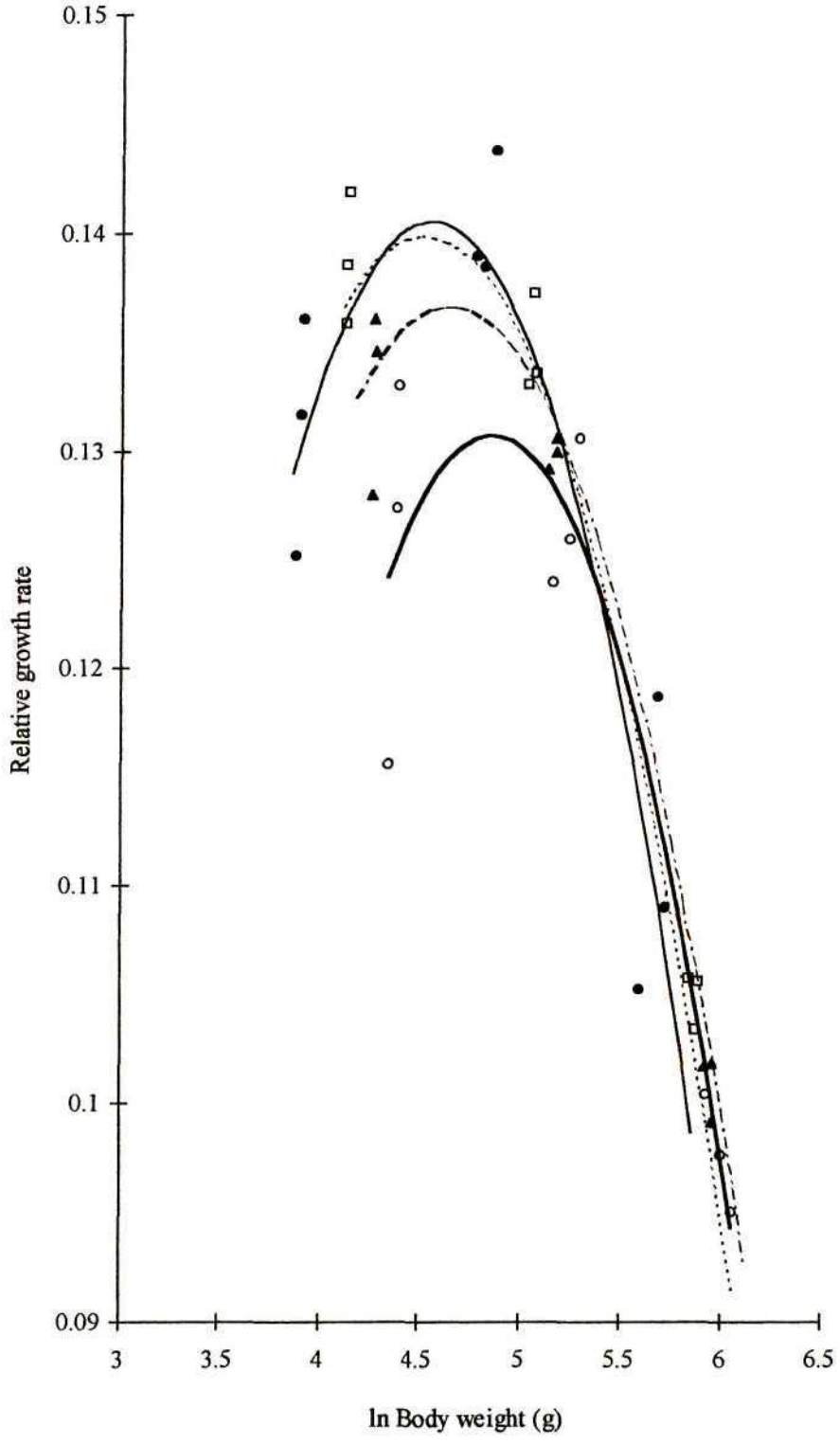

FIG. 4.9 —*Response in relative growth rate of the chicks within the small (—•—), medium (·····□·····), large (- -▲- -) and jumbo (—○—) egg size categories to ln body weight*

Food intake. Food intake throughout the post-natal phase, was influenced by initial egg weight (Table 4.16). Generally, the larger the chick, the more it was able to consume, regardless of the nutritional treatment imposed. These findings imply that the intake of nutrients by smaller chicks may be limited by reduced gut capacity, compared to bigger chicks. A greater amount of feed L was consumed each week compared to feed H. This can be explained by the theory of food intake regulation proposed by Emmans (1981) which suggests that a bird will increase its voluntary food intake when the concentration of a nutrient, in this case protein, is decreased in an otherwise well balanced diet.

Interestingly, chicks on the choice treatment consumed numerically more feed per day during the first week, and significantly ($P<0.05$) more feed thereafter, than those offered either L or H. When offered a choice of protein concentrations, chicks have been shown to consume an amount of feed intermediate between that recorded on the highest, and on the lowest protein concentrations, respectively (Shariatmadari and Forbes, 1990a; 1990b). However, the Effective Energy (Emmans, 1994) to AME ratio of the H feed was slightly lower than that of the L feed (0.945 compared to 0.993). This indicates that the heat produced on digestion and utilisation of the H feed was higher than that of feed L. Therefore, it can be speculated that food intake of the H feed was limited in the warm environment of the brooder by the necessity of the chicks to remain in thermal balance. Food intake of the low protein food may also have been constrained by limited space within the gastro-intestinal tract (Emmans, 1981). Therefore, by offering a choice between the feeds, it appears that the chicks were able to select a combination of the L and H feeds that presumably overcame the aforementioned restrictions. The increase in food intake would increase daily weight gains, and the consequent increase in gut capacity, would inturn allow for a greater food intake, and hence greater body weight gains etc.

TABLE 4.16

Mean food intake (g/bird d) of chicks produced from eggs of four weight categories, on three feed treatments for each week of the trial

Week 1[1]	Feed H	Feed L	Choice of H and L	Mean
Small[4]	12.10	10.62	13.80	11.55±0.55[a]
Medium[5]	15.59	16.36	14.38	15.04±0.55[b]
Large[6]	17.37	17.33	15.77	15.50±0.58[b]
Jumbo[7]	17.06	18.69	21.53	17.86±0.58[c]
Mean	15.53±0.51[a]	15.75±0.51[a]	16.37±0.51[a]	
Week 2[2]				
Small	23.68	25.51	29.47	26.22±0.80[a]
Medium	30.70	35.64	32.76	33.04±0.80[b]
Large	33.86	35.25	37.48	35.53±0.80[c]
Jumbo	34.44	34.68	40.38	36.50±0.84[d]
Mean	30.67±0.69[a]	32.77±0.72[b]	35.03±0.69[c]	
Week 3[3]				
Small	50.57	50.71	57.83	53.03±1.51[a]
Medium	63.59	70.87	65.88	66.78±1.43[b]
Large	66.02	64.01	67.57	65.87±1.43[b]
Jumbo	67.59	73.28	74.93	71.93±1.43[c]
Mean	61.94±1.24[a]	64.72±1.24[b]	66.55±1.29[c]	

[1]Residual S.E. (36df) 2.03
[2]Residual S.E. (35df) 2.78
[3]Residual S.E. (35df) 4.97
[4]40.5-49.5g; [5]50.5-59.5g; [6]60.5-69.5g; [7]70.5-79.5g
[a-d]Main effects with superscripts in common are not significantly different (P<0.05)
Size x diet interaction NS (P<0.05)

FCE. FCE was not affected by initial egg weight throughout the three week period (Table 4.17). Small chicks appeared to be as efficient as larger chicks in converting food to body weight gain. This is in accordance with previous findings (Gardiner, 1973; Morris et al., 1968; Proudfoot and Hulan, 1981; Wyatt *et al.*, 1985; Hearn, 1986). From the second week, food conversion efficiency was significantly ($P<0.01$) higher when chicks were maintained on the H feed. This trend was evident during the first week, but the differences in FCE between the chicks maintained on the three dietary treatments were not statistically significant. From the second week, chicks on L were significantly ($P<0.05$) less efficient in converting food into body weight gain than chicks within the remaining groups. This was expected since a greater proportion of L would have to be consumed in order for the chicks to obtain sufficient amino acids to grow at the same rate as those on H. In general, the FCE of the chicks offered a choice between H and L was lower than that of the H feed, but higher than the FCE of chicks on the L feed.

TABLE 4.17

Mean food conversion efficiency (g gain/kg food intake) of chicks produced from eggs of four weight categories, on three feed treatments for each week of the trial

Week 1[1]	Feed H	Feed L	Choice of H and L	Mean
Small[4]	541.2	574.3	541.5	543.3±20.03[a]
Medium[5]	576.0	521.5	597.4	565.0±20.03[a]
Large[6]	560.9	526.8	604.6	608.6±21.11[a]
Jumbo[7]	608.3	478.1	502.2	529.5±20.03[a]
Mean	571.6±17.34[a]	525.2±17.34[a]	554.7±18.05[a]	
Week 2[2]				
Small	728.5	649.6	633.7	670.6±14.26[a]
Medium	693.0	573.9	665.5	644.1±14.26[a]
Large	675.3	623.2	584.0	627.5±15.03[a]
Jumbo	691.5	590.6	639.3	640.5±14.26[a]
Mean	697.1±12.35[a]	609.3±12.85[b]	630.7±12.85[c]	
Week 3[3]				
Small	693.3	487.8	595.5	592.2±19.28[a]
Medium	597.6	522.3	559.9	560.0±18.29[a]
Large	598.1	595.5	567.5	587.0±19.28[a]
Jumbo	585.8	516.9	541.3	548.0±18.29[a]
Mean	618.7±15.84[a]	530.6±15.84[b]	566.1±17.11[c]	

[1]Residual S.E. (35df) 69.38
[2]Residual S.E. (34df) 49.39
[3]Residual S.E. (34df) 63.36
[4]40.5-49.5g; [5]50.5-59.5g; [6]60.5-69.5g; [7]70.5-79.5g
[a-c]Main effects with superscripts in common are not significantly different (P<0.05)
Size x diet interaction NS (P<0.05)

Proportion of protein selected. In order to assess the amount of H selected in relation to L, intake of H was expressed as a proportion of the total food consumed within each egg weight category and for each week of the post-natal period (Table 4.18). The small, medium and large chicks selected significantly ($P<0.05$) more of H than L over the three week period. The jumbo sized chicks chose to consume significantly ($P<0.05$) more of L. The proportion of protein selected was also dependent on time. After 21 days of growth, the chicks chose equal amounts ($P<0.05$) of the two feeds. Within egg weight categories, different proportions were chosen over time. The small chicks chose significantly ($P<0.05$) more of H until the third week when equal amounts of the feeds were selected. The medium and large sized chicks selected similar proportions of H and L from the second week. During the first week, jumbo chicks selected equivalent proportions of the two feeds, but significantly ($P<0.05$) more of L was consumed during the second week, and while the proportion selected in week three was not statistically significantly different from 0.5, it was numerically lower. The lack of significance is possibly due to the high degree of error within the group. The selection of equal amounts of H and L by the chicks of different sizes at different times after hatching is of interest, since this implies that the protein content of H is too high and the protein content of L is too low. Table 4.18 outlines the resultant content of protein chosen by the chicks within the categories for each week of the post-natal period. It is apparent that the concentration of protein selected by the small, medium and large chicks, decreased over time. From week two, the content of protein selected by the jumbo sized chicks, remained relatively constant. The difference in the content of protein selected by the chicks hatched out of eggs of different sizes may be related to gut capacity. Smaller chicks have a lower gut capacity, but presumably, similar daily protein requirements for maintenance and growth. Hence, they would need to eat a feed with a higher protein content than chicks that were bigger at day old in order to satisfy their daily amino acid or protein requirements.

TABLE 4.18

Proportion of the pre-starter feed chosen in relation to total food intake and the content of protein (g/kg) selected by the chicks within the four egg weight categories over the three week period

	Proportion of pre-starter selected			
	Week 1	Week 2	Week 3	Mean
Small[1]	0.5902 ± 0.04*	0.6658 ± 0.06*	0.4891 ± 0.08^{NS}	0.6015 ± 0.04*
Medium[2]	0.6095 ± 0.04*	0.6926 ± 0.06^{NS}	0.5333 ± 0.07^{NS}	0.6118 ± 0.04*
Large[3]	0.6866 ± 0.04*	0.5429 ± 0.06^{NS}	0.4823 ± 0.07^{NS}	0.5706 ± 0.04*
Jumbo[4]	0.5051 ± 0.04^{NS}	0.3712 ± 0.06*	0.3888 ± 0.07^{NS}	0.4217 ± 0.04*
Mean	0.5979 ± 0.018*	0.5681 ± 0.028*	0.4734 ± 0.04^{NS}	

	Content of protein selected			
	Week 1	Week	Week 3	Mean
Small	265.47	274.79	253.02	266.86
Medium	267.85	278.10	258.42	268.13
Large	277.6	260.19	252.17	263.05
Jumbo	254.98	238.47	240.64	244.70
Mean	266.42	262.75	251.07	

*Significantly different to 0.5 ($P<0.05$).
^{NS}Not significantly different to 0.5 ($P<0.05$)
[1]40.5-49.5g; [2]50.5-59.5g; [3]60.5-69.5g; [4]70.5-79.5g

Yolk-sac weight. The decline in yolk-sac weight after hatching is presented graphically (Fig. 4.6) and in Appendix Table 9. The significant differences ($P<0.05$) in weight between the four categories were no longer observed from the third day after hatching. The effects on yolk-sac weight due to the nutritional treatments imposed on the chicks, were non-significant. Therefore, a quadratic model was fitted to the data over the three day period using the initial egg weight categories as dummy variables. Table 4.19 details the regression coefficients. The intercepts and linear coefficients of the small and medium categories were of similar proportion, as were the intercepts and linear coefficients of the large and jumbo categories. The quadratic coefficients for the four groups were represented by one value. This indicates that the rate at which yolk-sacs were absorbed after hatching was dependent on initial egg size. The rate was slower for chicks hatched out of eggs between 40.5 to 59.5g compared to chicks hatched out of eggs between 60.5 to 79.5g. The fit was not significantly improved with the use of an exponential model.

TABLE 4.19

Regression coefficients relating yolk weight to age of chick for the four weight categories for a period of three days after hatching

Egg weight	Constant (b_0)	Linear coefficient (b_1)	Quadratic coefficient (b_2)
Small[1]	375.70 ± 63.75^a	-33.10 ± 5.76^a	0.73 ± 0.13^a
Medium[2]	386.02 ± 63.68^a	-33.55 ± 5.76^a	0.73 ± 0.13^a
Large[3]	402.60 ± 63.75^b	-34.28 ± 5.76^b	0.73 ± 0.13^a
Jumbo[4]	411.57 ± 63.69^b	-34.65 ± 5.76^b	0.73 ± 0.13^a
R^2		0.83	

[1]40.5-49.5g; [2]50.5-59.5g; [3]60.5-69.5g; [4]70.5-79.5g

[a-d]Regression coefficients within columns, with superscripts in common are not significantly different ($P<0.05$)

[NS]Not significantly different from zero($P<0.05$)

The rapid decline in yolk-sac weight 48 hours after hatching is in accordance with the findings of Chamblee *et al.* (1992), who determined that the initiation of growth in newly hatched chicks was correlated with yolk absorption. Although the role of residual yolk in the chick after hatching is debatable, it apparently has a crucial function for a brief period when it complements nutrient intake and fortifies efficient utilisation of dietary energy and protein (Murakami *et al.*, 1994; Nitsan *et al.*, 1995). The fact that performance in the post-hatch period may be limited by the reservoir of residual yolk was confirmed by Kulka and Duskin (1964), cited by Nitsan *et al.* (1995), and Daly and Peterson (1990). Latour *et al.* (1996) has suggested that yolk retention in chicks is directly related to parent age and may be associated with alterations in physiological and molecular processes, as was found during the pre-natal period. Therefore, apart from the lower body weight and lower yolk sac weight at hatching, the lower gut capacity and perhaps a higher temperature requirement, the smaller chicks had also to overcome the problem of a slower release of yolk material. As a result, it is not surprising that the growth rate of chicks within the smaller eggs was lower than that maintained by chicks in other groups during the first week post-hatch.

Therefore, in order to exploit the growth potential of the smaller chicks at day old, it would be advantageous for the broiler producer to segregate chicks according to initial egg size (parental flock age), and grow these smaller chicks on a higher protein feed than the starter feed typically used commercially. In addition, transportation after hatching should be limited as smaller chicks have a lower residual yolk supply. Smaller chicks are also more stressed initially and possibly have a higher energy requirement for maintenance of body temperature compared to larger chicks. Therefore, during the first week post-hatch, these chicks should either be fed a higher energy feed, or be brooded at a higher temperature, or both.

CHAPTER 5

A COMPARISON OF THE RESPONSE OF LAYING HENS AND BROILER BREEDERS TO TRYPTOPHAN INTAKE

5.1 INTRODUCTION

Although the responses of egg laying strains to amino acids, such as lysine and methionine, have been extensively investigated, production responses to another essential amino acid, tryptophan, have been neglected. In a review of the literature, McDonald and Morris (1985) derived pooled estimates of the tryptophan requirements per gram of egg output and per kg of body weight, the *a* and *b* coefficients of the Reading Model, respectively (Fisher *et al.*, 1973). However, the estimates were based on two data sources only (Morris and Wethli, 1978; du Preez and Duckitt, 1984), and involved one broiler breeder, and three laying hen genotypes. Since genetic selection of broiler breeders is based to a greater extent on broiler growth, rather than on egg production, breeders are heavier, their body fat content is considerably greater (Bowmaker and Gous, 1991) and they exhibit lower egg outputs than do commercial layers. Hence, doubts have been raised as to whether the *a* and *b* coefficients, estimated over a decade ago, can be confidently used to estimate simultaneously the optimum tryptophan intakes of both laying hens and modern-day broiler breeders.

Previously, and in all but one experiment (du Preez and Duckitt, 1984), the birds were housed in groups. This practice would confound amino acid response trials conducted on broiler breeders, since food intake is restricted in these birds and consumption might relate to the position of the bird in the pecking order, rather than to the bird's specific nutritional requirements. Therefore, to obtain comparable measures of food intake, and hence tryptophan intakes, both laying hens and breeders were housed individually in cages in the present study. Moreover, it would appear that measuring variables such as egg weight, rate of lay and body weight on a bird basis, would elicit a genuinely representative response of either strain to the dietary treatments imposed.

In addition, MacDonald and Morris (1985) estimated the response coefficients of the Reading Model using calculated total tryptophan intakes only. Both Morris and Wethli (1978) and Wethli and Morris (1978) based their analyses on calculated estimates of the concentration of available tryptophan in their experimental diets. Therefore, it can be assumed that optimum tryptophan intakes of laying birds have been based on inadequate measures of tryptophan in the feeds. Since the precision of measuring both total and available amino acids, specifically tryptophan, has improved in the last two decades, it was of particular interest to compare the response coefficients determined in the present experiment, with those determined previously by the various authors.

To produce a series of tryptophan limiting diets, the summit-dilution technique reported by Pilbrow and Morris (1974) was applied. Instead of diluting the summit feed, which is high in all amino acids but limiting in tryptophan, with a non-protein diluent, as described by Fisher and Morris (1970), a low protein diluent was used. A test trial, run concurrently with the experiment, determined whether tryptophan was indeed the first limiting amino acid.

The objectives of the experiments reported here were to compare the responses of laying hens and broiler breeders housed individually, and on a lipid-free basis, to increasing intakes of total and available dietary tryptophan.

5.2 MATERIALS AND METHODS

Test trial

Birds and management

64 Amberlink laying hens (50 weeks of age) were placed in an open-sided house (9 x 37m) on one side of a bank of cages two tiers high. Four birds were allocated per cage (55 x 53 x 37cm). Each cage was fitted with two nipple drinkers and the birds were fed *ad libitum*. The lighting pattern was 16L:8D.

Treatments and feeds

In order to determine whether the summit feed used in the response trial was first limiting in tryptophan, it was diluted by half with a non-protein diluent (Table 5.1). As a result, the diluted summit feed contained the same concentration of tryptophan as the dilution feed used in the response trial. The diluted summit feed and the dilution feed were fed on their own and with synthetic tryptophan added, which provided an extra 0.18g tryptophan/ kg of feed.

Each of the four feeds were offered to four groups of 16 laying hens (four cages x four replications). Randomisation was according to the Latin square experimental design.

Measurements

All four birds in one cage were weighed at the beginning and at the end of the test trial.

The number of eggs laid by the group of 16 birds was recorded daily and on three days of each week the eggs were weighed.

Food intake was measured weekly.

The trial ran for a period of four weeks.

Tryptophan limiting experiment

Birds and management

Laying hens 240 Amberlink hens aged 40 weeks were placed in an open sided house (9 x 13m). The birds were housed individually in wire cages (40 x 44 x 29cm) equipped with a nipple drinker and a feed trough (25 x 22 x 28cm). The cages were arranged in two banks, each bank consisting of 128 cages, made up as four rows (two tiers, back to back) of 32 cages per row. The lighting regime used throughout the experiment was 16L:8D.

Broiler breeders 240 Ross 788 broiler breeder hens aged 40 weeks were placed in a controlled environment house (10 x 7.7 x 3.5m). Cross ventilation was provided by six fans covered by baffles to ensure that the house was light tight. Birds were housed individually in wire cages (75 x 48 x 33cm) arranged in six rows, back to back. Each row consisted of two tiers of 48 cages respectively. Nipple drinkers and drip-cups were provided at the junctions of every cage, thus giving each bird access to two drinkers. A feed trough (10 x 30cm) and a wooden perch were supplied for each cage. The lighting regime used throughout the experiment was 16L:8D.

Treatments and feeds

Two basal feeds, a summit and dilution feed, were formulated to be first-limiting in tryptophan (Table 5.1) and blended in different proportions (Table 5.2) to produce eight feed treatments that were replicated 30 times with each of the two strains. The coefficients of response (a = 2.62mg tryptophan/g egg output; b = 11mg tryptophan/ kg body weight) determined by MacDonald and Morris (1985) were used to estimate the required intake of tryptophan for the most demanding bird in the population. For example, a 2kg hen producing 66g egg output/ d, would require 195mg tryptophan/d. This amount was converted to a dietary concentration, assuming the bird would eat about 110g feed/d, and taken to be the requirement. This value was multiplied by 1.2 to ensure that an adequate intake of tryptophan was assured for the most demanding hens in the population. Therefore, the summit feed was designed to provide tryptophan at 1.2 times, and the other amino acids at 1.6 times the estimated tryptophan requirement of laying hens. The low protein dilution feed provided tryptophan at 0.6 times, and the other amino acids at 0.8 times the estimated requirement for tryptophan. The eight diets were fed over a period of ten weeks.

TABLE 5.1
Composition (g/kg) of the tryptophan limiting diets

Ingredient	Summit		Dilution		Diluent
Yellow maize	248.0		529.0		-
Maize germ meal	154.0		229.0		-
Maize gluten 60	80.0		-		-
Soybean oilcake	15.0		-		-
Lupin	300.0		136.0		-
Gelatin	97.0		-		-
L-lysine HCL	1.5		3.2		-
DL-Methionine	3.6		1.6		-
Limestone	83.1		83.8		83.6
Monocalcium phosphate	10.8		10.8		14.3
Salt	4.3		4.2		5.1
Vitamin and mineral premix[1]	2.5		2.5		5.0
Sugar	-		-		238.9
Starch	-		-		238.9
Sunflower oil	-		-		95.6
Filler	-		-		318.6
Analysis	Calculated	Actual	Calculated	Actual	Calculated
AME (MJ/kg)	11.5	11.8	11.5	11.0	11.5
Protein	282.9	277.4	115.5	106.5	9.6
Tryptophan (total)	1.8	1.4	0.9	0.9	-
Tryptophan (available)	-	1.2	-	0.8	-
Lysine	12.3	13.4	6.6	6.6	-
Methionine	8.9	7.0	3.3	2.5	-
Methionine + Cystine	10.7	-	4.0	-	-
Threonine	8.9	8.6	3.9	3.4	-
Arginine	21.5	19.4	8.5	7.1	-
Histidine	6.1	5.1	2.9	2.8	-
Isoleucine	10.2	9.8	4.5	4.5	-
Phe + Tyr	20.6	17.8	5.6	7.5	-
Valine	11.5	11.4	5.2	4.0	-
Calcium	32.5	-	32.5	-	32.5
Phosphorus	3.2	-	3.5	-	3.5

[1]*Vitamin premix provided/kg diet*: 13000IU vitamin A, 3000IU vitamin D_3, 30mg ∝-tocopheryl acetate, 3mg menadione, 2mg thiamin, 6mg riboflavin, 5mg pyridoxine, 15 μg cyanocobalamin, 2mg folic acid, 100μg biotin, 15mg pantothenic acid, 60mg nicotinic acid *Mineral premix provided/kg diet*: 20mg Cu, 3mg I, 40mg Fe, 70mg Mn, 100mg Zn, 0.2mg Se, 0.5mgCo, 0.5mg Mo

TABLE 5.2

Mixing proportions of the summit and dilution feeds and the calculated concentration of the first limiting amino acid, tryptophan

Feed	Tryptophan content (g/kg)	Summit	Dilution
1	1.8	100	0
2	1.7	86	14
3	1.5	72	28
4	1.4	58	42
5	1.3	42	58
6	1.2	28	72
7	1.0	14	86
8	0.9	0	100

The two basal feeds were analysed for AME, protein, total amino acid, calcium and phosphorus content. Total tryptophan in the two basal feeds and six blends were determined by High Sensitivity Protein Hydrolysate Analysis using a Beckman 6300. Initially, the samples were hydrolysed with 4.2 N NaOH (Central Research Laboratories of Ajinomoto Co., Inc., 1984) at 110C for 18 hours to break the peptide bonds and to liberate the constituent amino acids (Hugin and Moore, 1972). An internal standard was added to the sample as a means of correcting for any inaccuracy that may have occurred during the sample preparation. The samples were then subjected to single-column ion-exchange chromatography which is an HPLC process (Biotext, 1991). Two micro flow pumps were used to control the analytical flow stream and included one buffer, and one reagent (ninhydrin) pump. The effluent/reagent mixture was then heated for a period prior to survey by visible photometry at wavelengths of 440 and 520 nanometres. The System Gold Software Package (Beckman Instruments Inc., 1983) was used to acquire the data from the detector channel and to produce the chromatographs.

Available tryptophan content of the summit and dilution feeds were calculated by analysis of the excreta of force-feed cockerels. 50g of each of the basal feeds were fed by tube (Sibbald, 1976) following a 48 h fasting period as described by McNab and Fisher (1981). The excreta voided during the following 48 h period of feeding were collected, weighed dry and analysed for tryptophan content as described above. Available tryptophan was estimated by correcting for endogenous tryptophan losses calculated from the determination of the tryptophan content of the excreta voided over a 48h period after force-feeding cockerels 50g glucose, using the following equation:

$$\text{Available tryptophan (\%)} = \frac{(F \times T_f) - ((E_m \times E_t) - (E_{m2} \times E_{t2}))}{F}$$

where F = amount of test feed force-fed (50g), T_f = tryptophan content of the test feed (%), E_m = excreta weight of the force-fed material (g), E_t = tryptophan content of the excreta (%), E_{m2} = weight of the excreta of the birds fed glucose and E_{t2} = tryptophan content of these excreta (%). The values used to calculate the available tryptophan contents of the summit and dilution feeds are given in Table 5.3.

TABLE 5.3

Parameters used to estimate the available tryptophan concentration of the summit and dilution feeds

Parameter	Summit	Dilution
F	50.00	50.00
T_f	0.15	0.09
E_m	18.87	16.10
E_t	0.13	0.12
E_{m2}	5.32	5.32
E_{t2}	0.28	0.28

F = Amount of test feed force-fed (g)
T_f = Tryptophan content of the test feed (%)
E_m = Excreta weight of the force-fed material (g)
E_t = Tryptophan content of the excreta (test feed) (%)
E_{m2} = Weight of the excreta of the birds fed glucose
E_{t2} = Tryptophan content of the excreta (glucose) (%)

Allocation of feeds

The laying hens were given *ad libitum* access to the diets. In the case of the broiler breeders, the daily food allocation (170g/bird) was weighed to within one g of the required weight and stored in plastic-coated cardboard containers. Feeding took place in the morning, and food remaining in the troughs at the end of the day was not discarded but allowed to accumulate for a week.

Measurements

Body weight was recorded at the beginning of the trial, and at three-weekly intervals thereafter.

Food intake of the laying hens was calculated by subtracting the weight of the trough at the end of the week from the weight of the full trough at the beginning of the week. Food intake of the broiler breeders was calculated by subtracting the amount remaining at the end of each week from the amount fed.

Egg production was recorded on each day of the week.

Egg weight was recorded twice daily on three days of each week, and on four days of each week, for the laying hens and broiler breeders, respectively.

At the end of the trial, five laying hens and five broiler breeders were randomly selected from each of three treatments, namely, treatments one, four and eight, and weighed. The birds were killed by means of carbon dioxide poisoning. After removal of the feathers, the birds were re-weighed and minced individually to form an homogenous blend. A sample from each carcass was then subjected to chemical composition analysis. All procedures conformed to those specified by the Association of Official Analytical Chemists (AOAC, 1975). Body water content was determined by freeze drying the samples. Body protein was determined on a water-free basis in a LECO nitrogen analyser as N x 6.25. Gross energies (MJ/kg dry matter) were determined and used to predict the lipid content of the birds using the following equation developed at the University of Natal:

$$L = -0.8756 \text{ (s.e. } 0.0527) + 0.04754 \text{ (s.e. } 0.0020) \times GE$$

where, L = lipid (g/g dry matter) and GE = gross energy (MJ/kg dry matter).

Lipid-free body weight (kg) was calculated as:

((1-Lipid content, g/g) x Feather-free body weight, kg) + Weight of the feathers, kg

Statistical analyses

The means and standard deviations of the measured variables were determined using the general linear model of Minitab Rel. 10.5 Xtra (Minitab Inc, 1995). The Reading Model (Fisher *et al.*, 1973) was applied to determine the coefficients of response to tryptophan, using the mean tryptophan intakes and egg outputs measured during the final four weeks of the trial, when it was assumed that the birds had equilibrated to the concentrations of tryptophan in the feeds. Linear regression analysis was used to determine the relationship between lipid-free body weight and live weight.

5.3 RESULTS AND DISCUSSION

Test trial

The mean responses in egg weight, rate of laying, egg output and food intake to the summit (diluted) and dilution feeds, on their own, and with supplemental tryptophan, are given in Table 5.4. The responses in the measured variables were similar on the two unsupplemented low protein feeds and significantly ($P<0.05$) improved on the feeds supplemented with tryptophan, confirming that tryptophan was the first limiting amino acid.

TABLE 5.4

Test trial responses in mean egg weight, rate of laying, egg output and food intake over the final three week period

	Egg weight (g)	Rate of laying (eggs/100 bird d)	Egg output (g/ bird d)	Food intake (g/ bird d)
Diluted summit	52.73[a]	36.50[a]	19.29[a]	81.09[a]
	±0.45	±2.63	±1.44	±3.85
Diluted summit + tryptophan	57.74[b]	76.71[b]	44.30[b]	122.28[b]
	±0.45	±2.63	±1.44	±3.85
Dilution	54.51[a]	46.85[a]	25.53[a]	88.49[a]
	±0.45	±2.63	±1.44	±3.85
Dilution + tryptophan	56.70[b]	72.84[b]	41.29[b]	115.97[b]
	±0.45	±2.63	±1.44	±3.85

[a-b] Means within columns with letters in common are not significantly different (P<0.05)

Tryptophan limiting experiment

Food intake

The mean food intakes of both the laying hens and broiler breeders, during the final four week period of the trial, on the eight concentrations of tryptophan, are shown in Table 5.5. The broiler breeders were allocated 170g of food per day on the assumption that these hens require about 2000kJ of AME per day (Bowmaker and Gous, 1991). The feeds used in the experiment were designed to contain 11.5MJ/kg, this being the optimum nutrient density of a feed for commercial laying hens under the prevailing circumstances. The broiler breeders did not consume all of the food allocated to them in all of the eight dietary treatments indicating that, for the level of performance of these birds, 2000kJ AME/d was more than required. Food intake by the laying hens decreased in a curvilinear manner as the concentration of tryptophan in the feeds decreased (Fig. 5.1). However, food intake by the broiler breeders was not

significantly ($P<0.05$) influenced by the concentration of tryptophan in the feeds. This suggests that the eight diets allocated to the breeders were not sufficiently limiting in tryptophan in order to procure a marked response.

In the case of laying hens, food intake has been shown to increase as the protein or amino acid concentration is decreased (Gous *et al.*, 1987), which implies that birds eat more in an attempt to compensate for marginal deficiencies of the first limiting amino acid (Boorman, 1979). The same trend has been observed previously in various tryptophan limiting trials (Morris and Wethli, 1978; Wethli and Morris, 1978; du Preez and Duckitt, 1984). However, in the case of the present trial, the effect was not as pronounced. It appears that food intake of the laying hens, at a tryptophan concentration of 1.32g/kg was greater than that on the highest tryptophan diet of the series, while egg output was lower. No distinct increase in food intake could be identified with the broiler breeders. However, it is doubtful whether this is a strain or tryptophan related difference.

The decline in food intake at low amino acid concentrations has been attributed to the necessity of animals to remain in thermal balance with their environment (Emmans, 1981). The more dilute diets had a notably higher energy to amino acid ratio compared to diets higher up in the dilution series. Therefore, the amount of energy that could possibly have been consumed by the birds on the dilute diets, would have been far in excess of the amount required to sustain egg production commensurate with the low available amino acid supply. Consequently, to prevent the substantial heat load that would be caused by the conversion of excess energy to body lipid, it was to the advantage of the hens to consume less. As a result, it appears that the prediction of food intake is far more complex than assuming that the bird has a requirement for energy that it seeks to satisfy.

TABLE 5.5

Response in mean egg weight, rate of laying, egg output, food intake and body weight change among laying hens and broiler breeders over the final four week period to dietary tryptophan content

	Laying hens					Broiler breeders				
Tryptophan content (g/kg)	Food intake (g/bird d)	Body weight change (g/bird d)	Rate of lay (eggs/ 100 bird d)	Egg weight (g)	Egg output (g/bird d)	Food intake (g/bird d)	Body weight change (g/bird d)	Rate of lay (eggs/ 100 bird d)	Egg weight (g)	Egg output (g/bird d)
1.39	110.99 ±2.86	-2.33 ±0.74	83.70 ±2.67	58.55 ±0.64	48.98 ±1.77	164.00 ±2.28	3.78 ±0.69	63.87 ±2.21	69.09 ±0.72	43.94 ±1.78
1.32	111.90 ±2.76	-2.19 ±0.70	82.86 ±2.62	57.24 ±0.63	47.25 ±1.71	161.80 ±2.24	3.91 ±0.69	65.05 ±2.13	68.08 ±0.72	43.60 ±1.68
1.24	107.36 ±2.76	-2.41 ±0.71	79.52 ±2.62	57.37 ±0.63	45.63 ±1.71	162.80 ±2.28	4.65 ±0.70	63.87 ±2.21	69.37 ±0.72	43.66 ±1.78
1.17	106.52 ±2.76	-1.75 ±0.73	77.02 ±2.62	56.09 ±0.63	43.01 ±1.71	161.50 ±2.28	5.06 ±0.70	61.99 ±2.13	68.85 ±0.72	42.70 ±1.71
1.10	101.11 ±2.86	-2.59 ±0.71	70.81 ±2.67	55.08 ±0.63	37.74 ±1.71	162.10 ±2.20	4.35 ±0.67	61.06 ±2.02	70.79 ±0.69	42.27 ±1.63
1.03	99.94 ±2.76	-2.76 ±0.71	67.38 ±2.62	55.35 ±0.63	36.62 ±1.71	161.30 ±2.24	4.16 ±0.69	61.35 ±2.13	69.71 ±0.72	42.37 ±1.71
0.96	92.99 ±2.86	-3.10 ±0.74	62.53 ±2.71	53.26 ±0.66	32.05 ±1.77	157.70 ±2.24	3.07 ±0.69	61.08 ±2.09	68.57 ±0.70	41.85 ±1.65
0.89	86.18 ±2.91	-4.17 ±0.75	49.23± 2.71	53.48 ±0.67	25.37 ±1.77	158.30 ±2.28	4.67 ±0.70	56.03 ±2.09	69.34 ±0.72	38.02 ±1.68

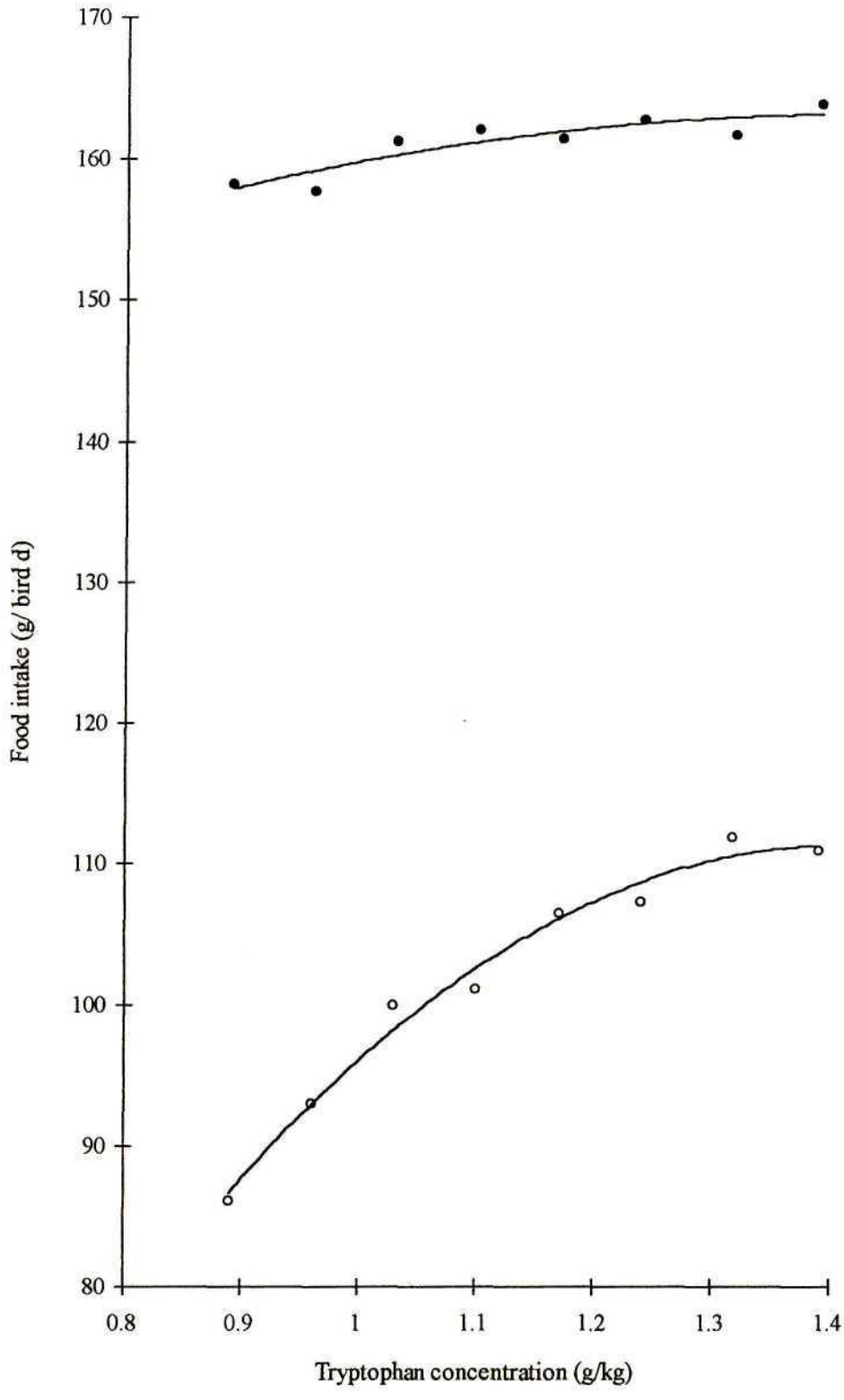

FIG. 5.1 —*The effect of dietary tryptophan concentration on food intake of the laying hens (o) and broiler breeders (•)*

Changes in body weight, and body composition

Over the eight dietary treatments, and over the final four week period, the laying hens lost weight, while the broiler breeders gained weight. The difference in weight loss of the laying hens over the eight tryptophan concentrations, was significant ($P<0.05$), while the relationship between the concentration of tryptophan, or food intake, and weight loss appeared to be curvilinear. Weight gain exhibited by the breeders was similar across the treatments.

Significant differences ($P<0.05$) in feather-free carcass composition, over the three tryptophan concentrations, for both the laying hens and broiler breeders, were evident at the end of the ten week trial period (Table 5.6). The body water and the body protein content of the broiler breeders were significantly lower ($P<0.05$) at the lowest concentration of tryptophan than at the higher concentrations, while body lipid content was significantly greater ($P<0.05$). A similar trend was evident with the laying hens. However, body protein content appeared to decline linearly as the concentration of tryptophan decreased. Therefore, at the lowest tryptophan concentration, the ratio of body lipid to body protein, of both the laying hens and broiler breeders, was increased. It is apparent that degeneration of body protein of both the strains occurred at the lowest tryptophan content since daily tryptophan intake was insufficient to maintain initial protein content and to produce eggs. The increase in body lipid could be attributed to the excess amino acids, which would have been deaminated and the resultant fraction stored as fat, as well as to the consumption of excess energy.

TABLE 5.6

Composition of the feather-free body weights of the laying hens and broiler breeders on three tryptophan concentrations at the end of the ten-week trial period

	Laying hens			Broiler breeders		
Tryptophan content (g/kg)	Body water (g/kg)	Body protein (g/kg)	Body lipid (g/kg)	Body water (g/kg)	Body protein (g/kg)	Body lipid (g/kg)
1.39	647.10[a]	159.40[a]	144.50[a]	577.90[a]	144.20[a]	230.70[a]
1.17	654.60[a]	153.20[b]	149.30[a]	572.20[a]	144.00[a]	234.40[a]
0.89	618.50[b]	145.80[c]	157.70[b]	558.10[b]	137.00[b]	257.30[b]

Body water: Residual S.E. (24df) 25.76
Body protein: Residual S.E. (24df) 5.02
Body lipid: Residual S.E. (24df) 32.25
[a-c]Means within columns with letters in common are not significantly different ($P<0.05$)

Rate of laying

The mean rates of laying for the laying hens and broiler breeders over the four week period and on the eight tryptophan concentrations are given in Table 5.5. Maximum egg production of the laying hens was achieved on the highest concentration of tryptophan, while that of the breeders was achieved on treatment two. Within the first four highest treatments, at least 29 out of 30 laying hens laid in closed cycles or above a rate of 0.5 eggs/ bird d. Within the remaining treatments, the numbers declined to 22, 28, 22 and 19, respectively. In the case of the broiler breeders, at least 29 out of 30 birds laid at a rate above 0.5 within the first two highest treatments. The numbers declined to 27, 25, 24, 26, 25 and 24, respectively, within the remaining treatments.

Egg weight

The mean weight of the eggs produced by the laying hens was significantly ($P<0.05$) related to dietary tryptophan content, while that of the broiler breeders was similar across the dietary treatments (Table 5.5). The response in egg weight was not

as pronounced as the response in rate of laying. In the case of the laying hens, egg weight declined to 92 percent of the maximum egg weight on the lowest tryptophan concentration, whilst rate of laying declined to 60 percent of the maximum observed value. These findings are confirmed by Morris and Gous (1988), who determined that at severe amino acid deficiencies, rate of laying is reduced to a greater extent than egg weight. However, they concluded that since the response in rate of laying to tryptophan continues beyond the point where egg weight reaches a maximum, the partitioning of egg output between egg weight and rate of laying, is not evenhanded at relatively marginal deficiencies of tryptophan intake. This trend, which was observed with the broiler breeders in the present trial, provides more evidence to suggest that the unequal partitioning of egg output in tryptophan-limiting diets, is actually a function of the limiting amino acid.

Response in egg output to tryptophan intake

The individual responses in egg output to total tryptophan intake of the laying hens and broiler breeders are presented graphically (Fig. 5.2) and in Table 5.5. On the lowest dietary tryptophan contents, egg output of the laying hens dropped to 50 percent of the maximum egg output achieved at the highest tryptophan content, while egg output of the broiler breeders dropped to only 87 percent of the maximum. The vertical displacement of the curve for the broiler breeders could be attributed to the higher body weight, and hence maintenance requirement, while the horizontal displacement could be attributed to the characteristically lower egg production of these birds, compared to the laying hens. It is apparent from Figure 5.2, that in comparison to the broiler breeders, the egg output of the laying hens did not approach the maximum at the highest tryptophan intakes in an asymptotic manner, while the slope of the response by the broiler breeders appeared to be more gradual than that by the laying hens.

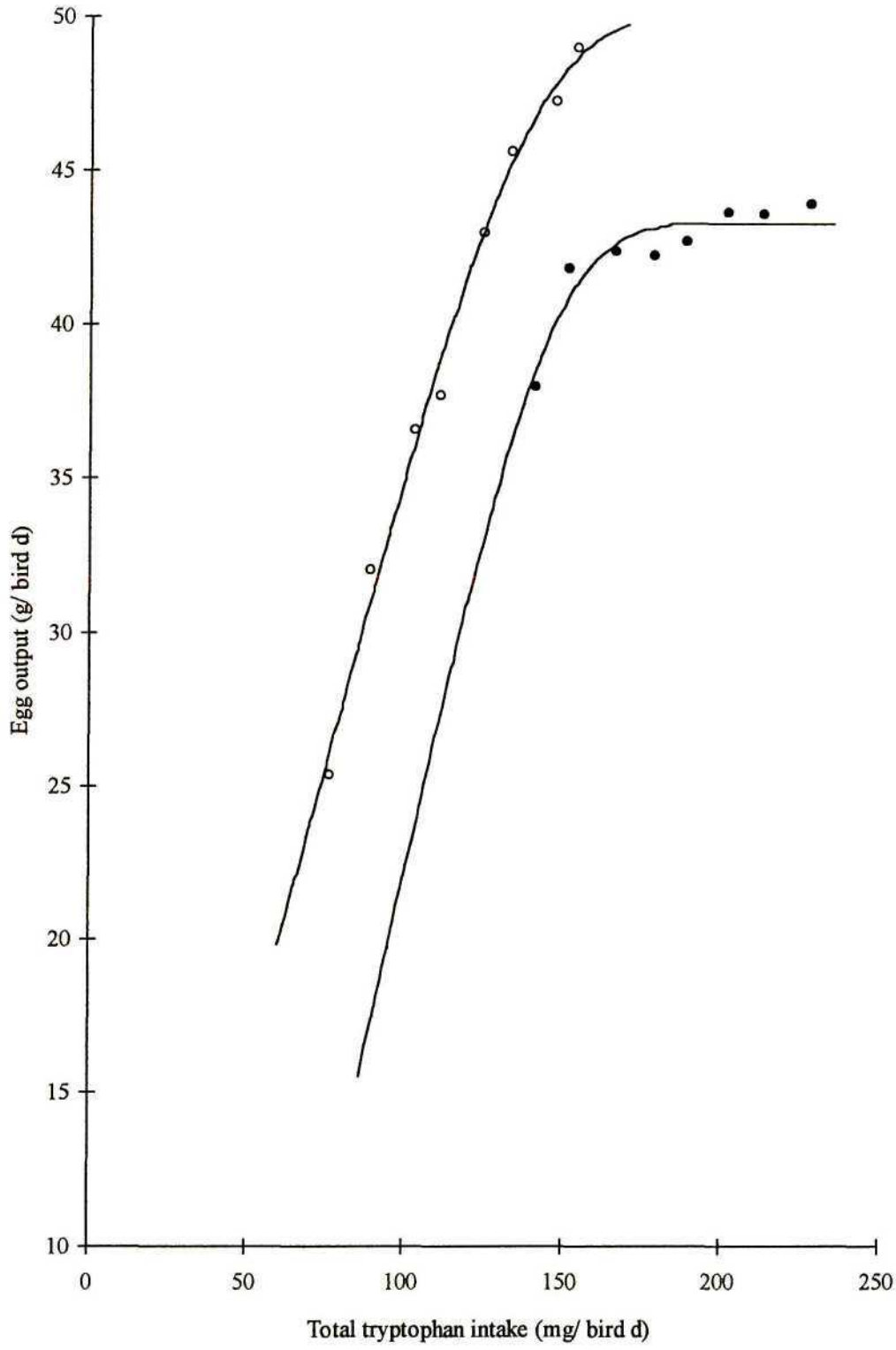

FIG. 5.2 —*The individual responses in egg output of the laying hens (○) and broiler breeders (●) to total tryptophan intake*

The separate response curves were derived by fitting the Reading Model to the treatment means using the parameters shown in Table 5.7. The coefficients of response, together with the residual mean squares (RMS), are given in Table 5.8. From the graph of the response of the broiler breeders to total tryptophan intake, it is obvious that the number of data points were sufficient in order to accurately fit the asymptote, while approximately two points only were used to estimate the slope and the intercept of the curve. As a result of the large degree of leverage exerted by the two points, it is doubtful whether the slope, or the *a* coefficient, is a true reflection of the amount of tryptophan required by the broiler breeders to produce a gram of egg output. Therefore, it is highly probable that the *b* coefficient is inaccurate since the *a* and *b* coefficients are negatively correlated (Fisher *et al.*, 1973). Table 5.8 shows that the *b* coefficient estimated for the broiler breeder data was more than double that estimated for the laying hens, while the *a* coefficient was lower. As a result, it is difficult to make accurate comparisons of the response coefficients between the strains. Therefore, in order to obtain a more precise fit, the results of both the laying hens and broiler breeders were pooled, and common *a* and *b* coefficients fitted, as was suggested by Morris and Wethli (1978). The common response in egg output to total tryptophan intake by the laying hens and broiler breeders is presented graphically (Fig. 5.3). Table 5.8 indicates that the common *a* and *b* coefficients were intermediate between those *a* and *b* coefficients estimated separately. Significantly more of the total variation in egg output was accounted for by fitting the individual response curves than by fitting parallel curves. This implies that there were differences between the two strains in the rates of tryptophan utilisation for maintenance, for egg production, or both (Morris and Wethli, 1978).

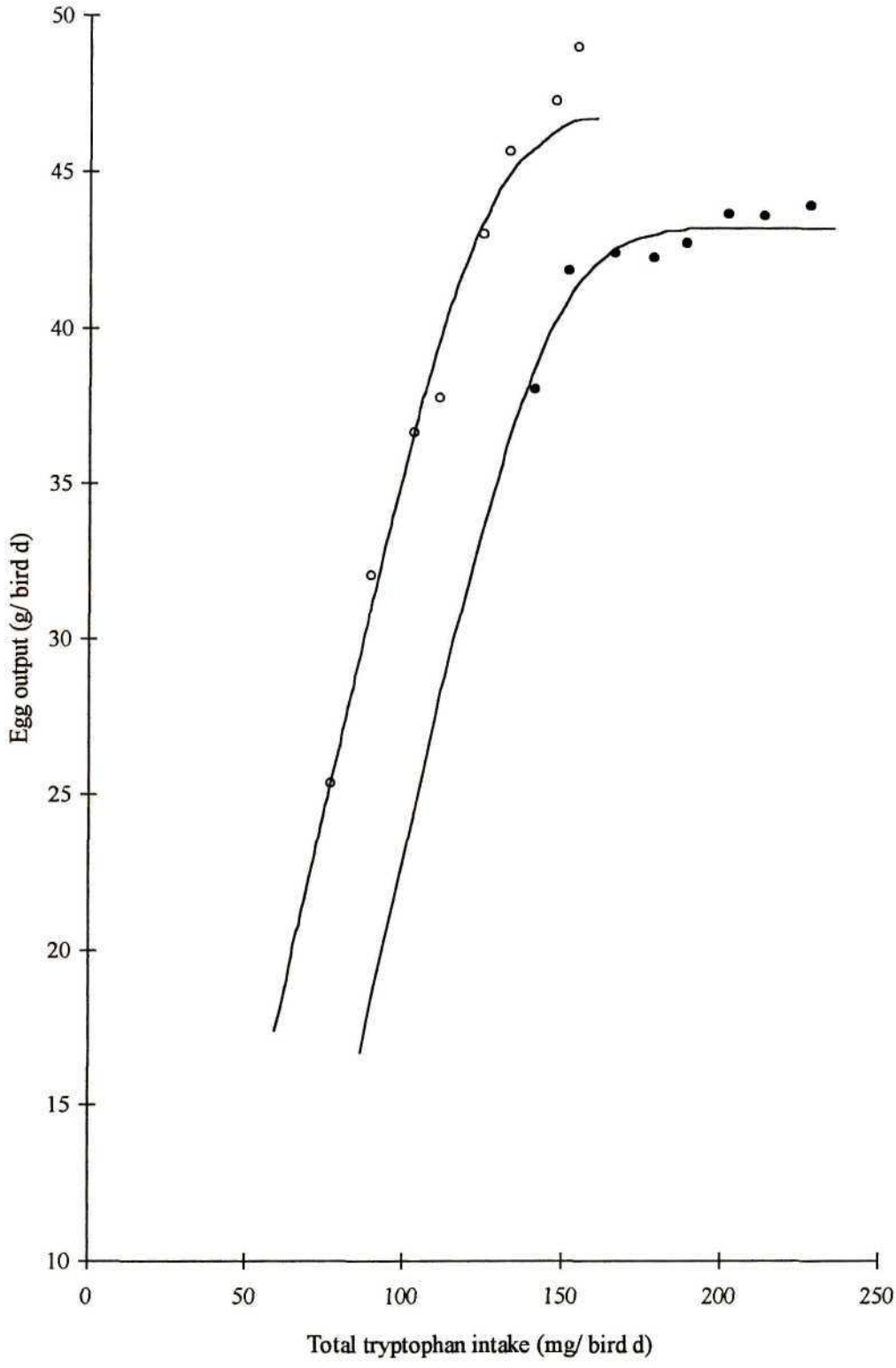

FIG. 5.3 —*The common response in egg output of the laying hens (o) and broiler breeders (•) to total tryptophan intake*

TABLE 5.7

Parameters used to estimate the coefficients of response for the laying hens and broiler breeders for both total and available tryptophan intake

	Laying hens	Broiler Breeders
E_{max} (g/ bird d)	48.98	43.94
δE_{max} (g/ bird d)	9.34	9.06
W (kg)	1.73	4.10
δW (kg)	0.19	0.30
r_{EW}	0.00	0.00

E_{max} = Mean maximum egg output of the flock
δE_{max} = Standard deviation of individual maxima for members of the flock
W = Mean body weight of the flock
δW = Standard deviation of mean body weight (single observation)
r_{EW} = Correlation between egg output and body weight

TABLE 5.8

Coefficients of response estimated for the laying hens and broiler breeders for both total and available tryptophan intake on a live weight basis

	Total tryptophan intake			Available tryptophan intake		
	a	*b*	RMS[1]	*a*	*b*	RMS[1]
Laying hens	2.60	5.00	0.017	2.19	5.94	0.019
Broiler breeders	2.14	12.87	0.015	2.88	1.02	0.014
Pooled	2.20	11.99	0.059	1.89	11.20	0.057

[1]Curves fitted individually, are determined by three parameters: an intercept (*b*W), a slope (*a*) and an asymptote (E_{max}) (Pilbrow and Morris, 1974). Therefore, three degrees of freedom were assigned to curves fitted individually. When both of the response curves were fitted with a common *b* value, the two intercepts, one common slope and two asymptotes effectively absorbed five degrees of freedom

The individual response curves relating egg output of the laying hens and the broiler breeders to available tryptophan intake, appeared to converge at the lower tryptophan intakes (Fig. 5.4). The difference in the shape of the individual response curves for the two strains, in comparison to those determined for total tryptophan intake, could be attributed to the fact that there were too few data points at the lower available tryptophan intakes that could be used to fit the slope and intercept of the broiler breeder response curve precisely.

The steeper slope of the response curve determined for the laying hens, in comparison to the slope of the response to total tryptophan intake, is indicative of the 84 percent availability of dietary tryptophan. This value was estimated by comparing the *a* coefficients determined for both total and available tryptophan intakes (Table 5.8). However, since the tryptophan content of eggs is 2.096mg tryptophan/ g (Lunven *et al.*, 1973), it appears that the conversion of available tryptophan in the diet to tryptophan in the egg is 96 percent efficient. The *b* coefficient increased proportionately with the reduction in the value of the *a* coefficient.

The response coefficients determined using available tryptophan intake for the broiler breeders did not reflect the availability of total tryptophan in the feed. In comparison to the coefficients fitted using total tryptophan intake, the *a* coefficient increased, while the *b* coefficient decreased significantly.

As a result of the imprecision in estimating the *a* and *b* coefficients of the broiler breeder response curve, it was not possible to compare the response coefficients estimated separately for the two strains. Therefore, the data from both the laying hens and broiler breeders were pooled and common *a* and *b* coefficients were fitted using the Reading Model (Table 5.8). The response curves are presented graphically (Fig. 5.5). Again, it is apparent that significantly more of the total variation in egg output was accounted for by fitting the individual response curves than by fitting parallel curves. However, the *a* coefficient was lower than the reported tryptophan content of eggs, indicating that fitting a common slope for the two strains is grossly inaccurate.

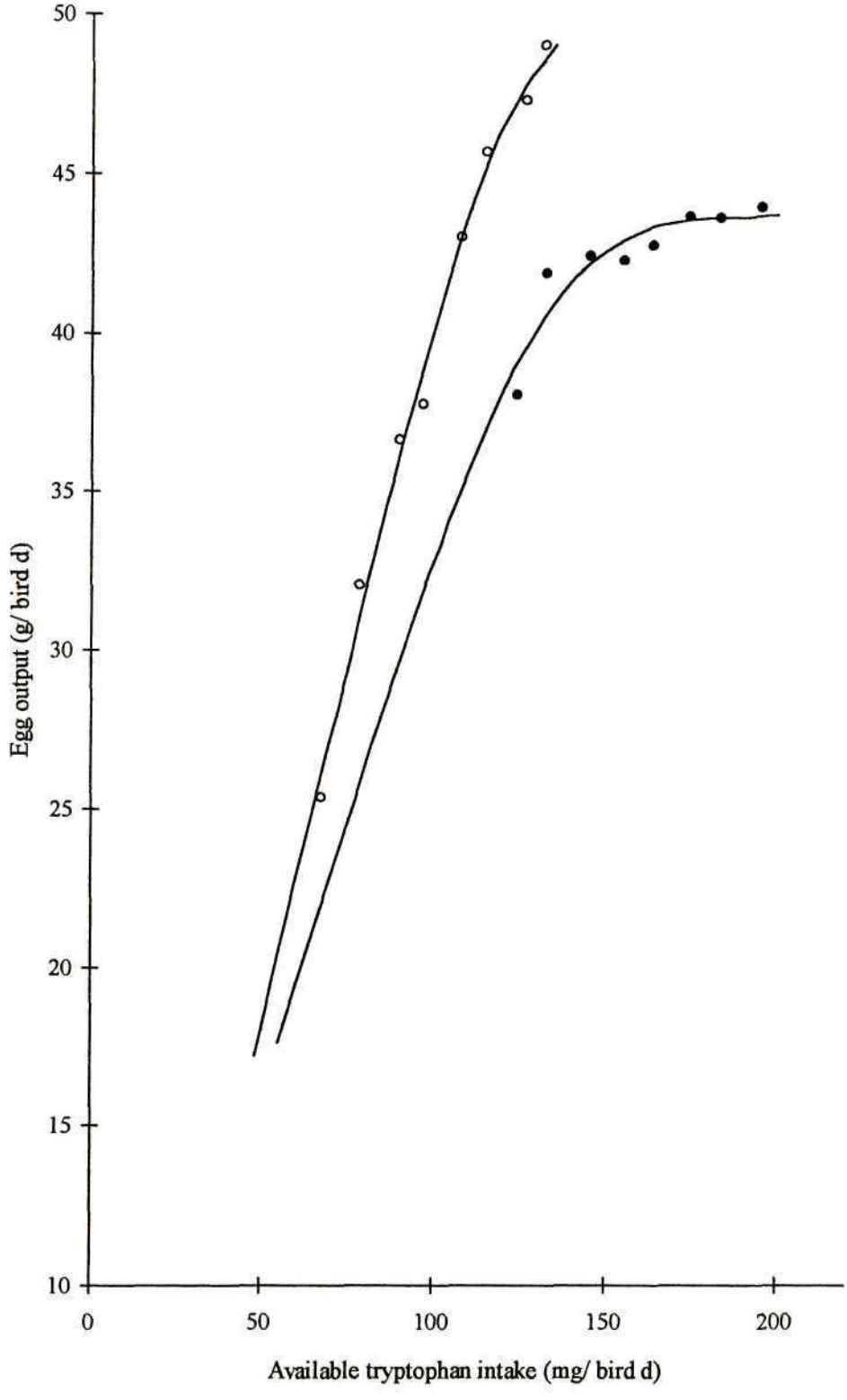

FIG. 5.4 —*The individual responses in egg output of the laying hens (o) and broiler breeders (•) to available tryptophan intake*

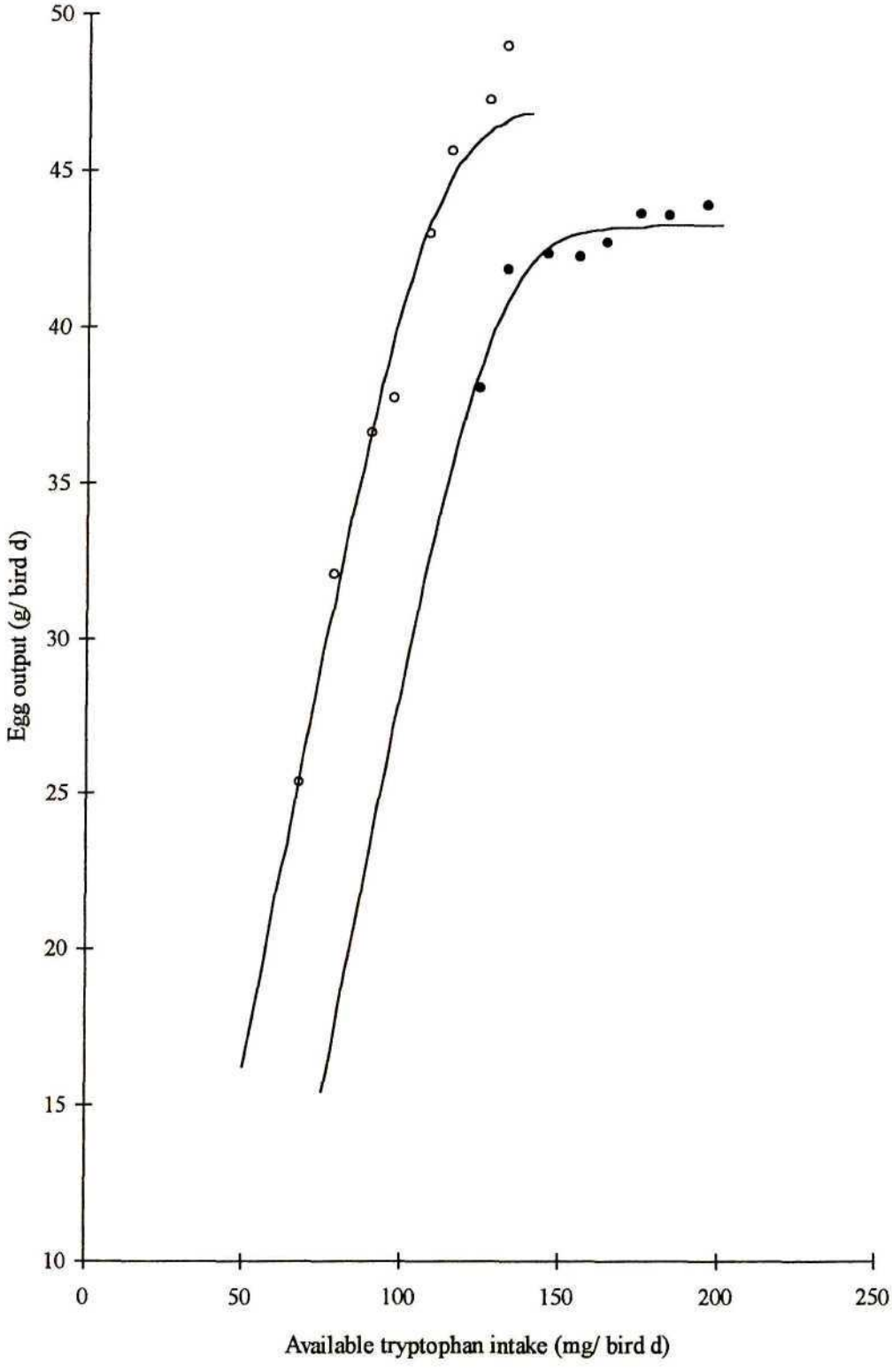

FIG. 5.5 —*The common response in egg output of the laying hens (○) and broiler breeders (●) to available tryptophan intake*

In order to compare accurately the response coefficients determined in the present study with the response coefficients determined previously (Table 5.9), the tryptophan intakes reported by various authors were recalculated using a common matrix of tryptophan contents of the feedstuffs reported in the published papers. In order to calculate the available tryptophan intakes, the digestibilites of tryptophan in the various feedstuffs were determined using the Rhodimet Nutrition Guide (1993) and the resultant values included in the matrix. Individual response curves were fitted using both total and available tryptophan intake. Common *a* and *b* coefficients were subsequently fitted to the data from the six sources. Table 5.10 outlines the resultant response coefficients and residual mean square values. The variation in both the *a* and *b* values estimated for total and available tryptophan intake, among various experiments, was significant.

TABLE 5.9

Coefficients of response for tryptophan previously estimated by various authors

Author	Experiment	Strain	*a*	*b*
Morris and Wethli (1978)	1	Shaver	2.20	17.06
		Warren	1.89	19.77
	2	Shaver	2.26	4.34
		Arbor Acres	2.09	13.08
Wethli and Morris (1978)	4	Shaver	2.12	4.51
du Preez and Duckitt (1984)		Amberlink	2.02	8.26
McDonald and Morris (1985)[1]			2.62	11.20

[1]Coefficients were derived by pooling the data collected by the aforementioned authors

TABLE 5.10

Coefficients of response for both total and available tryptophan intake determined using a common matrix

			Total			Available		
Author	Experiment	Strain	*a*	*b*	RMS	*a*	*b*	RMS
Morris and Wethli (1978)	1	Shaver	2.38	15.91	0.038	1.96	9.78	0.032
		Warren	1.96	21.13	0.007	1.69	13.83	0.006
	2	Shaver	2.26	6.59	0.002	1.90	2.05	0.001
		Arbor Acres	2.13	14.33	0.007	1.83	8.86	0.008
Wethli and Morris (1978)	4	Shaver	1.60	22.00	0.012	1.36	15.53	0.012
du Preez and Duckitt (1984)		Amberlink	2.11	8.79	0.007	1.49	11.69	0.006
	Pooled[1]		2.23	9.93	0.065	1.81	8.57	0.091
	Pooled[2]		2.22	10.34	0.059	1.84	8.93	0.094

[1]Six response curves fitted with a common slope (13DF)

[2]Eight response curves (Experimental data included) fitted with a common slope (17DF)

Pooling the data from the six sources for total tryptophan intake, resulted in *a* and *b* coefficients that were lower than those estimated by MacDonald and Morris (1985), using a similar procedure. This could be due to the additional data source (Wethli and Morris, 1978) which was used in the present study. It is also possible that the computer programme used to fit the response curves in the present study may have been more accurate than the programme developed to fit the Reading Model over a decade ago since a more sensitive criterion was used in determining when the minimum was reached in the iterative procedure. Including the laying hen data did not alter the coefficients or the residual mean square value significantly. Interestingly, including the broiler breeder data decreased the residual mean square value.

The coefficients derived by fitting common *a* and *b* coefficients to the six, and then the eight data sources, respectively, for available tryptophan intake, were not significantly different.

The parameters outlined in Table 5.11 and the coefficients derived by pooling all the data sets were then used to calculate the optimum daily total and available tryptophan intakes of a population of laying hens and a population of broiler breeders. The cost of supplying tryptophan was estimated by formulating a series of least-cost diets for layers, using current (1998) ingredient prices, with tryptophan contents of 10-40g/kg. A linear relationship was obtained between price/kg and tryptophan content, the slope of which, or the marginal cost of tryptophan, was 24.51c/g. The marginal revenues for the laying hens and broiler breeders were calculated to be 0.955c/g and 1.528c/g, respectively. Optimum intakes were calculated by applying the equation explained by MacDonald and Morris (1985):

$$I = aE_{max} + bW + x\sqrt{a^2\delta^2_{Emax} + b^2\,\delta^2_{W} + 2ab\; r_{EW}\delta_{Emax}\,\delta_{W}}$$

where I = total or available tryptophan intake (mg/ bird d), *a* = mg tryptophan/g egg output, *b* = mg tryptophan/kg body weight, E_{max} = mean maximum egg output, δE_{max} = variance of maximum egg output, W = mean body weight, δW = variance of body weight, *x* = standard normal deviate corresponding to an area *ak* in one tail of the normal distribution, where *ak* is the marginal cost of 1mg of amino acid/marginal

revenue of 1g egg output and $r_{EW} = 0$ (correlation between egg output and body weight).

TABLE 5.11

Parameters used to determine the optimum total and available tryptophan intakes of laying hens and broiler breeders

	Laying hens	Broiler Breeders
a	2.22	2.22
b	10.34	10.34
E_{max} (g/ bird d)	55.0	55.0
δE_{max} (g/ bird d)	9.3	9.0
W (kg)	1.7	4.1
δW (kg)	0.2	0.3
r_{EW}	0.0	0.0

a = mg tryptophan/ g egg output
b = mg tryptophan/ kg body weight
E_{max} = Mean maximum egg output of the flock
δE_{max} = Standard deviation of individual maxima for members of the flock
W = Mean body weight of the flock
δW = Standard deviation of mean body weight (single observation)
r_{EW} = Correlation between egg output and body weight

Optimum total tryptophan intakes for a flock of laying hens and broiler breeders were estimated to be 173 and 200 mg/ bird d, respectively. At daily food intakes of 110 and 160g/ bird d, the concentrations of tryptophan in the diets of laying hens and broiler breeders, would be 1.6 and 1.3g/kg, respectively, which are similar to the values typically used in formulating feeds at the University of Natal. The optimum available intakes for the flock of laying hens and broiler breeders, were 145 and 170mg/ bird d, respectively. It appears that the higher marginal revenue for broiler breeder eggs influenced the optimum tryptophan intake. In comparison to the optimum intakes of the laying hens, the high value of broiler breeder eggs increased the optimum total and available tryptophan intakes by approximately two percent.

Calculations based on lipid-free body weight. It has been suggested that a more accurate estimate of the amount of an amino acid required for maintenance, when comparing hens of different size and body composition, would be related to the protein content (Emmans and Fisher, 1986), or the lipid-free content of the body. Table 5.12 details the live weights, feather-free body weights and body lipid contents of the two strains on the three treatments, from which the lipid-free body weights were calculated. The lipid-free body weights of the laying hens and broiler breeders in the remaining treatments were estimated by linear interpolation of lipid-free body weight and live weight measured for the three tryptophan treatments.

In order to reduce error variation, mean lipid-free body weight of treatments one and four only were substituted for live weight in the Reading Model and the response curves fitted as described previously. The parameters : W = 1.49kg; δW = 0.15kg and W = 3.24kg; δW = 0.32kg, for the laying hens and broiler breeders respectively. Table 5.13 details the resultant response coefficients for both strains of hens and for total and available tryptophan intake.

TABLE 5.13

Coefficients of response estimated for the laying hens and broiler breeders for both total and available tryptophan intake on a lipid-free weight basis

	Total tryptophan intake			Available tryptophan intake		
	a	*b*	RMS[1]	*a*	*b*	RMS[1]
Laying hens	2.60	5.78	0.017	2.19	6.88	0.019
Broiler breeders	3.31	1.04	0.015	2.96	0.26	0.014
Pooled	2.13	15.68	0.057	1.82	14.72	0.057

[1]Curves fitted individually, are determined by three parameters: an intercept (bW), a slope (a) and an asymptote (E_{max}) (Pilbrow and Morris, 1974). Therefore, three degrees of freedom were assigned to curves fitted individually. When both of the response curves were fitted with a common b value, the two intercepts, one common slope and two asymptotes effectively absorbed five degrees of freedom

TABLE 5.12

Mean live weight, feather-free body weight, body lipid content and lipid-free body weight of the laying hens and broiler breeders measured on three tryptophan concentrations, and the lipid-free body weights estimated by linear regression with live weight for the remaining treatments, at the end of the ten-week trial period

		Laying hens				Broiler breeders			
Treatment	Tryptophan content (g/kg)	Live weight (kg)	Feather-free body weight (g/kg)	Body lipid content (g/kg)	Lipid-free body weight (kg)	Live weight (kg)	Feather-free body weight (g/kg)	Body lipid content (g/kg)	Lipid-free body weight (kg)
Measured[1]									
1	1.39	1.80[a]	1.70[a]	144.50[a]	1.55[a]	4.18[a]	4.06[a]	230.70[a]	3.24[a]
4	1.17	1.69[a]	1.60[a]	149.30[a]	1.45[a]	4.19[a]	4.06[a]	234.40[a]	3.24[a]
8	0.89	1.53[b]	1.46[b]	157.70[b]	1.29[b]	4.016[b]	3.89[b]	257.30[b]	3.01[b]
Estimated[2]									
2	1.32	1.75			1.49	4.02			3.09
3	1.24	1.70			1.45	4.08			3.13
5	1.10	1.70			1.45	4.14			3.17
6	1.03	1.68			1.44	4.04			3.10
7	0.96	1.63			1.40	3.92			3.01
Constant			0.147 ± 0.102 [NS]				0.186 ± 0.529 [NS]		
Linear coefficient			0.767 ± 0.060				0.722 ± 0.128		
R^2			0.93				0.71		

[1]Live weight: Residual S.E. (24df) 0.24; Feather-free body weight: Residual S.E. (24df) 0.23; Body lipid content: Residual S.E. (24df) 32.25; Lipid-free body weight: Residual S.E. (24df) 0.18; [2]Live weight: Residual S.E. laying hens (226df) 0.19, broiler breeders (231df) 0.30

[a-b]Means within columns with letters in common are not significantly different ($P<0.05$); [NS]Not significantly different from zero ($P<0.05$)

In the case of the laying hens, the *a* coefficient estimated using live weight was the same as that estimated on a lipid-free basis. This indicates that it is in fact the maintenance requirement that is altered depending on the basis of weight used. The *b* coefficients estimated for both total and available tryptophan were 14 percent lower compared to the respective coefficients determined on a live weight basis. Therefore, it appears that more tryptophan was required per kg lipid-free body weight than per kg live weight.

The change in the body weight parameter had a profound influence on both of the response coefficients estimated for the broiler breeders. The *a* coefficients estimated for total and available tryptophan intake increased, whilst the *b* coefficients decreased, in comparison to those estimated on a live-weight basis.

The pooled results indicated that the requirement for tryptophan per gram of egg output was independent of the basis of weight used while the amount of tryptophan required to maintain body weight, was greater when expressed on a lipid-free basis than when expressed on a live weight basis. However, the *a* coefficient determined for available tryptophan was lower than the reported tryptophan content of eggs. Therefore, it appears that the response by the broiler breeders was erroneous, and that the response coefficients determined for the laying hens should be applied to the broiler breeders until further research can be conducted.

As a result of substituting lipid-free body weight for live weight in the Reading Model, the maintenance requirements per kg weight increased for both total and available tryptophan. The subsequent increases in the optimum total and available tryptophan requirements of the laying hens were substantial. Total intake increased to 188mg/ bird d, while available intake increased to 160mg/ bird d. The concentrations of tryptophan increased to 1.7 and 1.5g/kg, respectively. The optimum total and available intakes of the broiler breeders increased marginally to 203 and 177mg/ bird d, respectively. Since the lipid content of the broiler breeders was significantly greater than that of the laying hens, it appears that the small change in the optimum intakes were not a truly representative response.

Net efficiency of tryptophan utilisation

The net efficiency of tryptophan utilisation (NE) was calculated using the data for each of the laying hens and broiler breeders in the trial, respectively according to the equation:

$$NE = \frac{E}{(I - bW)/a}$$

where E = egg output (g/ bird d), I = available tryptophan intake (mg/ bird d), b = mg of available tryptophan required per kg lipid-free weight, W = lipid-free body weight and a = mg of available tryptophan required per gram egg output. The a and b coefficients used for the laying hens were 2.19mg available tryptophan/ g egg output and 6.88mg available tryptophan/ kg lipid-free weight, respectively. Lipid-free body weight for each of the hens in the trial, was determined by substituting the individual body weights for the dependent variable *(x)* in the linear models outlined in Table 5.12. Since the response coefficients determined for the broiler breeders were erroneous, the a and b coefficients determined on a live weight basis for available tryptophan, by pooling the data sources listed in Table 5.9 with the experimental data, were substituted into the equation for the breeders. As this b coefficient refers to body weight and not lipid-free body weight, the calculation of net efficiency of tryptophan utilisation, in this case, was based on live weight. The values were 1.84mg available tryptophan/ g egg output and 8.93mg available tryptophan/kg weight. Net efficiency was then plotted against rate of laying (Figs. 5.6 and 5.7). Efficiency of the laying hens reached a maximum value of 85 percent which was the same as that determined by MacDonald and Morris (1985), for birds laying in closed cycles. Below a rate of laying of 0.5eggs/ bird d, efficiency of utilisation declined to approximately zero at zero egg production. The 13 data points with efficiencies of utilisation above 1, can be regarded as outliers. It is possible that these values resulted from inaccuracies in the measuring of either food intake or body weight. The net efficiency of utilisation of tryptophan by the broiler breeders was approximately 65 percent. Again, the six data points with efficiencies of utilisation above 1, can be regarded as outliers. Therefore, it would seem that broiler breeders require a greater amount of tryptophan per gram of

egg output, than laying hens in order to maximise laying performance. However, the use of the response coefficients determined on a live weight basis to estimate the efficiency of utilisation of tryptophan is inaccurate. Broiler breeders consist of a higher concentration of body lipid than laying hens. Since the lipid fraction has no amino acid requirement for maintenance (Emmans and Oldham, 1989), the use of body weight as an indicator of the maintenance requirement for tryptophan results in artificially elevated estimates. Since the *a* and *b* coefficients are negatively correlated, the *a* coefficient is lowered.

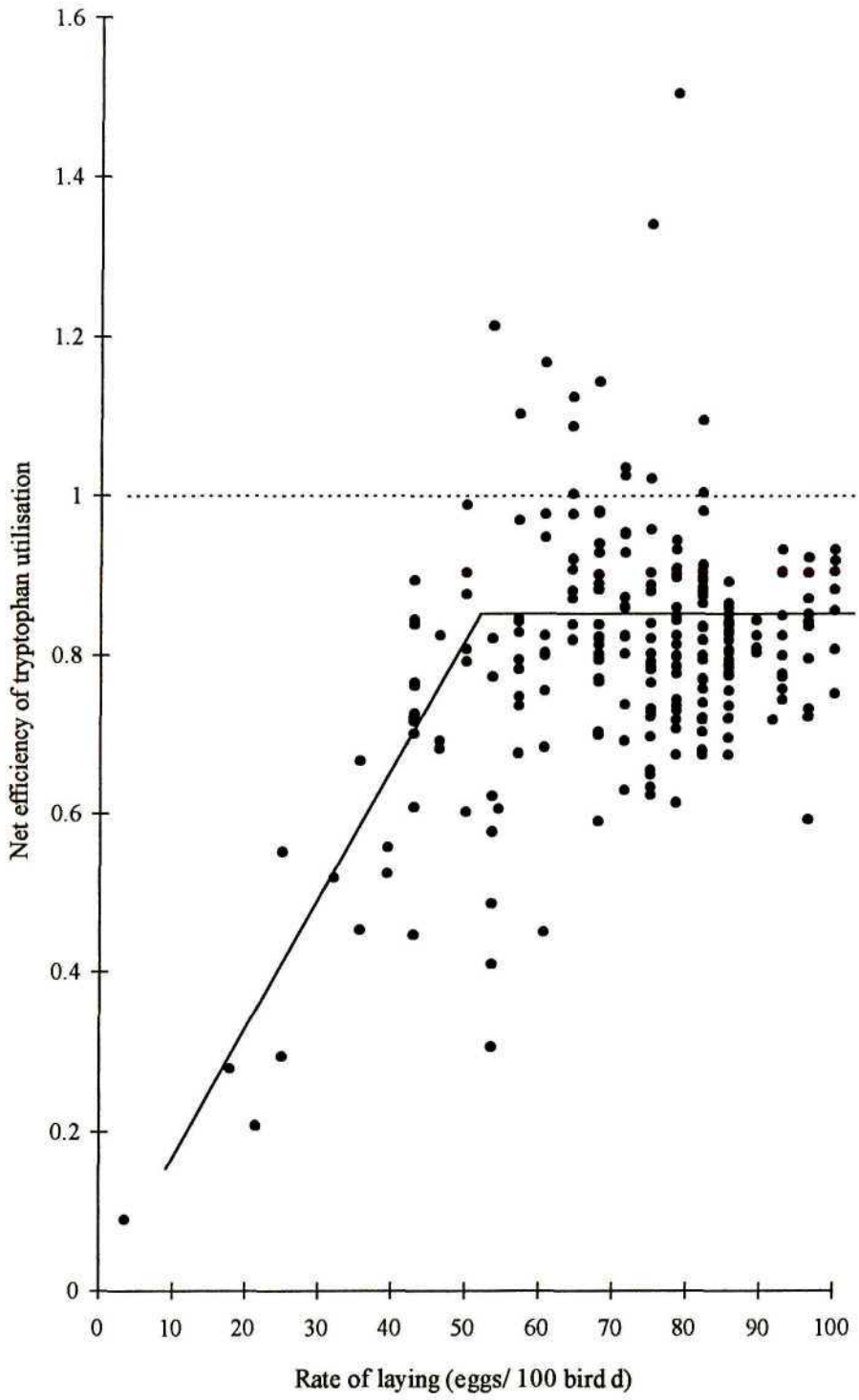

FIG. 5.6 —*Net efficiency of tryptophan utilisation of the laying hens calculated on a lipid-free basis*

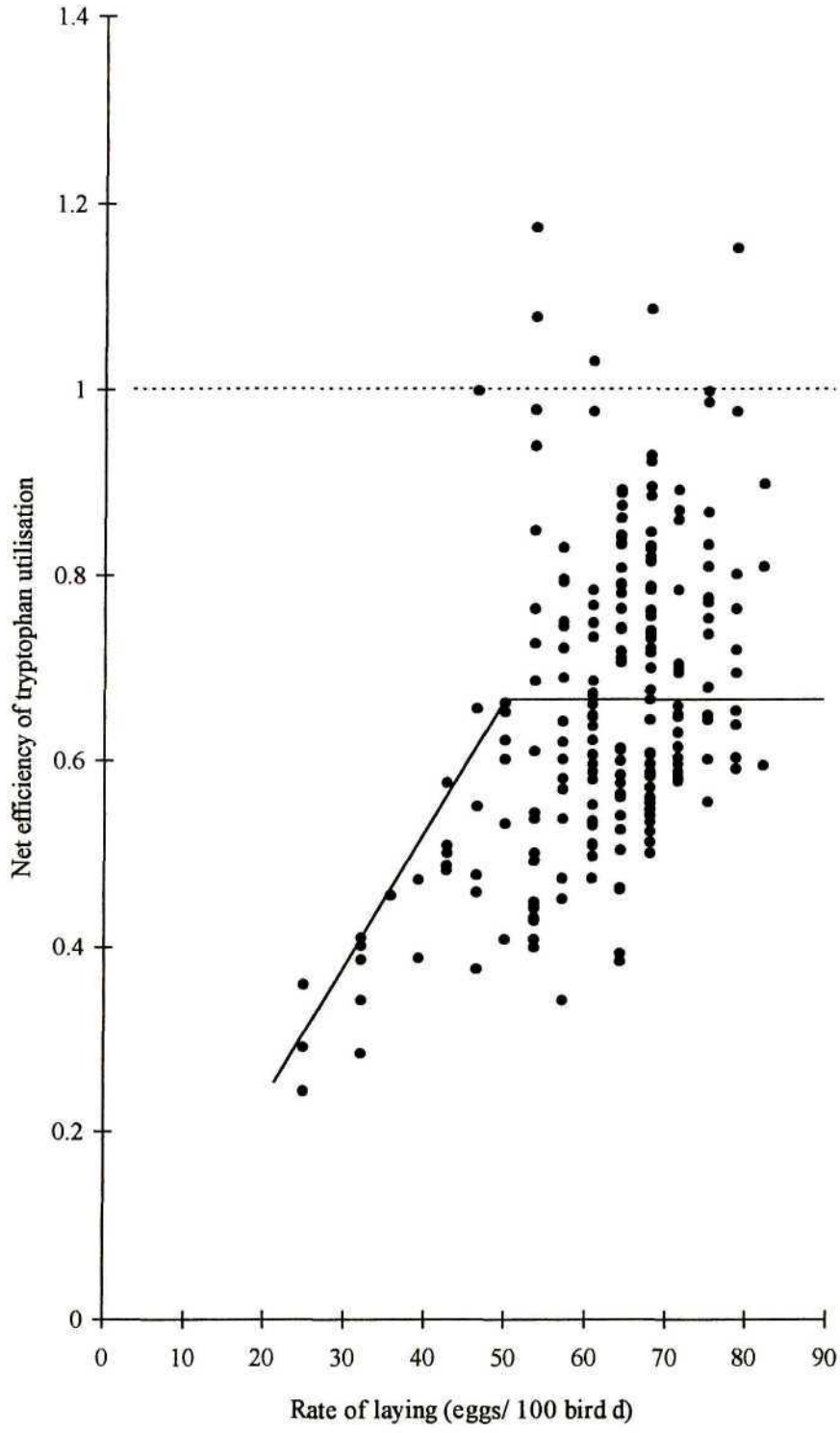

FIG. 5.7 —*Net efficiency of tryptophan utilisation of the broiler breeders calculated on a live weight basis*

CHAPTER 6

GENERAL DISCUSSION

The use of linoleic acid intake to manipulate egg weight

As a result of the rapidity of the response in egg weight to linoleic acid intake, the adjustment of the linoleic acid content of laying rations, as a means of manipulating egg weight, may be a viable option. By comparing the present experimental results with those estimated by quantitative analysis of the published data, it was determined that egg weight may be altered by 0.8g for each gram change in daily linoleic acid intake, regardless of strain or age of the hen. Adjusting linoleic acid concentrations is made easy by the fact that sunflower or maize oil is extremely rich in the fatty acid, as is rice pollard (Balnave, 1982). However, the differences in the response in rate of laying to linoleic acid intake between the two strains emphasises the danger in extrapolating the results of research carried out on laying hens to broiler breeders, without confirmatory experimentation. The observation that rate of laying in broiler breeders is increased by an additional two eggs/ 100 bird d for a 1g increase in linoleic acid intake/ bird d, has not been reported previously and warrants further investigation.

The present commercial trend is to bring both egg laying strains and broiler breeders into lay at a relatively young age with the concomitant production of small, sometimes unmarketable eggs. Increasing the proportion of linoleic acid in the diets of these birds could reduce the proportion of small eggs that are produced, and increase the number of settable eggs respectively. In addition, egg production of broiler breeders would appear to be improved by increasing dietary linoleic acid intake. However, since it appears that only the albumen content of eggs is influenced by linoleic acid intake, further research needs to be conducted to establish whether hatchability is indeed improved. A reduction in linoleic acid content late in the laying cycle of broiler breeder hens would reduce egg size and possibly improve overall hatchability, but may also reduce rate of laying.

The influence of nutrient intake and 20 week body weight on early laying performance

The poor laying performance of broiler breeder hens during the early laying period appears to be linked to nutrient intake. It was shown that major discrepancies exist between the energy and amino acid requirements recommended by various breeding companies, and those calculated theoretically, in the amounts and in the patterns of change of nutrient requirement with age. During the pre-peak production period, the calculated requirements for lysine, methionine and energy were shown to be substantially lower than the recommended requirements. The overfeeding of nutrients has possibly lead to a high incidence of multiple ovulations, defective eggs and erratic ovipositions (Yu *et al.*, 1992). Therefore, feeding broiler breeders in excess of the daily food intakes stipulated in the production manuals in order to achieve set peak production goals, or even feeding the recommended amounts, seem to be contributing to the poor peak performance observed in the industry.

Over the post-peak period, it was illustrated that the calculated energy requirements increased, while the calculated amino acid requirements remained constant with the respective requirements recommended by the breeding companies. Therefore, adjusting food intake according to the level of production or age of the bird, as is stipulated in the production manuals, would appear to be unjustified since this assumes that the requirements for energy and protein change concomitantly. Therefore, it is evident that the change in the energy and amino acid requirements of broiler breeder hens with age warrants further investigation.

It was determined experimentally that it is possible to improve substantially the laying performance of young broiler breeders. However, the finding that 20 week body weight does not influence performance parameters such as peak production and post-peak egg weight, egg production, egg output and body weight gain has important implications for the broiler breeder industry. Much emphasis is placed on rearing broiler breeders to a standard weight with a low flock coefficient of variation at 20 weeks of age. However, it appears that birds weighing between 1900 and 2500g lay equally well up to 36 weeks of age, even though the range in age at sexual maturity between the lightest and heaviest birds was five days. These observations confirm

those of Robinson *et al.* (1995) and Fisher (1998 b), who found that an increase in body weight of 30% at 20 weeks had little effect on reproductive performance. Therefore, it is plausible that increased body weight targets, within limits, could be introduced to improve bird welfare and to simplify management (Hocking, 1996).

The nutrient density of the feeds allocated to the broiler breeders influenced all the measured variables other than age at peak production and oviduct weight. The high nutrient density feed advanced age at sexual maturity and increased body weight at point of lay. Regardless of the amount of food allocated, the hens consistently performed better on a higher nutrient density feed. However, this was associated with an increase in body weight, in body lipid gain, and in ovary weight.

Although the amount of food allocated only exerted an effect on the birds from 31 weeks of age, when differences in allocation were marked, the high peak production achieved indicates that it is not necessary to feed in excess of 150g/ bird d during this time. During the post-peak period, an increase in food allocation increased egg production, egg output, body weight and body weight gain in a positive and linear manner. As a result, maximum production in this experiment was achieved by feeding 180g/ bird d of H, but the increase in performance over intakes lower than this were so small that the highest intakes are unlikely to be justified economically.

The responses in egg output, egg weight and rate of laying to both energy and amino acid intake were positive and linear. The mean egg output of 52.1g/ bird d during the post-peak period was attained on a constant energy intake of 1898kJ/ bird d, a lysine intake of 1047mg/ bird d and a methionine intake of 434mg/ bird d. However, it can be argued, that since the experiment was carried out on birds in cages, the high egg production rate achieved would not be replicable in a commercial situation. This is due mainly to the fact that birds housed on the floor are more active and have a potentially higher nutrient requirement. Nevertheless, birds in individual cages loose more heat to the environment than when housed together on the floor and would, therefore, require a greater energy intake to maintain their core temperatures. However, it was beyond the scope of the experiment to recommend nutrient requirements of broiler breeders. This is especially in light of the fact that the energy and amino acid intakes were highly correlated due to the nature of the nutritional

treatments imposed. Of greater importance is the fact that the breeders performed consistently well during the post-peak period on a constant, and not a diminishing daily energy intake, and on a substantially lower mean lysine and methionine intake as is recommended by Ross Breeders Ltd. (1995).

Nutrition is of vital importance to the successful onset of laying and to subsequent performance. Even though it appears that high reproductive rates are associated with concomitant body weight or specifically body lipid gains, it must be emphasised that the subsequent effects on fertility and egg output later in the laying cycle were not determined. In other words, a certain amount of caution should be exercised when extrapolating the effects of the nutrient intake on performance presented here, to birds older than 36 weeks of age.

The influence of broiler breeder age, or egg size, on pre- and post-natal growth of broilers

Although the genetic potential of pre-natal broilers within differently sized eggs is similar, the results of the experiment have shown that small pre-incubation egg size does have a slowing effect on embryonic growth midway through the incubation process. It is doubtful whether this growth restriction can be attributed soley to the space within the egg shell, as was suggested by Wiley (1950). However, it appears that the proportionally lower weight of the residual yolk supply, and the slower rate at which this is was absorbed were important contributing factors. As a result, the chicks hatched out from the small eggs were significantly lighter than chicks hatched out from the larger eggs.

The smaller chicks were incapable of attaining the same rate of growth, and hence body weight, as the larger chicks throughout the three week period. However, if the growth results of the small chicks on H and the jumbo chicks on L were extrapolated to six weeks, the difference in weight would be about 110 g. Since the smaller chicks would be growing at approximately 70 g/d, a one and a half day delay before slaughter would presumably equal out the total weight differences which became evident during embryonic development.

Therefore, during times of chick shortage, it would be advisable to set small

eggs and for broiler producers to grow chicks weighing between 25 and 40g at day-old. This is especially in light of the fact that smaller chicks are as efficient in converting nutrient intake to body weight gain as larger chicks. However, there are some managerial implications during both the pre- and post-natal periods that are worth considering.

Since the embryo *per se* appears to have a limited ability to compensate for low water loss (Swann and Brake, 1990), small eggs should be set separately and water loss, as a proportion of initial egg weight, carefully monitored in order to optimise embryonic growth and the rates of hatchability. It has been suggested that a lower wet-bulb temperature would be able to overcome the water loss resistance of the thicker cuticle-eggshell-membrane complex, combined with the thicker albumen of the small eggs laid by young flocks (Vick *et al.*, 1993). Removal of the thick cuticle would also enable an increase in the proportion of water lost from the eggs (Peebles and Brake, 1987). However, incubator hygiene would need to be improved to prevent bacterial infection.

From day-old, the small chicks should remain segregated from the larger chicks. This is in order to prevent unequal competition for feed among the differently sized chicks throughout the growing period. Small chicks would also have to be placed as soon after hatching as possible as they have a proportionally lower residual yolk or nutrient supply. Transportation in excess of a day after hatching could have a detrimental effect on subsequent growth performance. Smaller chicks are also more stressed initially and possibly have a higher temperature requirement. Therefore, greater managerial input would be essential in order to ensure that growth performance of the chicks was not further limited by environmental factors. Lastly, uniformity between broilers hatched out of differently sized eggs would be improved if the small broilers were maintained on a higher protein feed than the starter feed typically used commercially.

The scope for future research is vast. Of particular interest would be the manipulation of the yolk or nutrient supply of the embryos within eggs laid within the first few weeks of production. Increasing the size of the yolk significantly by increasing the protein or amino acid intake of the young female broiler breeder beyond the optimum would not be a viable option. However, since it is possible to influence

the yolk fatty acids through dietary manipulation of broiler breeders (Latour *et al.*, 1994), it may be possible to increase the proportions of the vital lipid fractions and influence yolk fatty acid metabolism (Latour *et al.*, 1998) in embryos and in newly hatched chicks from small eggs. The effects on pre- and post-natal growth and hatchability would need to be established. Further research also needs to be conducted during the first week post-hatch in order to improve the relative growth rates of the small chicks. Temperature trials in a controlled environment would determine whether growth performance could be improved at higher temperatures. Amino acid and energy response trials conducted during the first three weeks post-hatch would fine-tune the nutritional requirements of the smaller chicks and lead to the optimisation of growth performance.

A comparison of the response of laying hens and broiler breeders to tryptophan intake

The objective of the tryptophan response trial, to compare the responses of individually housed broiler breeders and laying hens to total and available tryptophan intake, on a live weight basis and on a lipid-free basis, was not met. This is because there were too few data points at the lower tryptophan intakes that could be used to fit precisely the slopes and the intercepts of the respective broiler breeder response curves. As a result, the coefficients of response determined for the broiler breeders by means of the Reading Model (Fisher *et al.*, 1973) appear to be erroneous. It is evident that an additional broiler breeder response trial needs to be conducted in which a far wider range of tryptophan intakes is used, in order to compare the tryptophan requirements and coefficients of response among commercial laying hens and broiler breeders.

By pooling the experimental data with those previously published by various authors, a reasonable estimate of the response coefficients for total and available tryptophan were determined. The *a* coefficients obtained were 2.22 and 1.84mg tryptophan/g egg output, while the *b* coefficients were 10.34 and 8.93mg tryptophan/kg body weight, respectively. Optimum total tryptophan intakes for a flock of laying hens and broiler breeders with egg outputs of 55g/ bird d, and weighing 1.7 and 4.1kg, respectively, were estimated to be 173 and 200mg/ bird d. The

optimum intakes of available tryptophan were 145 and 170mg/ bird d, respectively.

In the case of the laying hens, it was shown that maintenance requirements for total and available tryptophan were dependent on the basis of weight used. As much as 14 percent more tryptophan was required per kg lipid-free body weight than per kg live weight. Since the lipid content of broiler breeders is significantly higher than that of laying hens, of particular interest would be to establish the maintenance requirements of broiler breeders for tryptophan on a lipid-free basis.

The net efficiency of available tryptophan utilisation of laying hens, calculated on a lipid-free basis, was 85 percent, which is the same as that estimated by McDonald and Morris (1985). However, the pooled response coefficients used to estimate the net efficiency of tryptophan utilisation for broiler breeders were based on live weight, since those estimated for the breeders on a lipid-free basis were erroneous. As a result, it is doubtful whether the value of 65 percent is a true reflection of the efficiency with which broiler breeders utilise available tryptophan. Again, it is obvious that the response in egg output of broiler breeders to a broader range of tryptophan intake warrants further investigation.

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Relative growth rates the chicks produced from eggs of four weight categories, on three feed treatments, for each week of the trial

Week 1[2]	Feed H	Feed L	Choice of H and L	Mean
Small	0.132	0.125	0.136	0.131
Medium	0.142	0.136	0.139	0.139
Large	0.135	0.128	0.136	0.133
Jumbo	0.127	0.116	0.133	0.125
Mean	0.133	0.126	0.136	
Week 2[3]				
Small	0.139	0.139	0.144	0.410
Medium	0.134	0.133	0.137	0.135
Large	0.130	0.129	0.131	0.130
Jumbo	0.126	0.124	0.131	0.127
Mean	0.132	0.131	0.136	
Week 3[4]				
Small	0.119	0.105	0.109	0.111
Medium	0.106	0.106	0.103	0.105
Large	0.102	0.102	0.099	0.101
Jumbo	0.098	0.100	0.095	0.098
Mean	0.106	0.103	0.102	

[2]Residual S.E. (35df) 0.01
[3]Residual S.E. (35df) 0.007
[4]Residual S.E. (35df) 0.004
Size x diet interactions NS for weeks 1 and 2; ($P<0.05$) week 3
[1] Refer to Chapter 4

APPENDICES

[1]APPENDIX TABLE 1

Mean rate of lay (eggs/ 100 bird d) of birds laying in closed cycles between 31 and 36 weeks of age

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	83.61
Density									L	80.70
Weight									Weight	
1	79.76	78.91	81.94	80.32	86.98	80.95	85.71	83.50	82.26	
2	85.08	80.78	84.18	80.32	82.23	80.36	87.07	81.46	82.69	
3	81.03	76.19	81.38	80.79	81.50	82.38	86.39	82.48	81.52	
Allocation	80.29		81.49		82.40		84.44			

Residual S.E. (311) 3.24
[1] Refer to Chapter 3

[1]APPENDIX TABLE 2

Mean egg output (g/ bird d) of birds laying in closed cycles between 31 and 36 weeks of age

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	53.42
Density									L	50.87
Weight									Weight	
1	50.21	48.56	52.81	49.54	55.71	51.04	54.71	53.97	52.07	
2	52.92	51.03	54.53	51.57	53.18	49.63	54.90	51.52	52.41	
3	51.67	48.41	51.14	51.49	52.99	52.42	56.27	51.22	51.95	
Allocation	50.47		51.85		52.50		53.76			

Residual S.E. (307) 3.24
[1] Refer to Chapter 3

[1]APPENDIX TABLE 3

Mean body weight change (g/ bird d) of birds laying in closed cycles between 31 and 36 weeks of age

	Allocation (g)									
	150		160		170		180			
Nutrient	H	L	H	L	H	L	H	L	H	11.54
Density									L	5.77
Weight									Weight	
1	8.74	3.63	11.44	4.08	11.03	7.19	14.35	9.67	8.76	
2	9.34	2.32	11.22	3.06	12.03	7.06	13.82	11.12	8.75	
3	7.60	3.10	10.91	1.94	12.83	6.87	15.15	9.15	8.44	
Allocation	5.79		7.11		9.50		12.21			

Residual S.E. (313) 3.24

[1] Refer to Chapter 3

[1]APPENDIX TABLE 4

The chemical composition (g/kg) of embryos from three flock ages during incubation

Age (weeks)	Time (days) 10	12	14	16	18	20
Body lipid						
30	4.69	9.60	14.97	30.49	37.44	62.34
35	3.83	9.71	16.56	29.89	40.56	77.12
52	6.73	9.63	16.40	27.88	44.83	76.823
Body water						
30	926.1	910.	878.3	831.	823.4	760.3
35	924.4	909.8	874.1	828.9	820.6	739.3
52	925.0	909.1	876.9	835.40	816.80	749.10
Body protein						
30	49.21	60.13	83.70	114.26	111.82	142.94
35	50.88	60.53	86.23	115.52	112.74	146.65
52	47.33	61.16	83.72	111.21	113.74	138.95
Body ash						
30	8.86	10.47	11.16	12.52	13.44	15.39
35	9.12	10.19	11.39	12.53	14.05	15.53
52	8.30	9.96	10.99	12.10	13.71	15.48

[1] Refer to Chapter 4

[1]APPENDIX TABLE 5

Mean weights of embryos (g) dissected out from differently sized eggs during the incubation period

	Egg weight				
Time (days)	Small	Medium	Large	Jumbo	S.E.
3	0.01	0.02	0.02	0.02	0.01
4	0.05	0.05	0.09	0.08	0.02
5	0.23	0.25	0.27	0.23	0.05
6	0.43	0.53	0.58	0.53	0.08
7	0.83	0.99	1.05	1.12	0.09
8	1.40	1.60	1.54	1.69	0.12
9	2.01	2.23	2.32	2.32	0.21
10	2.88	3.11	3.36	3.48	0.23
11	4.01	4.65	4.86	4.92	0.42
12	6.16	6.60	7.05	7.56	0.41
13	8.39	8.72	9.76	9.96	0.60
14	11.35	13.05	13.64	13.58	0.57
15	13.03	15.41	16.78	17.22	0.97
16	16.90	19.35	20.22	21.23	0.52
17	20.10	24.10	25.15	28.56	0.85
18	21.88	26.92	32.34	33.70	0.82
19	23.90	30.01	33.86	36.81	1.34
20	28.65	33.33	37.88	41.46	1.09

[1] Refer to Chapter 4

[1]APPENDIX TABLE 6

Mean embryo weight (g) expressed as a proportion of initial egg weight (g) for the four egg size categories during the incubation period

	Egg weight				
Time (days)	Small	Medium	Large	Jumbo	S.E.
3	0.0002	0.0003	0.0003	0.0002	0.0001
4	0.001	0.001	0.001	0.001	0.0004
5	0.005	0.004	0.004	0.003	0.0009
6	0.100	0.009	0.009	0.007	0.0012
7	0.018	0.018	0.016	0.015	0.0011
8	0.032	0.028	0.023	0.024	0.0012
9	0.045	0.040	0.035	0.031	0.0030
10	0.060	0.058	0.050	0.047	0.0030
11	0.089	0.081	0.074	0.066	0.0066
12	0.128	0.120	0.110	0.104	0.0086
13	0.178	0.154	0.145	0.136	0.0084
14	0.246	0.234	0.205	0.187	0.0110
15	0.283	0.274	0.252	0.233	0.0156
16	0.373	0.341	0.315	0.283	0.0168
17	0.431	0.431	0.388	0.386	0.0147
18	0.494	0.468	0.480	0.449	0.0139
19	0.540	0.530	0.519	0.500	0.0170
20	0.623	0.591	0.575	0.558	0.0212

[1] Refer to Chapter 4

[1]APPENDIX TABLE 7

Relative growth rates of the embryos in the four egg size categories during the incubation period

	Egg weight				
Time (days)	Small	Medium	Large	Jumbo	S.E.
7	0.504	0.644	0.622	0.812	0.201
8	0.448	0.514	0.385	0.457	0.081
9	0.359	0.342	0.410	0.273	0.120
10	0.375	0.315	0.376	0.435	0.108
11	0.377	0.416	0.372	0.346	0.128
12	0.501	0.331	0.374	0.437	0.117
13	0.271	0.282	0.321	0.277	0.095
14	0.338	0.402	0.336	0.302	0.065
15	0.171	0.198	0.207	0.255	0.072
16	0.208	0.176	0.146	0.220	0.053
17	0.196	0.247	0.218	0.297	0.031
18	0.057	0.086	0.258	0.166	0.046
19	0.082	0.108	0.060	0.088	0.057
20	0.169	0.117	1.054	0.117	0.047

[1] Refer to Chapter 4

[1]APPENDIX TABLE 8

Mean egg weight (g) within the four egg size categories over the incubation period

	Egg weight			
Time (days)	Small	Medium	Large	Jumbo
0	46.49	56.83	66.33	74.59
1	46.01	56.23	65.60	73.76
2	45.64	55.79	65.04	73.11
3	45.25	55.32	64.77	72.46
4	44.88	54.89	64.19	71.97
5	44.56	54.49	63.46	71.52
6	44.21	54.06	62.89	70.40
7	43.87	53.62	62.43	70.40
8	43.49	53.30	61.98	69.91
9	43.24	52.88	61.45	69.32
10	42.89	52.48	60.95	68.77
11	42.52	52.03	60.40	68.17
12	42.17	51.61	59.88	67.59
13	41.87	51.24	59.42	67.27
14	41.49	50.80	58.88	66.70
15	41.13	50.38	58.37	66.12
16	40.77	50.15	57.86	65.58
17	40.45	49.57	57.36	65.07
18	40.13	49.19	56.92	64.57
19	39.78	48.78	56.38	64.03
20	39.03	48.01	55.70	63.28

Residual S.E. (162df) 2.83

[1] Refer to Chapter 4

[1]APPENDIX TABLE 9

Mean yolk-sac weight (g) of the embryos and chicks for the four egg size categories during the pre- and post-hatch periods, respectively

	Egg weight			
Time (days)	Small	Medium	Large	Jumbo
15	9.43	13.80	17.14	19.97
16	8.91	12.49	16.56	18.47
17	8.91	11.58	14.17	16.90
18	6.91	11.36	13.33	15.61
19	6.90	8.66	11.49	13.73
20	4.93	6.89	9.50	11.16
21	2.95	3.67	5.36	6.42
22	1.33	1.83	2.25	3.14
23	1.10	1.00	1.03	1.41
24	0.54	0.56	0.61	0.59
25	0.41	0.43	0.40	0.62
26	0.13	0.14	0.25	0.27
28	0.04	0.05	0.47	0.42
33	0.03	0.06	0.07	0.13

Residual S.E. (9df) 3.60
Residual S.E. (24df) 0.29
[1] Refer to Chapter 4

[1]APPENDIX TABLE 10

Body water content (g/kg) of the chicks produced from eggs of four weight categories, on three feed treatments, on nine occasions during the course of the trial

	Small			Medium			Large			Jumbo		
Time (days)	H	L	Choice	H	L	Choice	H	L	Choice	H	L	Choice
1												
2	803.3	786.9	790.0	781.8	801.1	783.1	800.5	762.7	787.1	761.7	771.9	787.0
3	803.5	786.3	802.5	784.6	768.3	789.6	789.8	763.6	757.7	790.5	777.6	771.3
4	782.3	802.6	801.7	776.2	796.6	781.0	789.4	780.0	773.9	785.7	769.3	777.6
5	824.6	783.8	776.9	789.1	754.3	791.1	771.3	772.1	783.2	765.7	753.1	797.6
6	777.3	777.2	797.3	787.4	813.1	797.0	784.4	765.9	773.0	787.7	757.0	767.2
8	811.7	786.2	783.0	784.1	753.3	794.8	796.3	755.9	791.5	781.5	760.2	757.4
12	794.1	752.7	767.3	769.4	739.3	767.3	772.1	736.9	757.2	748.2	719.2	752.1
22	745.4	718.6	740.8	720.1	717.6	728.9	735.5	709.0	723.9	739.5	712.4	731.5