RESPONSE OF BROILER CHICKENS TO DIETARY LYSINE, METHIONINE AND METABOLISABLE ENERGY CONCENTRATIONS

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I hereby certify that this research is the result of my own investigation

R G Maclachlan

A C K N O W L E D G E M E N T S

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GENERAL INTRODUCTION

Modern broiler production has continued to make broad advances in a number of areas including genetics, nutrition and management. Today's broiler grows faster and eats less per unit of gain than birds marketed just a few years ago. Since no single factor can be responsible for these developments, constant re-evaluation and revision of the nutritional requirements and general management practises is necessary. The genetic improvement is in the hands of the geneticist, and, since birds adapt to a wide range of sound management practices, it behoves the nutritionist to keep pace with the nutrient requirements of the modern fast-growing broiler chicken.

The various breeds of domesticated fowl, each with its own requirements, are raised for extended periods during which the nutrient requirements change continuously. Thus, the number of variables in the empirical determination of the requirements for energy and amino acids is rather overwhelming and the value of general equations for such purposes becomes obvious. The requirements for energy and amino acids of chickens vary as functions of the rate of growth and production determined by the interaction of genotype and environment. The need for energy and amino acid in growing chickens is the sum of the requirements for maintenance of basic functions, and that for growth. It is assumed that there are no interactions between these two components of the requirements. Furthermore the requirement for each of the amino acids under study is assumed to be independent of others, with no interactions taken into account.

The discovery that energy concentration of the diet is of primary importance in determining the amount of food ingested by chickens is the key which has unlocked the door to a scientific understanding of the proper relationships among all nutrients in the diet. Birds eat primarily to satisfy an inner craving for energy, when this hunger is satisfied the bird stops eating. Therefore the amino acids, vitamins and minerals must be present in the diet in a very definite ratio to energy so that the chicken will receive enough of all essential nutrients whilst satisfying its hunger for energy. The absolute amount consumed depends upon the needs of the bird, which vary depending upon its size, its activity, its environmental temperature, whether it is growing or laying eggs, or simply maintaining itself. It is of the utmost importance, therefore, that we know the energy requirements of the chickens during each stage of their growth and development and that we have precise information covering the metabolisable energy values of the feedstuffs used to formulate their diets. With this information it is possible to closely predict the food consumption of any flock of chickens in a particular environment and thus to set the level of amino acids, vitamins and minerals such that all nutrients will be provided in adequate amounts for optimum daily growth and production.

At present an energy sytem, based on metabolisable energy (ME) values is used for evaluating feedingstuffs in poultry diet formulation. Research results have shown that the growing chicks can utilise the ME of fats and proteins respectively more and less efficiently compared with carbohydrates. A system based on ME values considerably underestimates fats and fat-rich feedingstuffs and overestimates protein-rich feedingstuffs in comparison with carbohydrates. The logical development therefore, has been the determination of a net energy (NE) system for evaluating poultry diet ingredients. The NE system is a compensatory one, and is based on the ME value plus the net availabilities of the feedingstuffs. The two different energetic evaluation systems have a considerable effect on least-cost broiler diet formulation, efficiency of food utilisation, broiler performance and economic efficiency, and there is little or no doubt that poultry diets will soon be based on the NE system.

It is an accepted principle that the amount of food consumed by chcikens is regulated by the energy concentration of the diet provided the diet is adequate in other essential nutrients. Therefore, the amino acid requirements of broilers should be expressed as a function of energy. The amino acid requirements of broilers expressed as a percentage of the diet is highest during the first week of age, and then decreases until the bird is marketed. Traditionally the feeding regimes have been divided into a starter period (0 - 28 days), a finisher period (29 - 49 days) and a withdrawal period (50 days onwards). Although the reliability of the amino acid profiles has improved, there is little information about the availability of these amino acids in the ingredients. There is little doubt that once accurate and reliable methods have been perfected to determine available amino acid values, these will be eagerly accepted by nutritionists for use as constraints in formulating least-cost poultry diets.

Least-cost broiler diets have been a major tool of feed companies and of integrated broiler firms for improving broiler performance and maximising profits. Although the least-cost diets minimise the cost of diets of pre-specified nutrient content, they do not determine which nutrient levels are most profitable. The focus of attention has now centred on the next step - least-cost gain or maximum profit diets. Ever increasing and highly variable feed ingredient prices have contributed to uncertainty amongst nutritionists as to the most profitable energy and amino acid levels of broiler diets, and therefore more variations than usual have been found in the nutrient density of diets in the market place. Depressed broiler meat prices and spiralling food costs have stressed the need for broiler performance models which will determine the most profitable diets for a given set of circumstances.

The object of the present study was to examine aspects of the dietary energy and amino acid requirements of the broiler chicken, followed by an attempt to determine the optimum nutrient density of practical broiler diets. Lysine and methionine are the major limiting amino acids in practical broiler diets, therefore the amino acid aspect will be confined to these two important amino acids. This study, therefore, has the following major objectives:

1 To evaluate the dilution technique of Fisher and Morris (1970) for measuring the response of broiler chickens to increasing dietary lysine and methionine concentrations.

2 To examine the relationship between the first-limiting amino acid and total protein *per se*, and to measure its effect on performance. The amino acids under scrutiny will be lysine and methionine.

3 To determine the optimum lysine and methionine intakes for the starter and finisher periods.

4 To determine the optimum lysine/energy and methionine/energy ratios for the starter and finisher periods.

5 To develop broiler performance models which determine the optimum energy and amino acid concentrations in the diet for specific nutrient and broiler meat prices.

P A R T   O N E

EVALUATION OF THE DILUTION TECHNIQUE FOR MEASURING THE RESPONSE TO DIETARY AMINO ACID CONCENTRATIONS

CHAPTER 1

EVALUATION OF A DIET DILUTION TECHNIQUE FOR MEASURING THE RESPONSE OF BROILER CHICKENS TO LYSINE INTAKE DURING THE STARTER PERIOD

INTRODUCTION

Most of the information available on the amino acid requirements of the growing chick stems from the determination of the requirements for single amino acids. The method most commonly used to determine amino acid requirements of broilers is one in which a basal diet is chosen which is adequate and balanced in all essential amino acids except the one under study, which will be first-limiting. Graded levels of this amino acid in synthetic form are then added to the basal diet and the level of response in body mass gain recorded. A response curve is derived, and various methods are then used to ascertain the exact requirement of the broiler for that amino acid from the response curve (Griminger and Scott, 1959; Dean and Scott, 1965; Combs, 1968; Hewitt and Lewis, 1972).

D'mello and Lewis (1970) presented evidence that the requirements for an amino acid may depend on the level of another amino acid, suggesting that all the amino acids should be considered together. Such interdependence may mean that in the determination of the requirement for a single amino acid the results may be influenced by the level of a second amino acid. Application of the results of such experiments should perhaps be restricted to dietary situations that are similar to those used in the determination.

The question also arises whether to use intact proteins or synthetic amino acids in the formulation of the experimental diets. Pure amino acid diets have been used in the determination of the amino acid requirements, but the variable results recorded have cast doubt on their general acceptance. Dean and Scott (1965) who considered all the essential amino acids, obtained reasonable results with synthetic amino acids, but the validity of applying the results directly to diets composed of natural ingredients is questionable.

There are a number of disadvantages to the basal diet method, namely: that each diet does not have the same amino acid balance, which could be expected to influence the results; at the high levels of supplementation the amino acid under study may be no longer first-limiting, but might be capable of giving an additional response if the new first-limiting amino acid were added to the diet. Whatever the method of diet formulation has been used, there is the general difficulty to pinpoint the requirements from the response curve with any degree of accuracy thereby leading to the widely divergent results obtained by various authors for the requirements by broilers for each amino acid (Zimmerman and Scott, 1965; National Research Council, 1971; Hewitt and Lewis, 1972). Fisher and Morris (1970) presented a summit-dilution technique for measuring the response of laying hens to dietary methionine intake, and subsequently Pilbrow and Morris (1974) used a modified version of this technique to measure the responses of laying hens to lysine intake. This method consists essentially of feeding a series of diets made by serial dilution of a summit diet with a dilution diet. The summit diet is formulated to contain all amino acids in excess of the "requirement", for example 200 percent of the assumed requirement, except for the amino acid under test which is kept at, for example, 130 percent of the "requirement". The dilution diet is formulated in the same way, with all amino acids at approximately 120 percent of the requirement, and the test amino acid at about 50 percent of the requirement. In this way it is ensured that the summit and dilution diets are first-limiting in the test amino acid, and that all serial dilutions of the two experimental diets will also be first-limiting in that amino acid. An added advantage is that all test diets will have a similar amino acid balance. This is shown diagramatically in Figure 1.1, and is compared with the more usual method of determining amino acid requirements. The imbalanced nature of the diets in the "classical" method is also evident.

The results of Thomas, Twining and Bossard (1975) have generally shown that increasing the protein level of the diet will improve performance. However, the question has been posed whether it is better to add the limiting amino acid to a diet or to meet the amino acid requirement by raising the protein level thereby increasing the amount of the amino acid in the diet. Generally speaking, opinions are divided whether or not increasing the protein level of the diet increases the overall amino acid requirement. If we accept that the amino acid requirements are related to protein level, then increasing the protein level will increase the amino acids required to produce optimum response in performance. In determining the amino acid requirements of broilers using the classical technique, Woodham and Deans (1975) used diets containing 18 percent protein throughout their trials in order to overcome the confounding effect of protein level on amino acid requirement.

Because the protein content of all diets in the dilution series are not the same, nor is the ratio of protein to energy constant throughout the series, the validity of such a technique may be questioned. In order to ensure that responses to dietary amino acid concentrations can be measured by this technique, i.e. to prove that the response in mass gain is determined by the most-limiting nutrient and not by the protein content of the diets *per se*, a series of experiments was conducted using methionine and lysine in the starter and finisher periods, using the dilution technique of Pilbrow and Morris (1974) to provide additional information regarding the response of broilers to increasing concentrations of amino acids. The results of the first experiment express the response of lysine intake on growth during the starter

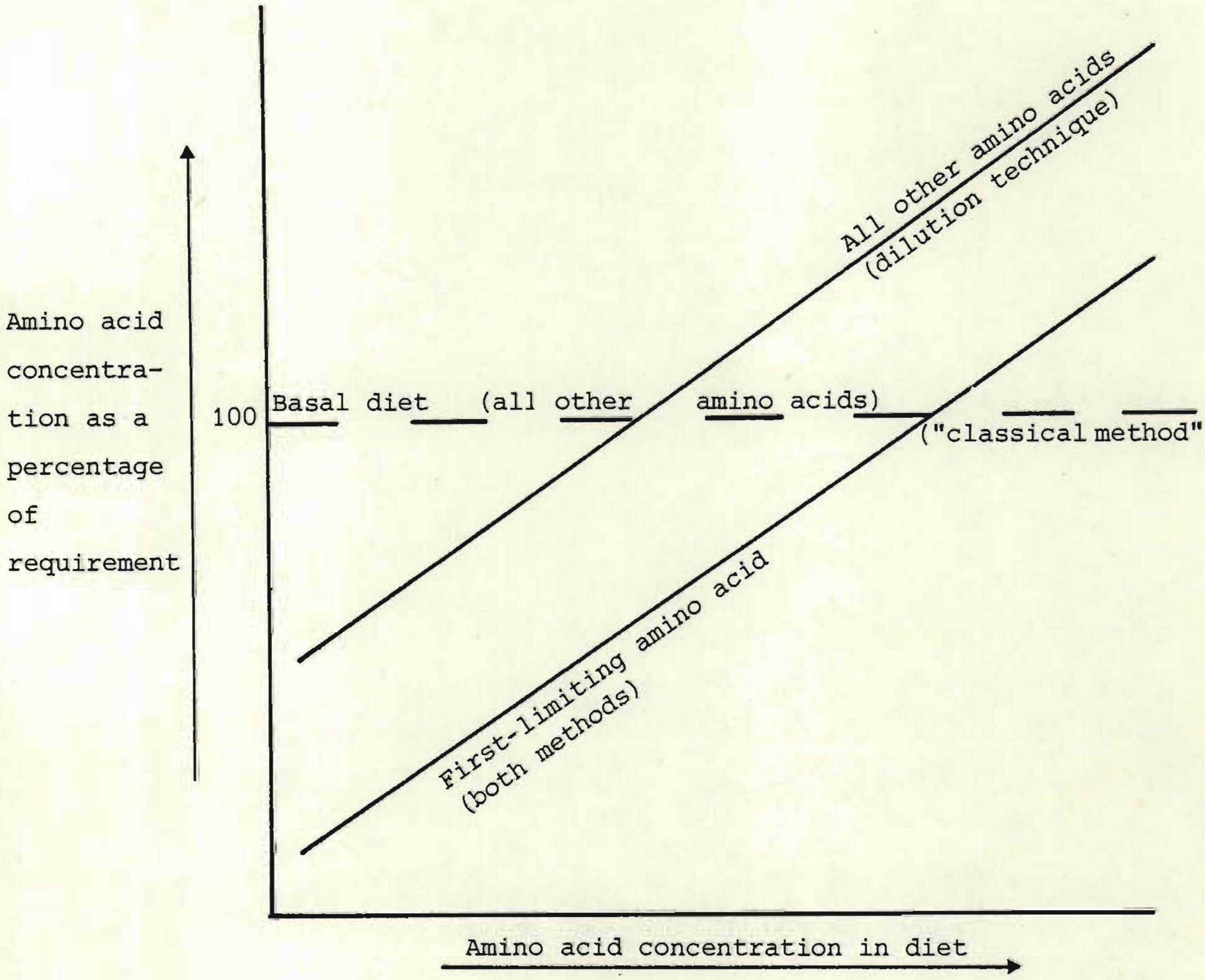

Figure 1.1 Schematic illustration of the difference in approach in determining amino acid responses using a dilution technique, and the "classical" method using a basal diet supplemented with increasing concentrations of the test amino acid

period, as a response curve, from which an equation can be derived for the estimation of the lysine requirement of broilers for the starter period.

MATERIALS AND METHODS

Ross male broiler chickens were reared in wire-floored tier brooders to seven days of age on a commercial starter diet. Food and water were fed *ad lib.* and artificial light was supplied for 24 hours per day. Each tier was divided into four pens, and the brooder temperatures were maintained at a comfortable level for the chickens throughout the 28 day experimental period.

The body mass of each chicken was determined. The lightest and heaviest chickens which represented approximately 10 percent of the total were discarded to reduce the standard deviation of body mass in each pen and the remaining chicks were allocated to the experimental pens such that the mean mass was kept as uniform as possible. Each pen contained 10 chicks, there being four replications of each treatment.

In choosing the range of lysine contents of the experimental diets it was intended that as wide a range as was practically feasible would be used in order that a comprehensive response curve could be obtained. The required range of lysine contents was obtained by formulating a summit diet calculated to contain 13,7 g/kg lysine and a dilution diet calculated to contain 5,7 g/kg lysine. The intermediate lysine contents were obtained by blending the two basal diets in appropriate proportions as shown in Table 1.3. This diet dilution technique is based on that of Fisher and Morris (1974). The composition of the summit and dilution diets is shown in Table 1.1. Specific protein contents were not used in formulation but the minimum contents of all essential amino acids except

TABLE 1.1 Composition (g/kg) of the summit and dilution diets

Ingredient	Summit diet	Dilution diet
Yellow maize meal	305,0	717,3
Maize gluten meal (60%)	107,0	4,0
Sunflower meal	229,0	180,0
Groundnut meal	183,0	50,0
Fish meal	100,0	
Bone meal		15,0
Monocalcium phosphate	2,0	9,0
Limestone flour	14,2	10,0
Salt	1,0	4,0
Sunflower oil	51,0	6,0
DL - Methionine	4,8	1,7
Vitamins and trace minerals *	3,0	3,0
Calculated analysis		
Arginine	23,38	11,70
Histidine	7,85	3,77
Isoleucine	11,89	5,30
Leucine	29,01	14,11
Lysine	12,36	4,94
Methionine	6,72	3,24
Cystine	5,50	3,67
Phenylalanine	15,51	7,24
Tyrosine	10,10	4,99
Threonine	11,44	5,51
Tryptophan	3,44	1,84
Valine	16,10	7,38
Calcium	10,00	10,00
Phosphorus	7,00	7,00
Crude protein (gN x 6,25/kg)	321,80	158,20
Metabolisable energy (MJ/kg)	12,97	
Net energy (MJ/kg)	9,25	9,25

*Supplies per kg of diet: Vit A 7 027 IU, Vit D_3 2 000 IU, Vit E 20 IU, Hetrazine 3 ppm, Thiamine hydrochloride 0,985 ppm, Riboflavin 8 ppm, Calcium pantothenate 7,846 ppm, Niacin 29,823 ppm, Folic acid 0,95 ppm, Biotin 0,08 ppm, Choline chloride 300 ppm

Copper 125 ppm, Iron 40 ppm, Zinc 28 ppm, Iodine 2 ppm, Manganese 78 ppm, Selenium 0,1 ppm

TABLE 1.2 Calculated amino acid contents of the summit and dilution diets relative to the requirements of broilers during the starter period

	Requirements according to Thomas *et al.* (1978) (g/kg)*	Amino acid contents expressed as multiples of requirement	
		Summit diet	Dilution diet
Arginine	12,40	1,89	0,94
Histidine	4,51	1,74	0,84
Isoleucine	8,45	1,41	0,63
Leucine	15,78	1,84	0,89
Lysine	12,67	1,10	0,45
Methionine	5,17	1,30	0,85
Cystine	3,80	1,45	0,97
Phenylalanine	7,89	1,97	0,92
Tyrosine	6,76	1,49	0,74
Threonine	7,47	1,53	0,74
Tryptophan	2,25	1,53	0,82
Valine	9,58	1,68	0,77

* Requirements are expressed in g/kg for a diet containing 12,97 MJ ME/kg (equivalent to 9,25 MJ NE/kg). Amino acid contents as multiples of requirements are expressed in terms of the requirements at the corresponding energy level of the diet

TABLE 1.3 Summary of dilution technique and calculated analysis of the experimental diets

Diet code	Blending ratio				Lysine supplementation (g/kg)	Calculated dietary lysine (g/kg)	Calculated dietary protein (gN x 6,25/kg)
	Summit diet		Dilution diet				
Series 1							
1	100	+	0			13,7	322,0
2	80	+	20			12,1	289,0
3	60	+	40			10,5	256,0
4	40	+	60			8,9	224,0
5	20	+	80			7,3	191,0
6	0	+	100			5,7	158,0
Series 2							
7	80	+	20	+	1,2	13,3	289,0
8	60	+	40	+	1,2	11,7	256,0
9	40	+	60	+	1,2	10,1	224,0
10	20	+	80	+	1,2	8,5	191,0
11	0	+	100	+	1,2	6,9	158,0
Series 3							
12	60	+	40	+	2,4	12,9	256,0
13	40	+	60	+	2,4	11,3	224,0
14	20	+	80	+	2,4	9,7	191,0
15	0	+	100	+	2,4	8,1	158,0

lysine were set at 2,0 times (for the summit) and 1,0 times (for the dilution), the requirements proposed by Thomas, Twining, Bossard and Nicholson (1978). The extremes of the range of lysine contents used, namely 13,7 g/kg and 5,7 g/kg were 1,10 and 0,45 times the estimated requirement of Thomas *et al.* (1978).

Six serial dilutions of the summit diet were made (series 1 Table 1.3), concentrations of lysine in each diet being 1,10; 0,96; 0,83; 0,70; 0,58 and 0,45 times the requirement for lysine respectively. A second series of dilution diets was prepared in the same manner as that above. Synthetic lysine was then added to diets 2 to 6, the amount added (1,2 g/kg) being equal to 0,75 times the difference in lysine content between two adjacent diets in the first series. The lysine contents of the second series were then 1,05; 0,92; 0,80; 0,67 and 0,54 times the requirement respectively. A third series was prepared in which the level of lysine was increased by the addition of lysine, the amount added amounting to 1,5 times the difference in lysine content between two adjacent diets in the first series. The level added (2,4 g/kg) to diets 3 to 6 in this series increased the lysine to 1,02; 0,89; 0,77 and 0,64 times the requirement. The net result of this diet blending and supplementation technique is that there are identical dilutions in each series, with similar protein and amino acid balances, except for increasing lysine levels as shown in Table 1.3.

The experimental diets were fed from 7 to 28 days of age, and the criteria studied were growth rate and food intake during the three week experimental period. The experimental period was divided into two periods, viz:

7 to 21 days - Period 1

7 to 28 days - Period 2.

RESULTS

The objective of this experiment was to establish whether a protein factor was important in chick nutrition, or whether the first-limiting amino acid was of greater significance in regulating chick growth. The results of the first period are shown in Table 1.4. The response to supplemented lysine in the second and third series of diets clearly indicated that lysine was the most limiting amino acid in the first series of diets. The favourable response in body mass gain recorded after the higher level of lysine addition in the third series of diets confirms the calculation that lysine remained first-limiting in all cases. Had the response waned in the lower dilutions of the third series, then it would have been indicative that an amino acid other than lysine had become limiting. The results recorded in the two supplemented series indicate that the magnitude of the responses elicited by the added lysine was proportional to the level of lysine added.

A multiple regression analysis was performed on this data to test the relative importance of lysine intake and protein intake on body mass gain. The terms tested in the analysis covering the two periods were the following:

Lysine, $lysine^2$

Protein, $protein^2$

Lysine, $lysine^2$, protein

Lysine, $lysine^2$, protein, $protein^2$

Lysine, $lysine^2$, protein, $protein^2$, lysine $\times$ protein

The regression analysis of the first period indicated that both lysine intake and protein intake were important in determining body mass gain. The t-values for testing the significance of lysine intake (7,593) and the squared term for lysine intake (5,773) were highly significant (t-value for significance at $P < 0{,}01$ is 2,671), whereas the t-value for protein intake (2,148) and the squared term

TABLE 1.4 Response of broiler chickens to dietary lysine and protein intakes from 7 - 21 days of age

Diet code	Calculated dietary lysine (g/kg)	Calculated dietary protein (gN x 6,25/kg)	Gain in mass (g/bird d)	Lysine intake (mg/bird d)	Protein intake (g/bird d)	Food intake (g/bird d)
Series 1						
1	13,7	322,0	29,5	577,0	13,6	42,1
2	12,1	289,0	28,3	519,0	12,4	42,9
3	10,5	256,0	26,9	451,0	11,0	43,0
4	8,9	224,0	23,2	376,0	9,5	42,3
5	7,3	191,0	16,3	317,0	8,3	43,4
6	5,7	158,0	12,1	225,0	6,2	39,4
Series 2						
7	13,3	289,0	29,8	567,0	12,3	42,6
8	11,7	256,0	28,8	517,0	11,3	44,2
9	10,1	224,0	26,1	460,0	10,2	45,5
10	8,5	191,0	23,3	375,0	8,4	44,1
11	6,9	158,0	19,0	297,0	6,8	43,0
Series 3						
12	12,9	256,0	28,4	570,0	11,3	44,2
13	11,3	224,0	27,7	511,0	10,1	45,2
14	9,7	191,0	25,1	450,0	8,9	46,4
15	8,1	158,0	22,9	371,0	7,2	45,8

TABLE 1.5 Response of broiler chickens to dietary lysine and protein intakes from 7 - 28 days of age

Diet code	Calculated dietary lysine (g/kg)	Calculated dietary protein (gN x 6,25/kg)	Gain in mass (g/bird d)	Lysine intake (mg/bird d)	Protein intake (g/bird d)	Food intake (g/bird d)
Series 1						
1	13,7	322,0	31,2	705,0	16,6	51,5
2	12,1	289,0	31,6	622,0	14,9	51,3
3	10,5	256,0	31,0	548,0	13,4	52,2
4	8,9	224,0	26,0	452,0	11,4	50,8
5	7,3	191,0	20,9	370,0	9,7	50,7
6	5,7	158,0	13,2	266,0	7,4	46,6
Series 2						
7	13,3	289,0	32,8	697,0	15,1	52,4
8	11,7	256,0	31,2	616,0	13,5	52,6
9	10,1	224,0	30,0	551,0	12,2	54,6
10	8,5	191,0	26,8	468,0	10,5	55,0
11	6,9	158,0	21,8	350,0	8,0	50,7
Series 3						
12	12,9	256,0	31,1	671,0	13,3	52,0
13	11,3	224,0	29,1	583,0	11,6	51,6
14	9,7	191,0	28,1	535,0	10,5	55,1
15	8,1	158,0	26,8	442,3	8,6	54,7

for protein intake (2,251) were only significant at the lower level (t-value for significance at $P < 0,05$ is 2,015). The variance inflation factor for protein intake (241) and the squared term for protein intake (216) were both high, indicating that this result is very unreliable and should be viewed with caution.

The responses in livemass gain to lysine and protein intakes for the period 7 to 28 days are presented in Table 1.5. These results were subjected to the same multiple regression analysis as outlined in the first period. In this period, as in period 1, highly significant t-values resulted when testing separately the significance of lysine and protein intake, and their respective squared term, (Table 1.6). However, when all four terms were subjected to analysis the t-value for testing the significance of lysine intake (7,651) was highly significant, whereas the t-value for protein intake (1,698) and the squared term for protein intake (1,903) were non significant. The regression coefficients and their t-values for testing their significance for both periods are presented in Table 1.6.

The mean values for growth rate and lysine intake were used to calculate an equation relating growth rate and mean body mass to lysine intake. Use was made of the Reading model (Fisher and Morris, 1970), and the parameters used, together with the coefficients for the average individual in the flock are given in Table 1.7 and illustrated in Figures 1.4 and 1.5. The Reading model is represented by the following equation:

$$I = a\Delta\bar{W} + b\bar{W} + x(\sqrt{a^2\sigma^2\Delta\bar{W} + b^2\sigma^2\bar{W} - 2abr\sigma\Delta\bar{W}\sigma\bar{W}}). \qquad (1.1)$$

where

I = amino acid intake (mg/bird d)

a = amount of amino acid required/unit of output (mg/g$\Delta\bar{W}$)

b = amount of amino acid required/unit of body mass (mg/g $\bar{W}$)

TABLE 1.6 Regression coefficients describing response of broiler chickens to increasing dietary concentrations of lysine and protein

Lysine		Protein	
linear coefficient	quadratic coefficient	linear coefficient	quadratic coefficient
7 - 21 days			
0,1343	-0,0001052	---	---
[1](9,155)**	(6,042)**	---	---
---	---	7,2474	-0,2637
---	---	(5,213)**	(3,734)**
0,1336	-0,0001065	-0,0937	---
(8,990)**	(5,965)**	(0,395)^{NS}	---
0,1737	-0,0001506	-2,8859	0,1454
(7,593)**	(5,773)**	(2,148)*	(2,251)*
7 - 28 days			
0,1406	-0,0001030	---	---
(13,401)**	(9,931)**	---	---
---	---	7,5308	-0,2496
---	---	(7,173)**	(5,654)**
0,1400	-0,0001059	0,1801	---
(13,316)**	(9,837)**	(0,990)^{NS}	---
0,1677	-0,0001318	-1,7404	0,07867
(9,399)**	(7,651)**	(1,698)^{NS}	(1,903)^{NS}

[1]t - value for testing significance of regression coefficients is shown in brackets. ^{NS}denotes non-significant, $^{*}P < 0,05$; $^{**}P < 0,01$

TABLE 1.7 Parameters used in fitting the Reading model to determine the relationship between mass gain and lysine intake using male chickens from 7 - 28 days of age

Parameter	7 - 21 days	7 - 28 days
$\bar{W}$	250,00	250,00
$\Delta\bar{W}$	30,50	31,32
a	15,40	16,55
b	0,03	0,65
$\sigma\bar{W}$	40,00	40,00
$\sigma\Delta\bar{W}$	7,50	3,47
r	0,60	0,60

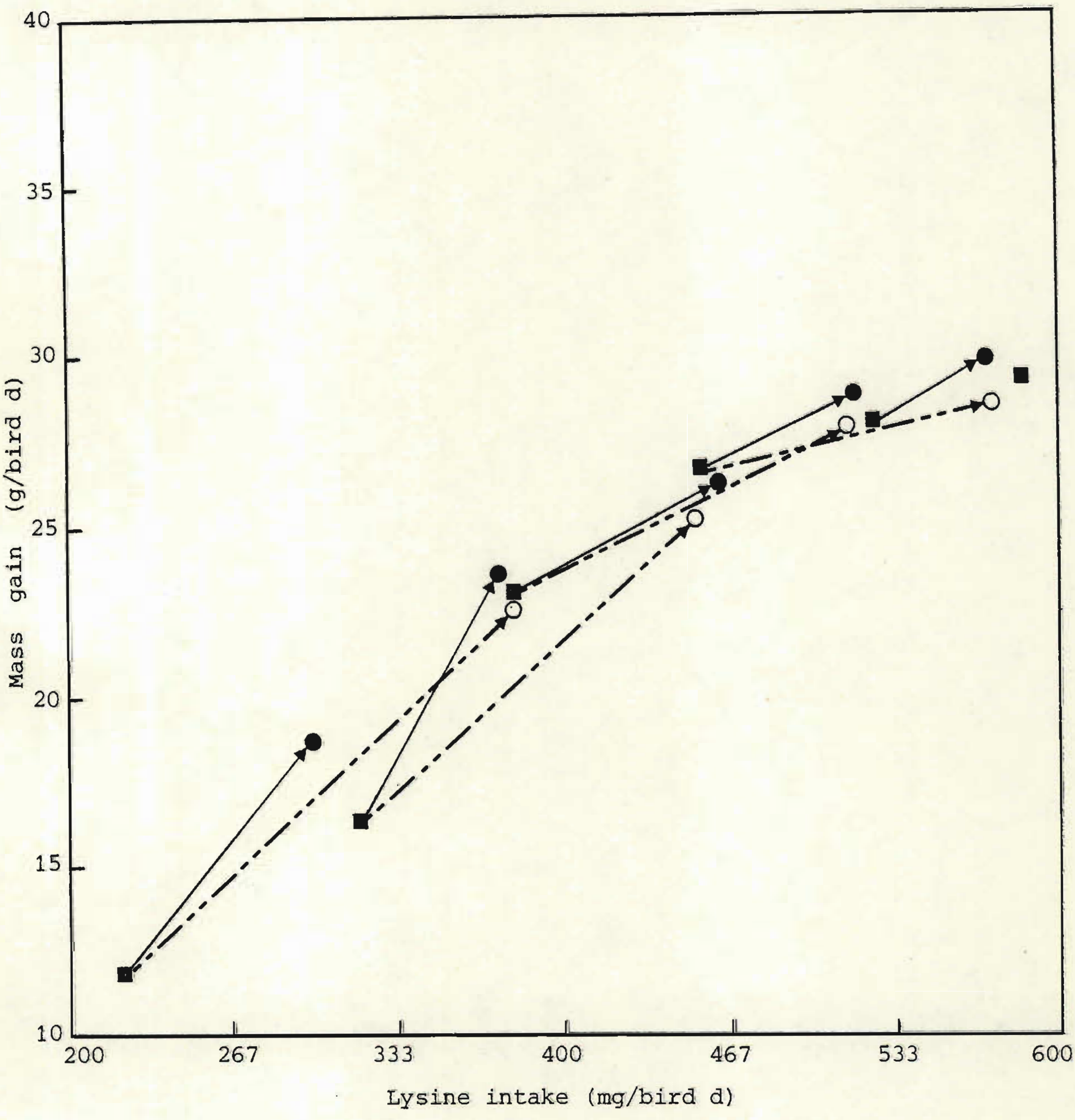

Figure 1.2 Response of male chickens to increasing lysine concentrations from 7 - 21 days of age

■ First dilution series (initial dilution)

● Second dilution series (lysine addition)

○ Third dilution series (lysine addition)

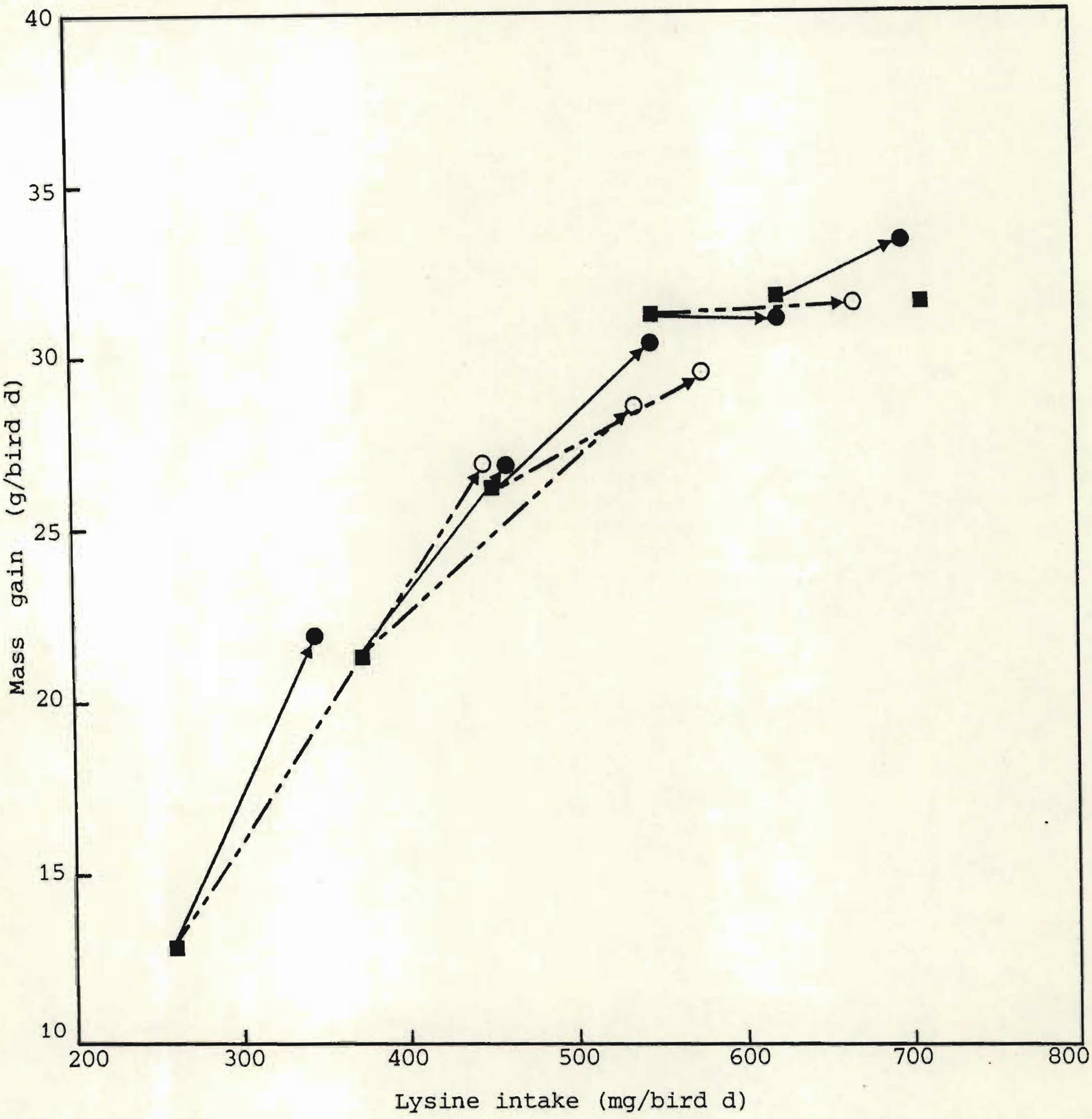

Figure 1.3 Response of male chickens to increasing lysine concentrations from 7 - 28 days of age

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● Second dilution series (lysine addition)

○ Third dilution series (lysine addition)

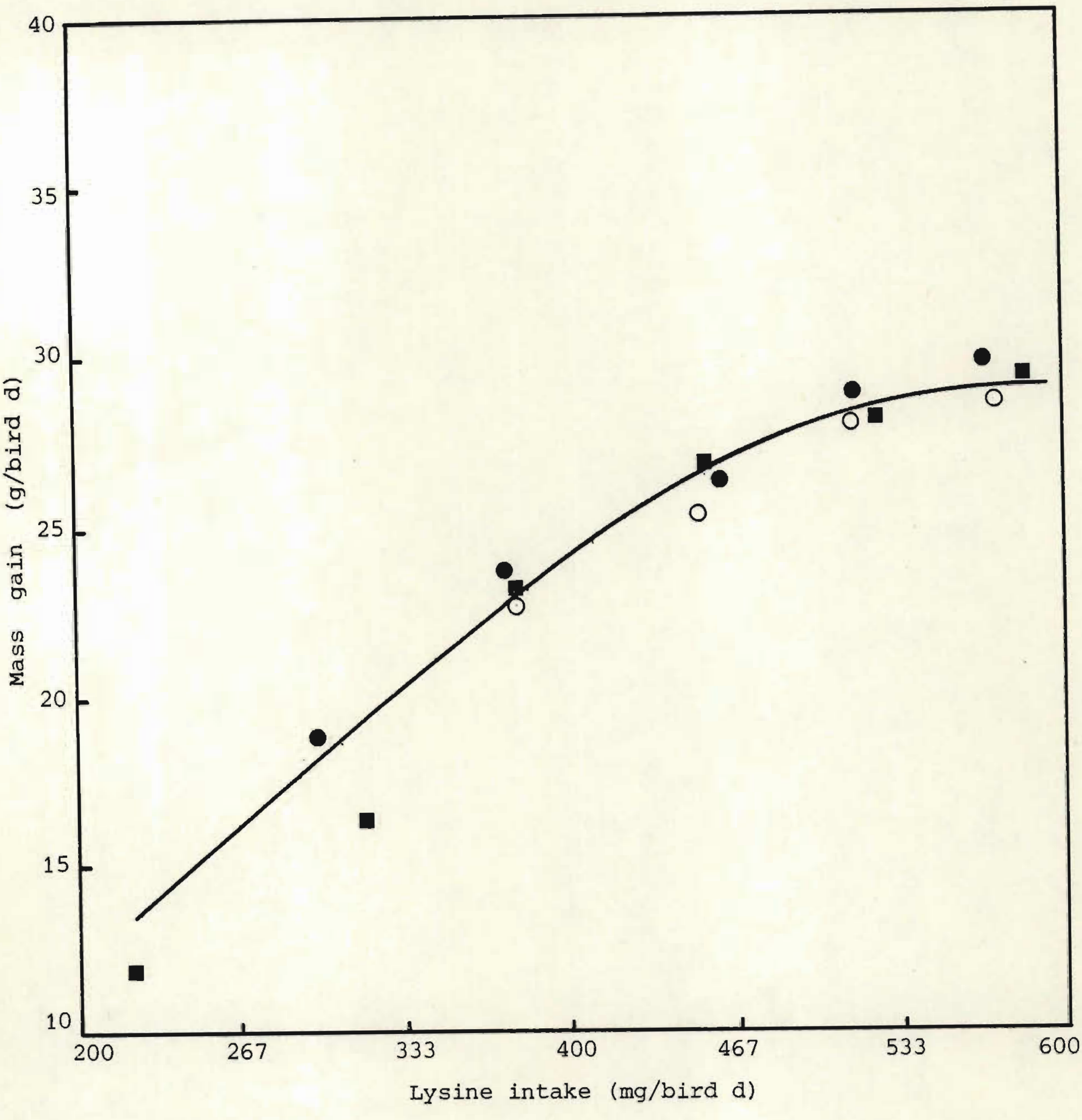

Figure 1.4 Response of male chickens to lysine intake and estimated response using the Reading model (Table 1.7) from 7 - 21 days of age

■ First dilution series (initial dilution)

● Second dilution series (lysine addition)

○ Third dilution series (lysine addition)

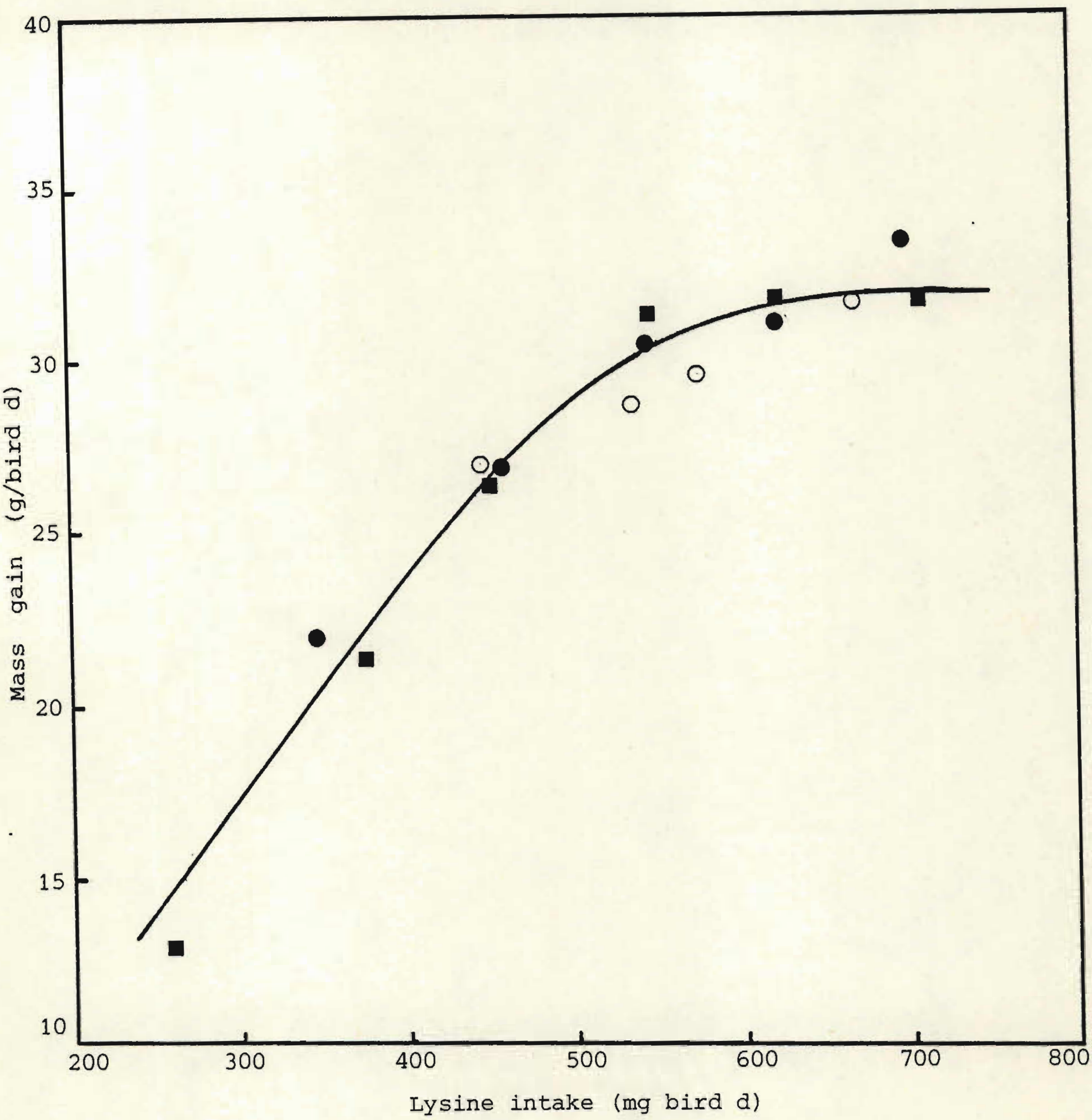

Figure 1.5 Response of male chickens to lysine intake and estimated response using the Reading model (Table 1.7) from 7 - 28 days of age

■ First dilution series (initial dilution)

● Second dilution series (lysine addition)

○ Third dilution series (lysine addition)

$\Delta\bar{W}$ = mean maximum body mass gain (g/bird d)

$\bar{W}$ = mean body mass (g/bird)

$\sigma\Delta\bar{W}$ = variation in mass gain

$\sigma\bar{W}$ = variation in body mass

r = correlation between mass gain and body mass ($r\Delta\bar{W}/\bar{W}$)

x = deviation from mean of a standard normal distribution which is exceeded with probability ak in one tail.

DISCUSSION

The major objective of this experiment was to evaluate a diet dilution technique for measuring the response of broiler chickens to increasing amino acid concentrations and to establish whether this dilution technique could be implemented as a means of determining the amino acid requirements of broiler chickens during the starter period.

The concept of using a dilution technique for determining the response of chickens to amino acid intakes has been presented in relation to the nutrition of laying hens (Fisher and Morris, 1970; Pilbrow and Morris, 1974; Morris and Wethli, 1978), and more recently for the determination of the available tryptophan requirements of broilers (Freeman, 1979). The major advantage of this technique over prior methods of determining amino acid requirements is the fact that the amino acid under study is first-limiting in all diets in the dilution series. If the addition of the test amino acid to the summit and dilution diets, or indeed to any of the intermediate dilutions, elicits an additional response, this is conclusive evidence that all the diets in the series are most limiting in the test amino acid.

This is not the case where increased levels of an amino acid are added to a basal diet which is balanced for all amino acids except the one under study as in the "classical" method of measuring amino acid responses. Where increasing doses of an amino acid are added in the latter method, a point is reached where the amino acid under study is no longer first-limiting, and any additional response which may have been forthcoming is suppressed by another amino acid which has subsequently become first-limiting.

In the formulation of the summit and dilution diets no specifications were set for the protein level in either diet. It is however evident in the first series of dilution diets that a wide range of protein values is covered in the experimental diets. Graded levels of lysine were then added to the second and third series of diets without altering the protein levels. The response in livemass gain to increasing lysine levels was then measured and the results subjected to multiple regression analysis. The regression analysis was performed on this data in order to test the relative importance of lysine intake and protein intake on body mass gain. Had the t-values for the protein term proved significant when all four terms were subjected to analysis then the dilution technique could not have been utilised to determine the response of broiler chickens to increasing amino acid levels, since cognisance had not been taken of the optimum protein levels in the summit and dilution diets. If protein intake was significant in determining the response to lysine and protein intake then the protein term would have to be included in the response equation.

The responses in livemass gain to increasing levels of lysine and protein were recorded for the two periods and subjected to multiple regression analysis. In spite of the relatively high concentrations of protein in the experimental diets, protein intake was less important in determining the response in body mass gain than was lysine intake. The response in livemass gain to protein intake was not significant in period 2, and was only just significant in period 1. Lysine intake, on the other hand, was responsible to a highly significant degree for the response in mass gain in both periods.

The significant response to protein intake in the first period should be viewed with caution. Because the protein intake and its squared term were only just significant, coupled with their very high variance inflation factors, it would be unwise to draw a definite conclusion in this regard from these results. Intake of the most limiting amino acid thus appears to be of greater importance than intake of protein in regulating livemass gain.

The results in Table 1.6 show that lysine and the lysine squared term have high t-values, indicating that they fit the data accurately. However, when protein, and the protein squared term were added the t-values for testing the regression coefficient were not significant, indicating that protein and its squared term are not contributing significantly to the model. When an attempt was made to improve the fit by including the lysine × protein term, the model became singular, in which case the lysine × protein regression coefficient cannot be measured.

The Reading models derived from this data measure the response of broiler chickens in livemass gain to lysine intakes for the two periods under study. These responses then allow a mathematical approach to the determination of the amino acid concentration in the diet yielding the optimum biological or economic response.

This experiment was not designed specifically to measure the response of broilers to increasing dietary concentrations of lysine, but rather to prove that this dilution technique could be used to measure such responses. Nevertheless, the results can be used for the purpose of measuring the response of broilers to lysine intake. Although a Reading model was used to describe the data obtained in this trial, little emphasis should be placed on the equation based on this data alone. In Chapter 8 the results from this and subsequent experiments are combined to formulate a general model to describe the response of broiler chickens to increasing concentrations of lysine.

CHAPTER 2

EVALUATION OF A DIET DILUTION TECHNIQUE FOR MEASURING THE RESPONSE OF BROILER CHICKENS TO LYSINE INTAKE DURING THE FINISHER PERIOD

INTRODUCTION

In Chapter 1 a diet dilution technique for measuring the response of broiler chickens to increasing dietary concentrations of an amino acid was evaluated. Chickens in the early stage of growth (0 to 28 days of age) were used in the experiment. In order to further evaluate this technique, broilers of 26 to 53 days of age were used in a subsequent trial designed primarily to evaluate the dilution technique but with the added objective of obtaining further data relating dietary lysine intake to body mass gain.

MATERIALS AND METHODS

One-day-old chicks of the Ross Broiler strain were allocated at random to 60 pens, such that 30 pens each contained 180 male chicks, and 30 pens contained 180 female chicks. The stocking was 19,4 birds/m^2. The pens were in an environmentally controlled broiler house of a commercial design and management procedures that conformed as closely as possible with commercial practise were adopted. Gas canopy brooders were used during the brooding period, and the photoperiod was 23 hours/day. The chicks were reared on a commercial broiler starter diet to 26 days of age, after which the experimental diets were introduced.

Summit and dilution diets based on the principles of Fisher and

Morris (1970) as modified by Pilbrow and Morris (1974), were formulated at a ME level of 13,39 MJ/kg with amino acid levels based on the recommendations of Thomas *et al.* (1978). The wide range of lysine contents required in the experimental diets was obtained by formulating the summit diet calculated to contain 12,60 g/kg lysine, and the dilution diet calculated to contain 5,30 g/kg lysine, and the intermediate lysine contents were obtained by blending these diets in appropriate proportions as shown in Table 2.3.

Specific protein contents were not used in formulation but the minimum contents of all essential amino acids except lysine were set at 2,0 times (for the summit) and 1,0 times (for the dilution), the requirements proposed by Thomas *et al.* (1978). In some instances it was impossible to reach the higher level set for some amino acids in the summit and dilution diets, with the raw ingredients at our disposal. In these cases the highest feasible levels were then accepted. The extremes of the range of lysine contents used, namely 12,60 g/kg and 5,30 g/kg were 1,10 and 0,45 times the requirements as estimated by Thomas *et al.* (1978).

Six serial dilutions of the summit diet were made as shown in Table 2.3, concentrations of lysine in each diet being 1,10; 0,97; 0,84; 0,71; 0,58 and 0,45 times the requirement for lysine respectively. A second series of dilution diets was prepared in the same manner as that above. Synthetic lysine was then added to diets 2 to 6, the amount supplemented (1,10 g/kg) being equal to 0,75 times the difference in lysine content between two adjacent diets in the first series. The lysine contents of the second series were then 1,05; 0,93; 0,80; 0,68 and 0,55 times the requirement respectively. A third series was prepared in which the level of lysine was increased by the addition of lysine, the amount being added amounting to 1,50 times the difference in lysine content between two adjacent diets in the first series. The level added (2,20 g/kg) to diets 3 to 6 in this series increased the lysine to 1,02; 0,90; 0,77 and 0,64 times the requirement. The net result

TABLE 2.1 Composition (g/kg) of the summit and dilution diets

Ingredient	Summit diet	Dilution diet
Yellow maize meal	302,2	677,5
Maize gluten meal (60%)	212,0	62,0
Sunflower meal	90,0	118,0
Groundnut meal	221,0	70,0
Fish meal	100,0	
Monocalcium phosphate	7,0	17,0
Limestone flour	13,0	15,0
Salt	2,0	4,0
Sunflower oil	46,0	32,0
DL - Methionine	3,3	1,0
Vitamins and trace minerals *	3,0	3,0
Anti-coccidial	0,5	0,5

Calculated analysis		
Arginine	22,46	11,28
Histidine	8,94	4,19
Isoleucine	12,95	5,98
Leucine	36,36	18,09
Lysine	12,60	5,30
Methionine	10,32	4,52
Cystine	5,51	3,70
Phenylalanine	17,75	8,58
Tyrosine	12,24	6,10
Threonine	12,58	6,10
Tryptophan	3,22	1,74
Valine	18,05	8,56
Calcium	10,00	10,00
Phosphorus	7,00	7,00
Crude protein (gN x 6,25/kg)	345,00	168,00
Metabolisable energy (MJ/kg)	13,39	
Net energy (MJ/kg)	9,49	9,49

* Supplies per kg of diet: Vit A 7 025 IU, Vit D_3 2 000 IU, Vit E 8,5 IU, Hetrazine 3 ppm, Thiamine hydrochloride 0,969 ppm, Riboflavin 8 ppm, Calcium pantothenate 7,837 ppm, Niacin 24, 42 ppm, Folic acid 0,95 ppm

Copper 125 ppm, Iron 40 ppm, Zinc 28 ppm, Iodine 2 ppm, Manganese 78 ppm, Selenium 0,1 ppm

TABLE 2.2 Calculated amino acid contents of the summit and dilution diets relative to the requirements of broilers during the finisher period

	Requirements according to Thomas *et al.* (1978) (g/kg)*	Amino acid contents expressed as multiples of requirement	
		Summit diet	Dilution diet
Arginine	12,08	1,86	0,93
Histidine	4,37	1,94	0,96
Isoleucine	8,29	1,56	0,72
Leucine	15,28	2,38	1,18
Lysine	11,64	1,10	0,45
Methionine	5,34	1,93	0,85
Cystine	3,64	1,51	1,02
Phenylalanine	7,71	2,30	1,11
Tyrosine	6,55	1,87	0,93
Threonine	7,71	1,63	0,79
Tryptophan	1,75	1,84	0,99
Valine	9,31	1,94	0,92

* Requirements are expressed in g/kg for a diet containing 13,39 MJ ME/kg (equivalent to 9,49 MJ NE/kg). Amino acid contents as multiples of requirements are expressed in terms of the requirements at the corresponding energy level of the diet

TABLE 2.3 Summary of dilution technique and calculated analysis of the experimental diets

Diet code	Blending ratio				Lysine supplementation (g/kg)	Calculated dietary lysine (g/kg)	Calculated dietary protein (gN x 6,25/kg)
	Summit diet		Dilution diet				
Series 1							
1	100	+	0			12,60	345,0
2	80	+	20			11,14	309,6
3	60	+	40			9,68	274,2
4	40	+	60			8,22	238,8
5	20	+	80			6,76	203,4
6	0	+	100			5,30	168,0
Series 2							
7	80	+	20	+	1,10	12,24	309,6
8	60	+	40	+	1,10	10,78	274,2
9	40	+	60	+	1,10	9,32	238,8
10	20	+	80	+	1,10	7,86	203,4
11	0	+	100	+	1,10	6,40	168,0
Series 3							
12	60	+	40	+	2,20	11,88	274,2
13	40	+	60	+	2,20	10,42	238,8
14	20	+	80	+	2,20	8,96	203,4
15	0	+	100	+	2,20	7,50	168,0

of this diet blending and supplementation technique is that there are identical dilutions in each series, with similar protein and amino acid balances, except for increasing lysine levels as shown in Table 2.3.

The ideal method of manufacturing the experimental diets when using the dilution technique is to prepare the total summit and dilution requirements and then to blend the intermediate diets proportionally. Because of the large volume of food required (in excess of 33 tonnes) this procedure could not be adopted in the manufacture of the experimental food. The 15 experimental diets were therefore mixed individually. Great care was taken to minimise any raw ingredient variation by blending the total requirements of each of the major ingredients, and then drawing from the same ingredient pools whilst mixing the experimental diets.

The 15 experimental diets were allocated such that there were two pens of each sex being fed each diet. Pelleted food, in tube feeders, and water were provided *ad lib*. The body mass of the birds was measured at 26,40 and 53 days of age; food consumption was recorded for intervals corresponding to the mass recordings; mortality was recorded daily and post-mortems were carried out at regular intervals to diagnose possible abnormalities. At 53 days of age a male and a female which were representative of the pen mean were drawn from each replicate for carcass analysis.

RESULTS

The results for the first period are shown in Table 2.4 and Figure 2.1. The response to supplemental lysine in the second and third series of diets, confirmed that lysine was the first-limiting amino acid in the first series of diets. The favourable response in body mass gain elicited after the higher level of lysine addition in the third series indicates that lysine was

TABLE 2.4 Response of broiler chickens to dietary lysine and protein intakes from 26 - 40 days of age

Diet code	Calculated dietary lysine (g/kg)	Calculated dietary protein (gN x 6,25/kg)	Gain in mass (g/bird d)	Lysine intake (mg/bird d)	Protein intake (g/bird d)	Food intake (g/bird d)
Series 1						
1	12,60	345,0	53,5	1325,6	36,3	105,2
2	11,14	309,6	49,7	1167,2	32,4	104,8
3	9,68	274,2	50,7	1015,1	28,8	104,9
4	8,22	238,8	48,9	898,7	26,1	109,3
5	6,76	203,4	41,2	703,1	21,2	104,0
6	5,30	168,0	27,8	476,8	15,1	90,0
Series 2						
7	12,24	309,6	54,1	1303,6	33,0	106,5
8	10,78	274,2	51,7	1134,7	28,9	105,3
9	9,32	238,8	53,9	1035,3	26,5	111,1
10	7,86	203,4	50,9	873,3	22,6	111,1
11	6,40	168,0	49,3	734,0	19,3	114,7
Series 3						
12	11,88	274,2	52,8	1274,6	29,4	107,3
13	10,42	238,8	53,9	1129,0	25,9	108,3
14	8,96	203,4	50,9	982,0	22,3	109,6
15	7,50	168,0	50,2	845,6	18,9	112,7

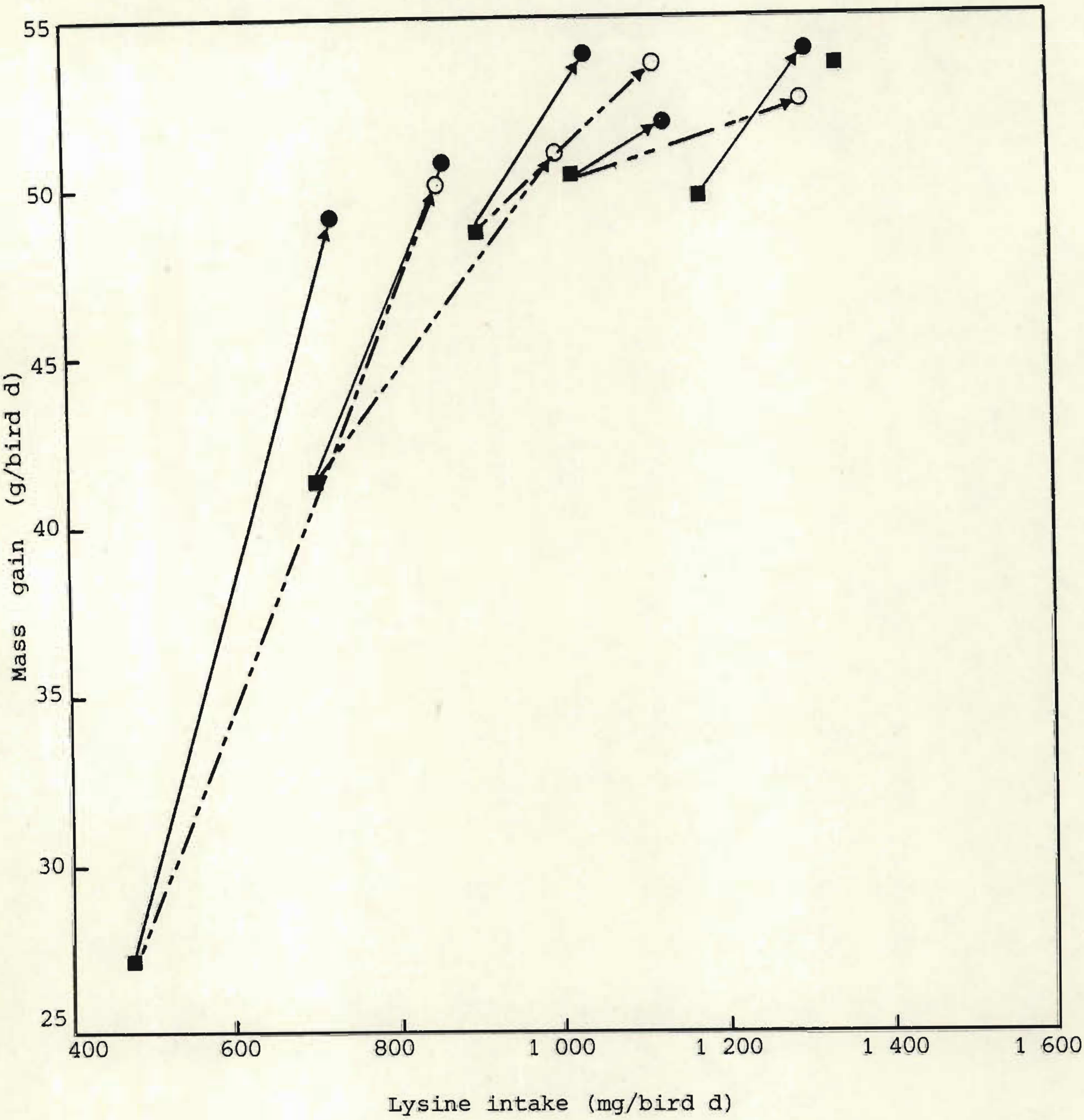

Figure 2.1 Response of male and female chickens to increasing lysine concentrations from 26 - 40 days of age

■ First dilution series (initial dilution)

● Second dilution series (lysine addition)

○ Third dilution series (lysine addition)

TABLE 2.5 Response of broiler chickens to dietary lysine and protein intakes from 26 - 53 days of age

Diet code	Calculated dietary lysine (g/kg)	Calculated dietary protein (gN x 6,25/kg)	Gain in mass (g/bird d)	Lysine intake (mg/bird d)	Protein intake (g/bird d)	Food intake (g/bird d)
Series 1						
1	12,60	345,0	49,0	1368,4	379,6	110,0
2	11,14	309,6	46,0	1215,0	337,7	109,1
3	9,68	274,2	47,9	1025,2	290,4	105,9
4	8,22	238,8	45,5	902,0	262,0	109,7
5	6,76	203,4	35,0	730,0	219,7	108,0
6	5,30	168,0	26,3	519,7	164,7	98,1
Series 2						
7	12,24	309,6	47,6	1340,6	339,1	109,5
8	10,78	274,2	47,2	1198,0	304,7	111,1
9	9,32	238,8	48,2	1075,5	275,6	115,4
10	7,86	203,4	45,6	876,5	226,8	111,5
11	6,40	168,0	45,7	745,2	195,6	116,4
Series 3						
12	11,88	274,2	48,2	1320,0	304,7	111,1
13	10,42	238,8	47,9	1163,6	266,7	111,7
14	8,96	203,4	47,7	989,1	224,5	110,4
15	7,50	168,0	45,0	840,8	188,3	112,1

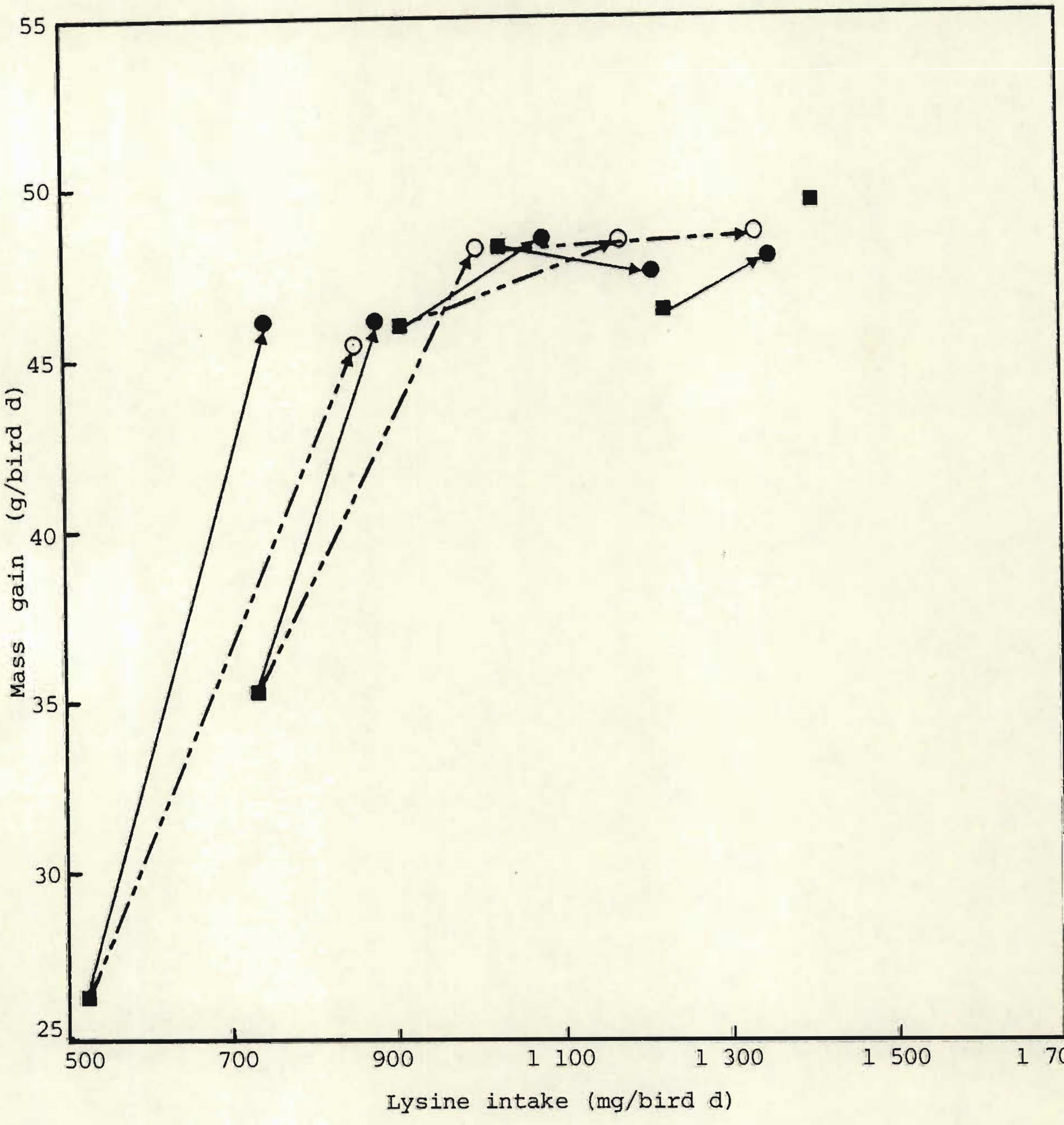

Figure 2.2 Response of male and female chickens to increasing lysine concentrations from 26 - 53 days of age

■ First dilution series (initial dilution)

● Second dilution series (lysine addition)

○ Third dilution series (lysine addition)

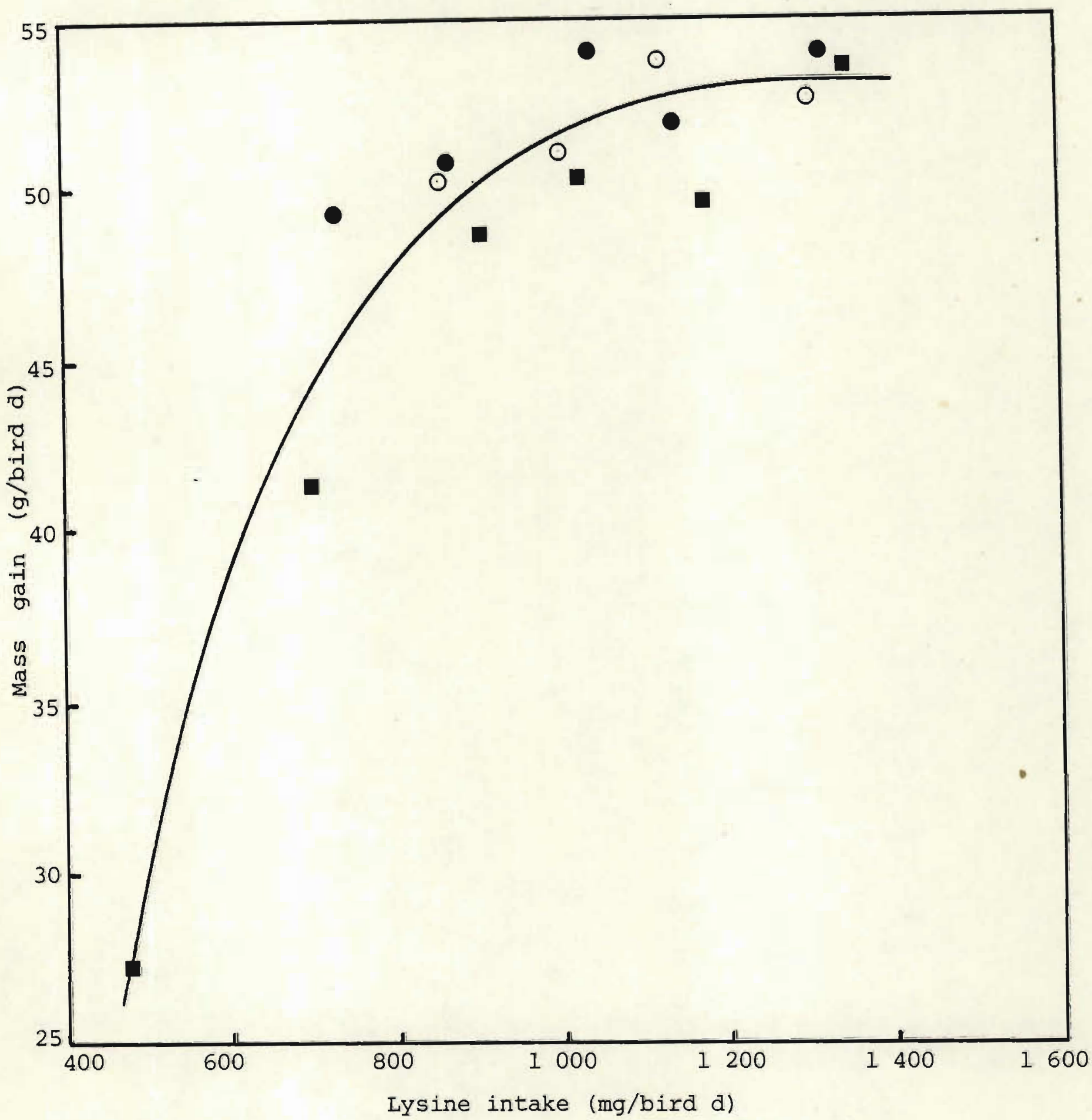

Figure 2.3 Response of male and female chickens to lysine intake and estimated response using Reading model (Table 2.7) from 26 - 40 days of age

■ First dilution series (initial dilution)

● Second dilution series (lysine addition)

O Third dilution series (lysine addition)

TABLE 2.6 Regression coefficients describing response of broiler chickens to increasing dietary concentrations of lysine and protein

Lysine		Protein	
linear coefficient	quadratic coefficient	linear coefficient	quadratic coefficient
26 - 40 days			
0,0697	-0,0000227	---	---
[1](3,495)**	(2,182)*	---	---
---	---	3,0501	-0,04129
---	---	(3,029)**	(2,127)*
0,0753	-0,0000204	-0,4404	---
(3,704)**	(1,941)^{NS}	(1,257)^{NS}	---
0,1261	-0,0000484	-2,5078	0,03976
(2,576)*	(1,735)^{NS}	(1,090)^{NS}	(0,871)^{NS}
26 - 53 days			
0,0829	-0,0000312	---	---
(4,735)**	(3,572)**	---	---
---	---	2,9900	-0,04231
---	---	(3,199)**	(2,451)*
0,0858	-0,0000289	-0,3289	---
(4,871)**	(3,239)**	(1,181)^{NS}	---
0,1261	-0,0000484	-2,5078	0,03976
(4,069)**	(3,181)**	(1,772)^{NS}	(1,570)^{NS}

[1]t - value for testing significance of regression coefficients is shown in brackets. ^{NS}denotes non significant, $^{*}P < 0,05$; $^{**}P < 0,01$

TABLE 2.7 Parameters used in determining the relationship between mass gain and lysine intake using the Reading model using male and female chickens from 26 - 53 days of age

Parameter	26 - 40 days	26 - 53 days
$\bar{W}$	630,00	630,00
$\Delta\bar{W}$	53,47	50,79
a	11,29	11,96
b	0,218	0,288
$\sigma\bar{W}$	52,50	52,50
$\sigma\Delta\bar{W}$	18,03	18,03
r	0,60	0,60

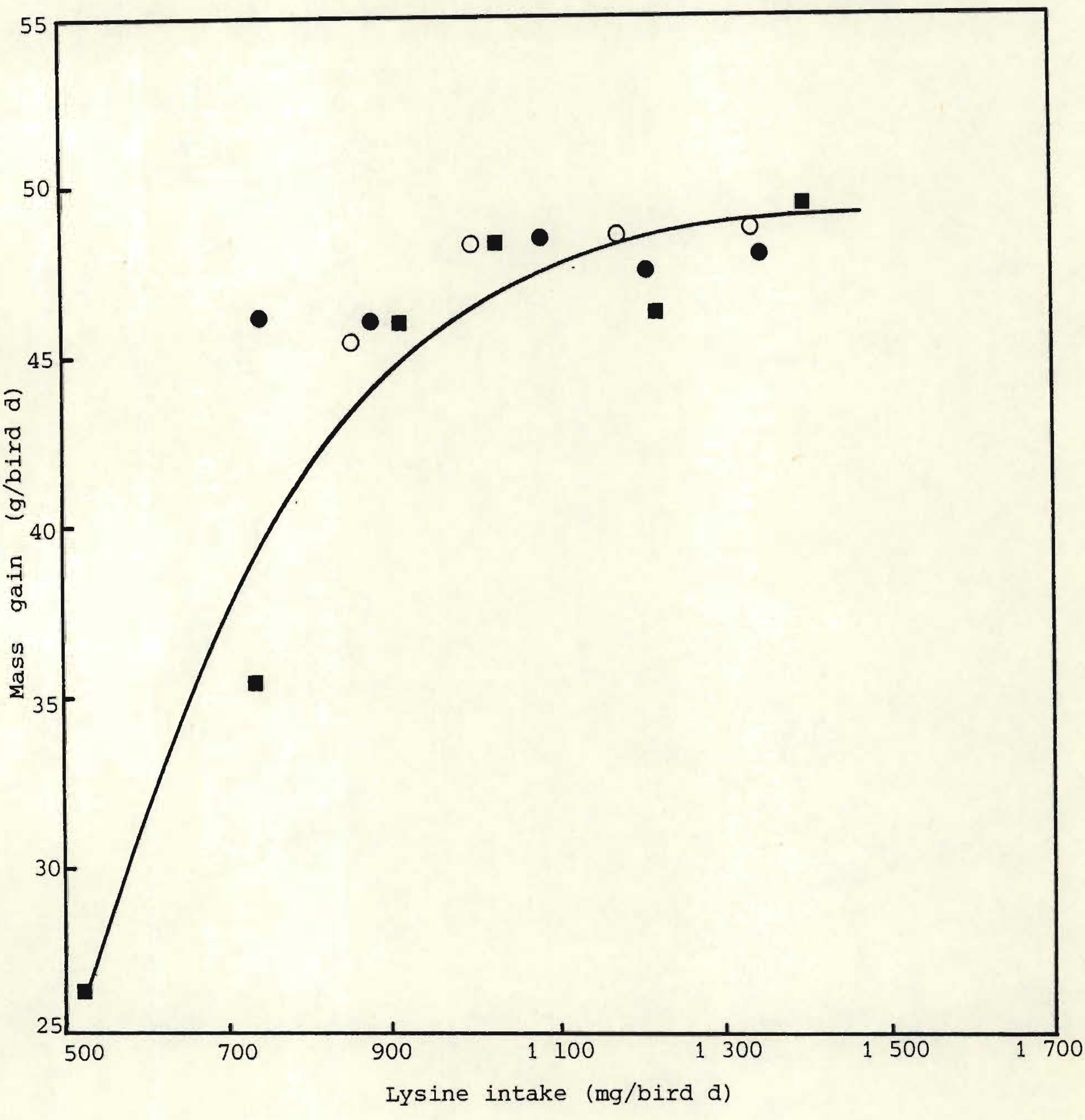

Figure 2.4 Response of male and female chickens to lysine intake and estimated response using Reading model (Table 2.7) from 26 - 53 days of age

■ First dilution series (initial dilution)

● Second dilution series (lysine addition)

○ Third dilution series (lysine addition)

limited in all cases, and that the magnitude of the response was proportional to the level of lysine added.

A multiple regression analysis was performed on this data to test the relative importance of lysine intake and protein intake on livemass gain. The terms tested in the analysis covering the two periods were the same as described in Chapter 1. The regression analysis of the first period (Table 2.6) indicates that lysine intake was more important than protein intake in determining livemass gain. The t-value for testing the significance of lysine intake (2,576) was significant, whilst the squared term for lysine intake (1,735) was non significant (t-value for significance at $P < 0,05$ is 2,015) whereas the t-value for protein intake (1,090) and the squared term for protein intake (0,871) were non significant.

The responses in livemass gain to lysine and protein intakes for the period 26 to 53 days are presented in Table 2.5 and Figure 2.2. These results were subjected to the same multiple regression analysis as outlined in the first period. In this period, as in the first period, highly significant t-values were recorded for testing the significance of lysine and protein intake, and their respective squared terms, when tested separately (Table 2.6). However, when all four terms were subjected to analysis the t-value for testing the significance of lysine intake (4,069) and the squared term for lysine intake (3,181) were highly significant, whereas the t-value for protein intake (1,772) and the squared term for protein intake (1,570) were non significant. The equation presented in Figure 2.4 thus represents a model describing the response of lysine alone on gain in body mass, derived from the results shown in Table 2.5.

The Reading model was used to describe the response in body mass gain to increasing concentrations of dietary lysine intake. The parameters used in fitting this model are given in Table 2.7 Data from both periods were used in fitting the model, and the

TABLE 2.8 Crude protein gain (ΔCP) and carcass fat gain (ΔFT) in relation to dietary lysine and metabolisable energy intakes for male and female broilers from 26 - 53 days of age

Diet code	Lysine intake (mg/bird d)	Energy intake (MJ/bird d)	Protein gain (g/bird d)	Fat gain (g/bird d)
Series 1				
1	1 440,0	1,53	9,65	10,07
2	1 262,0	1,52	9,23	10,16
3	1 065,0	1,47	9,37	12,17
4	937,0	1,53	8,84	11,22
5	758,0	1,50	6,58	9,24
6	539,0	1,36	4,38	8,02
Series 2				
7	1 392,0	1,52	9,43	11,20
8	1 244,0	1,55	8,96	10,40
9	1 117,0	1,60	9,11	11,57
10	910,0	1,55	8,41	12,29
11	774,0	1,62	8,37	12,52
Series 3				
12	1 371,0	1,55	9,00	11,58
13	1 208,0	1,55	9,62	10,23
14	1 027,0	1,53	8.55	11,63
15	873,0	1,56	8,10	11,40

TABLE 2.9 Parameters used in determining the relationship between crude protein gain (ΔCP) and lysine intake using the Reading model using male and female chickens from 26 - 53 days of age

Parameter	26 - 53 days
$\bar{W}$	113,40
$\Delta\bar{W}$	9,41
a	102,12
b	0,057
$\sigma\bar{W}$	10,00
$\sigma\Delta\bar{W}$	1,72
r	0,60

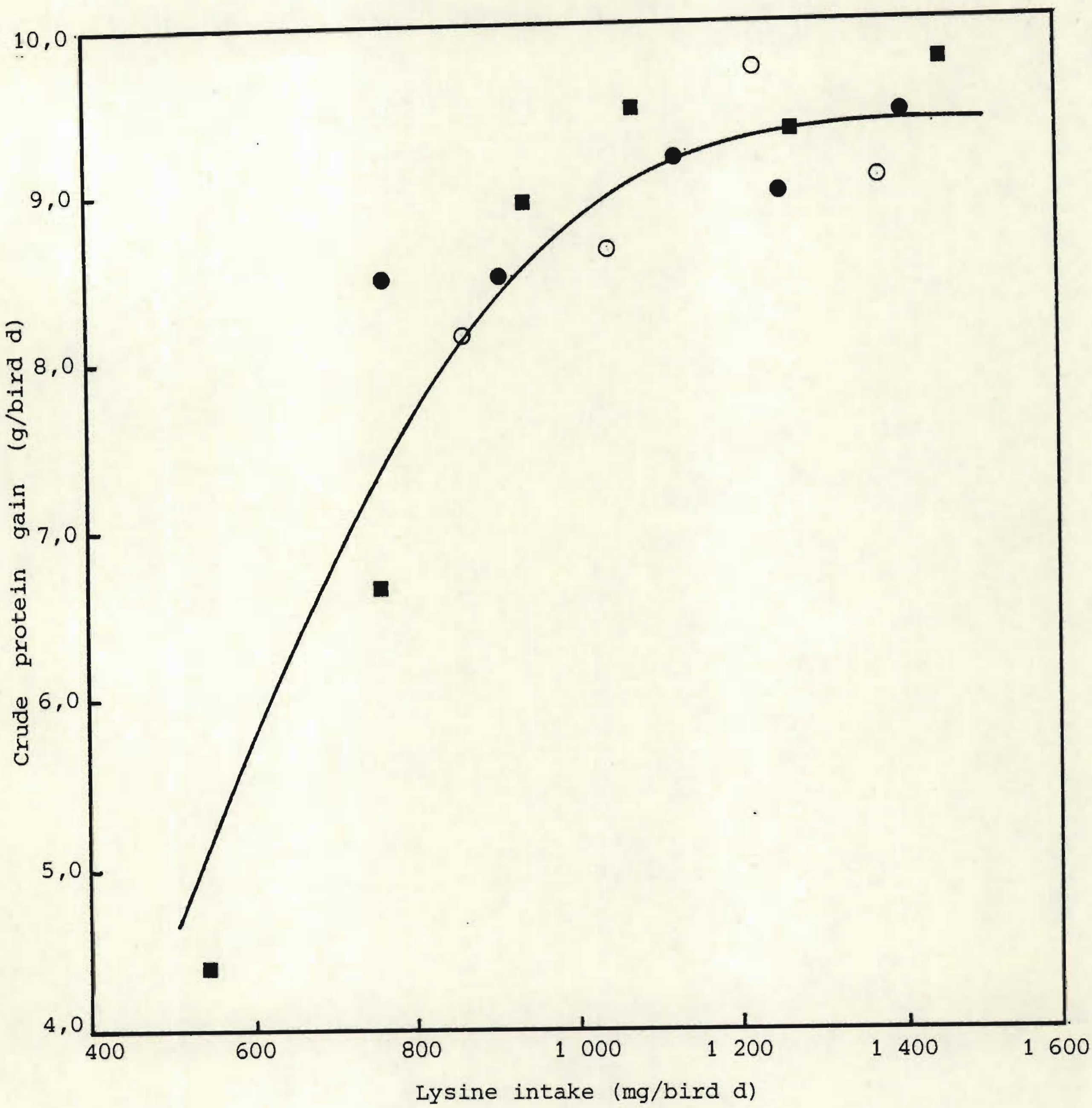

Figure 2.5 Response of male and female chickens to lysine intake and estimated response using Reading model (Table 2.9) from 26 - 53 days of age

■ First dilution series (initial dilution)

● Second dilution series (lysine addition)

○ Third dilution series (lysine addition)

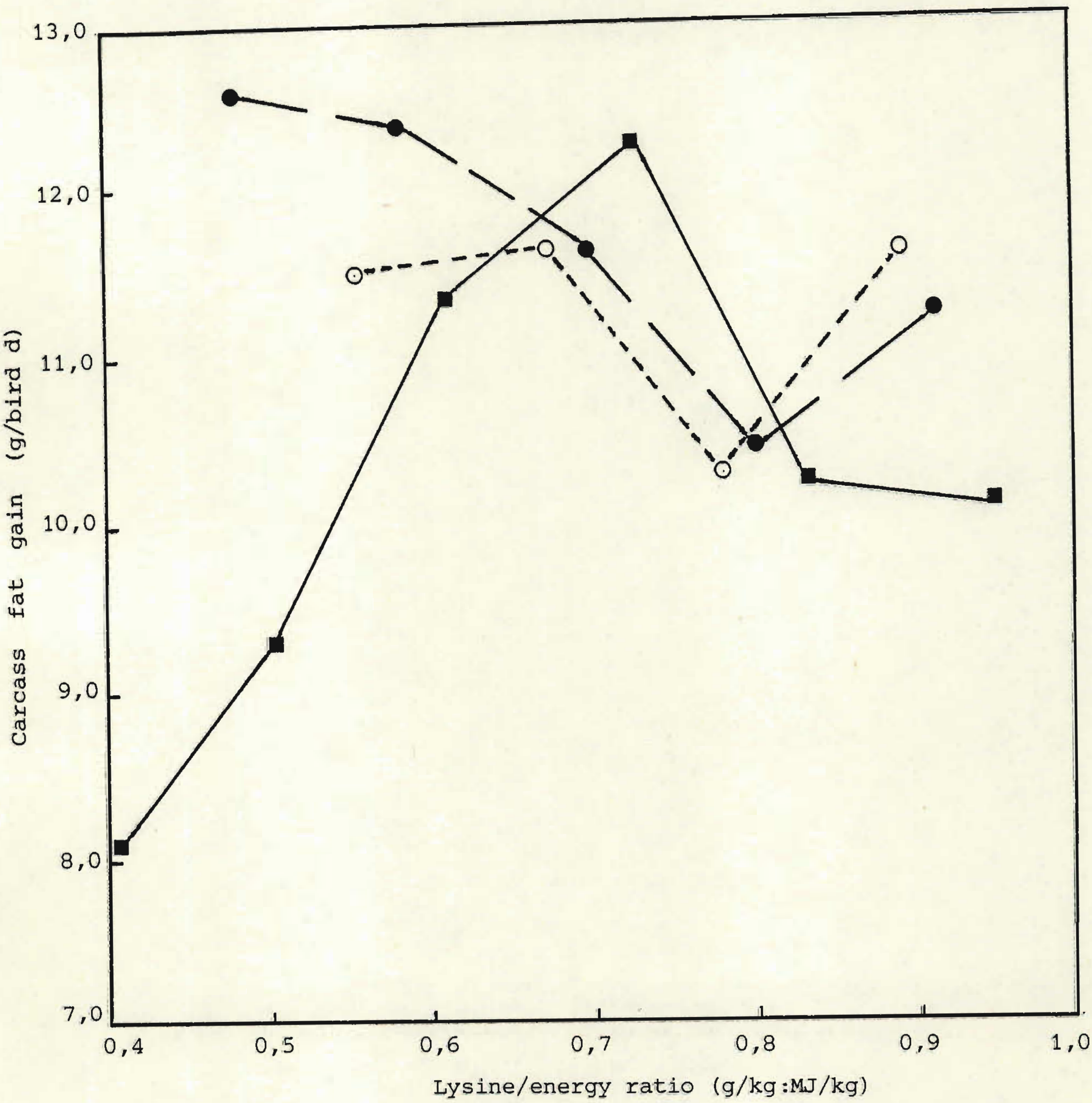

Figure 2.6 Response of male and female chickens to lysine/energy ratio from 26 - 53 days of age

■ First dilution series (initial dilution)

● Second dilution series (lysine addition)

○ Third dilution series (lysine addition)

results are illustrated in Figures 2.3 and 2.4 respectively.

The results of the carcass analysis on representative samples of broilers from the trial were used to calculate the gain in protein and fat that occurred during the experimental period. These results are shown in Table 2.8. The Reading model was used to describe the response in protein gain to increasing dietary intakes of lysine, and this is illustrated in Figure 2.5. The parameters used in fitting the model are given in Table 2.9. Figure 2.6 illustrates the effect of the lysine/energy ratio on the amount of carcass fat gained during the finisher period.

DISCUSSION

The major objective of this experiment was to evaluate a diet dilution technique for measuring the response of broiler chickens to increasing amino acid levels, and then to establish whether the dilution technique could be used for determining the amino acid requirements during the finisher period.

The responses in livemass gain to increasing levels of lysine and protein were recorded for the two periods and subjected to multiple regression analysis. In spite of the wide range of concentrations of protein in the experimental diets corresponding to the range of lysine levels used, protein intake was less important in determining the response in body mass gain than was lysine intake. The response in livemass gain to protein intake was non significant in both periods when all four terms were tested simultaneously. Lysine intake, on the other hand, was responsible to a significant degree for the response in mass gain in both periods.

The results in Table 2.6 show that lysine intake was of greater significance than protein intake in determining livemass gain. When protein and the protein squared term were added the t-values for testing the regression coefficient were non significant, indicating that protein and its squared term are not contributing significantly to the model.

Use was made of the Reading model to describe the relationship between lysine intake and mass gain. The results of all 15 treatments were used, since the responses to lysine supplementaion indicate that it would not be unreasonable to assume that the full response to lysine was obtained in all the experimental diets. The results presented in Figures 2.3 and 2.4 substantiate the dependence of body mass gain on lysine intake.

The effect of the lysine/DME ratio on carcass fat content is shown in Table 2.8 and Figure 2.6 respectively. It is evident that the fat content decreases as the lysine level of the diet increases and the optimum lysine/DME ratio is approximately, 8,0 g lysine/MJ. When this ratio increases beyond 8,0 g lysine/MJ there is an appreciable increase in carcass fat content. The higher carcass fat content resulting from treatments 1, 7 and 12 is possibly due to the excessive lysine concentration in these diets, as the increase appears to be proportional to the synthetic lysine content of the diets.

The equations derived from this study measure the response in body mass gain of broiler chickens to lysine intake, for the two periods under study. The optimum lysine intakes will be calculated only in Chapter 8 when the Reading model will be used to describe the responses in mass gain to lysine intake from the various lysine trails.

CHAPTER 3

EVALUATION OF A DIET DILUTION TECHNIQUE FOR MEASURING THE RESPONSE OF BROILER CHICKENS TO METHIONINE INTAKE DURING THE STARTER PERIOD

INTRODUCTION

It is almost axiomatic that the better the balance of the amino acids in the dietary protein, the lower the bird's requirement for the latter. The modern concept of formulating least-cost poultry diets based on minimum levels of essential amino acids emphasises the need to have reliable estimates of these requirements. The nutrient density in a diet has a direct bearing on the cost of the food, therefore, it is important from a biological and economic standpoint to ensure that the optimum amino acid balance is achieved in the diet.

An area receiving considerable attention is the possibility of adjusting the level of each nutrient so as to maximise profit margins rather than to achieve maximum levels of production. This implies abandoning the idea of a "fixed" requirement for a nutrient and replacing it by data relating rates of output to levels of input. Such data must be obtained from suitably designed feeding trials. Fisher and Morris (1970) described a method for the determination of amino acid requirements of laying hens which is of general applicability in this field. They considered the features of a desirable procedure to be:

(1) that a wide range of amino acid levels could be used;

(2) that the maximum response to the acid being studied should not be limited by the level of other amino acids in the diet;

(3) that the problems of amino acid balance should be minimised;

(4) that the experimental diets should be sufficiently inexpensive to permit feeding of large numbers of birds for long periods if necessary;

(5) that the maximum levels of performance obtained and, as far as possible, the ingredients used in the diets should reflect closely the commercial conditions under which the results might be applied.

The methods used previously for determining amino acid levels do not satisfactorily meet these requirements. When graded levels of an amino acid are added to a basal diet deficient in that amino acid it is possible to study only a narrow range of input levels unless very expensive mixtures of synthetic amino acids are used to supply part or all of the basal diet. It is also difficult to determine when the supply of other essential amino acids becomes limiting. If each level of the test amino acid is obtained by formulating a series of diets from practical raw materials the pattern and levels of all amino acids must change. That the responses obtained under such conditions can be attributed solely to the levels of a single amino acid is questionable. A technique which seems likely to meet all the requirements for a successful assay is one developed by Fisher and Morris (1970) in which a high protein diet is diluted with protein-free materials.

In the dilution technique all of the protein containing ingredients are displaced proportionally by a protein-free dilution mixture which is isocaloric with the summit diet. By a suitable definition of the amino acid make-up of the summit diet the amino acid under study is made first-limiting and the dilution technique then affords a method of measuring the response to one essential amino acid.

In Chapter 1 it was established that the dilution technique was a suitable method for measuring the response of broilers to increasing lysine concentrations during the starter period. In the experiment described below the response of broilers to increasing methionine levels will be determined using the dilution technique.

MATERIALS AND METHODS

Day-old Ross broiler chicks were allocated to 60 wire-floored tier brooder pens in a biological experimental unit. Each deck was divided into four pens and the brooder temperatures were maintained at a comfortable level for the chicks throughout the 21 day experimental period. Food and water were fed *ad lib.* and artificial light was supplied for 24 hours per day.

The chicks were allocated at random to the pens, but individuals that were abnormally small or large were removed in order that the mean was kept as uniform as possible. Each pen contained 10 chicks, there being four replications per treatment.

In choosing the level of methionine and the remaining essential amino acids of the summit diet it was estimated that as wide a range as was practically feasible would be used in order that a full response curve could be plotted. The required range of methionine contents was obtained by formulating a summit diet calculated to contain 1,0 times the methionine and 2,0 times the other amino acids of the requirements proposed by Thomas *et al.* (1978). The composition of the summit and dilution diets is shown in Table 3.1. When the summit diet is diluted with a protein-free energy source methionine will be first-limiting at all levels of dilution if the requirement standards used are sufficiently accurate and appropriate to all levels of protein intake. The method rests on the interpretation of the response to different levels of dilution or a response to the first-limiting amino acid, in this case methionine. To reduce as far as possible unwanted surpluses of amino acids, the

TABLE 3.1 Composition (g/kg) of the summit and dilution diets

Ingredient	Summit diet	Dilution diet
Soyabean unextracted	556,0	
Sunflower meal	117,0	
Groundnut meal	213,0	
Blood meal	50,0	
Monocalcium phosphate	10,0	33,0
Limestone flour	15,0	9,0
Salt	4,0	4,0
Sunflower oil	32,0	
Sugar		300,0
Sunflower husk		170,0
Starch		481,0
Vitamins and trace minerals *	3,0	3,0
Calculated analysis		
Arginine	30,9	
Histidine	10,3	
Isoleucine	14,5	
Leucine	29,2	
Lysine	21,7	
Methionine	5,2	
Cystine	6,4	
Phenylalanine	19,4	
Tyrosine	11,6	
Threonine	14,2	
Tryptophan	5,1	
Valine	19,2	
Calcium	10,00	10,00
Phosphorus	8,00	8,00
Crude protein (gN x 6,25/kg)	383,00	
Metabolisable energy (MJ/kg)	12,97	
Net energy (MJ/kg)	9,44	9,44

* Supplies per kg of diet: Vit A 7 027 IU, Vit D_3 2 000 IU, Vit E 20 IU, Hetrazine 3 ppm, Thiamine hydrochloride 0,985 ppm, Riboflavin 8 ppm, Calcium pantothenate 7,846 ppm, Niacin 29,823 ppm, Folic acid 0,95 ppm, Biotin 0,08 ppm, Choline chloride 300 ppm

Copper 125 ppm, Iron 40 ppm, Zinc 28 ppm, Iodine 2 ppm, Manganese 78 ppm, Selenium 0,1 ppm

TABLE 3.2 Calculated amino acid contents of the summit and dilution diets relative to the requirements of broilers during the starter period

	Requirements according to Thomas *et al.* (1978) (g/kg)*	Amino acid contents expressed as multiples of requirement	
		Summit diet	Dilution diet
Arginine	12,40	2,49	
Histidine	4,51	2,28	
Isoleucine	8,45	1,72	
Leucine	15,78	1,85	
Lysine	12,67	1,71	
Methionine	5,17	1,00	
Cystine	3,80	1,68	
Phenylalanine	7,89	2,46	
Tyrosine	6,76	1,72	
Threonine	7,47	1,90	
Tryptophan	2,25	2,27	
Valine	9,58	2,00	

* Requirements are expressed in g/kg for a diet containing 12,97 MJ/kg (equivalent to 9,44 MJ NE/kg). Amino acid contents as multiples of requirements are expressed in terms of the requirements at the corresponding energy level of the diet

TABLE 3.3 Summary of dilution technique and calculated analysis of the experimental diets

Diet code	Blending ratio					Methionine supple-mentation (g/kg)	Calculated dietary methionine (g/kg)	Calculated dietary protein (gN x 6,25/kg)
	Summit diet		Dilution diet					
Series 1								
1	100	+	0				5,17	383,0
2	86	+	14				4,45	329,0
3	72	+	28				3,72	276,0
4	58	+	42				3,00	222,0
5	44	+	56				2,27	169,0
6	30	+	70				1,55	115,0
Series 2								
7	86	+	14	+		0,54	4,99	329,0
8	72	+	28	+		0,54	4,26	276,0
9	58	+	42	+		0,54	3,54	222,0
10	44	+	56	+		0,54	2,81	169,0
11	30	+	70	+		0,54	2,09	115,0
Series 3								
12	72	+	28	+		1,08	4,80	276,0
13	58	+	42	+		1,08	4,08	222,0
14	44	+	56	+		1,08	3,35	169,0
15	30	+	70	+		1,08	2,63	115,0

crude protein content of the summit diet was kept as low as possible. In some cases the specified minima of essential amino acids could not be achieved, in which case the highest levels possible were accepted.

Six serial dilutions of the summit diet were made (series 1 Table 3.3), concentrations of methionine in each diet being 1,00; 0,86; 0,72; 0,58; 0,44 and 0,30 times the requirement for methionine respectively. A second series of dilution diets was prepared in the same manner as that above. Synthetic methionine was then added to diets 2 to 6, the amount supplemented (0,54 g/kg) being equal to 0,75 times the difference in methionine contents between two adjacent diets in the first series. The methionine contents of the second series were then 0,97; 0,82; 0,68; 0,54 and 0,40 times the requirment respectively. A third series was prepared in which the level of methionine was increased by the addition of methionine, the amount being added amounting to 1,50 times the difference in methionine content between two adjacent diets in the first series. The level added (1,08 g/kg) to diets 3 to 6 in this series increased the methionine to 0,93; 0,80; 0,65 and 0,51 times the requirement. The outcome of the diet blending and supplementation technique is that there are identical dilutions in each series, with similar protein and amino acid balances, except for increasing methionine levels as shown in Table 3.3.

The experimental diets were fed from 0 to 21 days of age, and the criteria studied were growth rate and food intake during the three week experimental period. At 21 days of age, two birds which were representative of the pen mean were drawn from each replicate for carcass analysis. The data on each variate were subjected to multiple regression analysis using the method of Rayner (1967).

RESULTS

The objective of this experiment was to evaluate the dilution technique for measuring the response of broilers to methionine intake, and to establish whether a protein factor was important in chicken nutrition, or whether the first-limiting amino acid was of greater significance in regulating chick growth.

The results of the first period are shown in Table 3.4. The response to supplemented methionine in the second and third series of diets clearly indicated that methionine was the most limiting amino acid in the first series of diets. A multiple regression analysis was performed on this data to test the relative importance of methionine intake and protein intake on body mass gain. The terms tested in the analysis covering the two periods were the following:

Methionine, methionine^2

Protein, protein^2

Methionine, methionine^2, protein

Methionine, methionine^2, protein, protein^2

The regression analysis of the first period indicated that when tested separately both methionine and protein intake were important in determining body mass gain. The t-values for testing the significance of methionine intake (7,036) and the squared term for methionine intake (3,753) were highly significant (t-value for significance at $P < 0,01$ is 2,671), as were the t-values for protein intake (12,930) and the squared term for protein intake (8,835). The multiple correlation coefficient for methionine on mass gain was 0,942 and that for protein 0,963.

When methionine and protein and their squared terms were tested simultaneously it was evident that protein was more important than methionine in controlling growth. The t-values for protein (8,076)

TABLE 3.4 Response of broiler chickens to dietary methionine and protein intakes from 0 - 14 days of age

Diet code	Calculated dietary methionine (g/kg)	Calculated dietary protein (gN x 6,25/kg)	Gain in mass (g/bird d)	Methionine intake (mg/bird d)	Protein intake (g/bird d)	Food intake (g/bird d)
Series 1						
1	5,17	383,0	16,1	110,0	8,1	21,2
2	4,45	329,0	17,2	101,0	7,4	22,4
3	3,72	276,0	17,4	87,4	6,5	23,6
4	3,00	222,0	14,8	69,8	5,2	23,3
5	2,27	169,0	10,8	47,9	3,5	20,8
6	1,55	115,0	7,3	31,0	2,2	19,4
Series 2						
7	4,99	329,0	18,5	120,5	7,9	24,1
8	4,26	276,0	17,7	98,8	6,3	23,0
9	3,54	222,0	15,7	81,9	5,2	23,4
10	2,81	169,0	12,6	60,9	3,7	21,8
11	2,09	115,0	8,3	40,1	2,2	19,1
Series 3						
12	4,80	276,0	18,0	114,8	6,6	23,9
13	4,08	222,0	16,7	97,7	5,3	23,8
14	3,35	169,0	13,5	83,6	3,9	23,2
15	2,63	115,0	8,7	52,2	2,3	20,1

TABLE 3.5 Response of broiler chickens to dietary methionine and protein intakes from 0 - 21 days of age

Diet code	Calculated dietary methionine (g/kg)	Calculated dietary protein (gN x 6,25/kg)	Gain in mass (g/bird d)	Methionine intake (mg/bird d)	Protein intake (g/bird d)	Food intake (g/bird d)
Series 1						
1	5,17	383,0	20,0	153,5	11,3	29,5
2	4,45	329,0	21,9	138,9	10,2	30,9
3	3,72	276,0	21,7	118,0	8,8	31,9
4	3,00	222,0	17,0	91,2	6,8	30,4
5	2,27	169,0	12,6	62,7	4,6	27,3
6	1,55	115,0	7,8	40,8	2,9	25,5
Series 2						
7	4,99	329,0	23,1	161,2	10,6	32,2
8	4,26	276,0	22,0	134,7	8,6	31,3
9	3,54	222,0	20,1	109,5	6,9	31,3
10	2,81	169,0	15,4	81,7	4,9	29,2
11	2,09	115,0	9,5	52,9	2,9	25,2
Series 3						
12	4,80	276,0	23,2	156,9	9,0	32,7
13	4,08	222,0	21,1	129,5	7,0	31,6
14	3,35	169,0	17,6	112,1	5,3	31,1
15	2,63	115,0	11,1	69,2	3,1	26,6

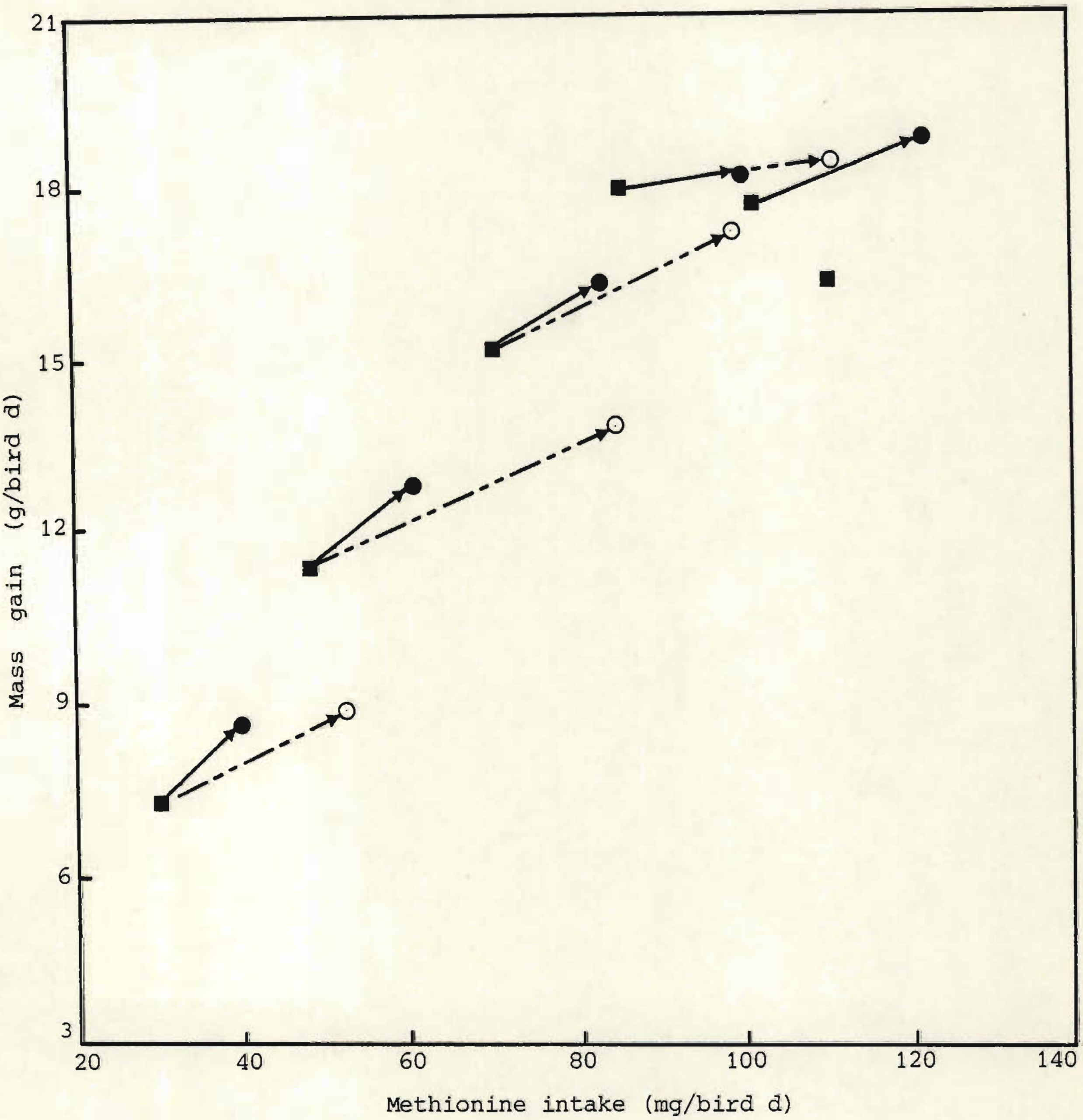

Figure 3.1 Response of male chickens to increasing methionine concentrations from 0 - 14 days of age

■ First dilution series (initial dilution)

● Second dilution series (methionine addition)

○ Third dilution series (methionine addition)

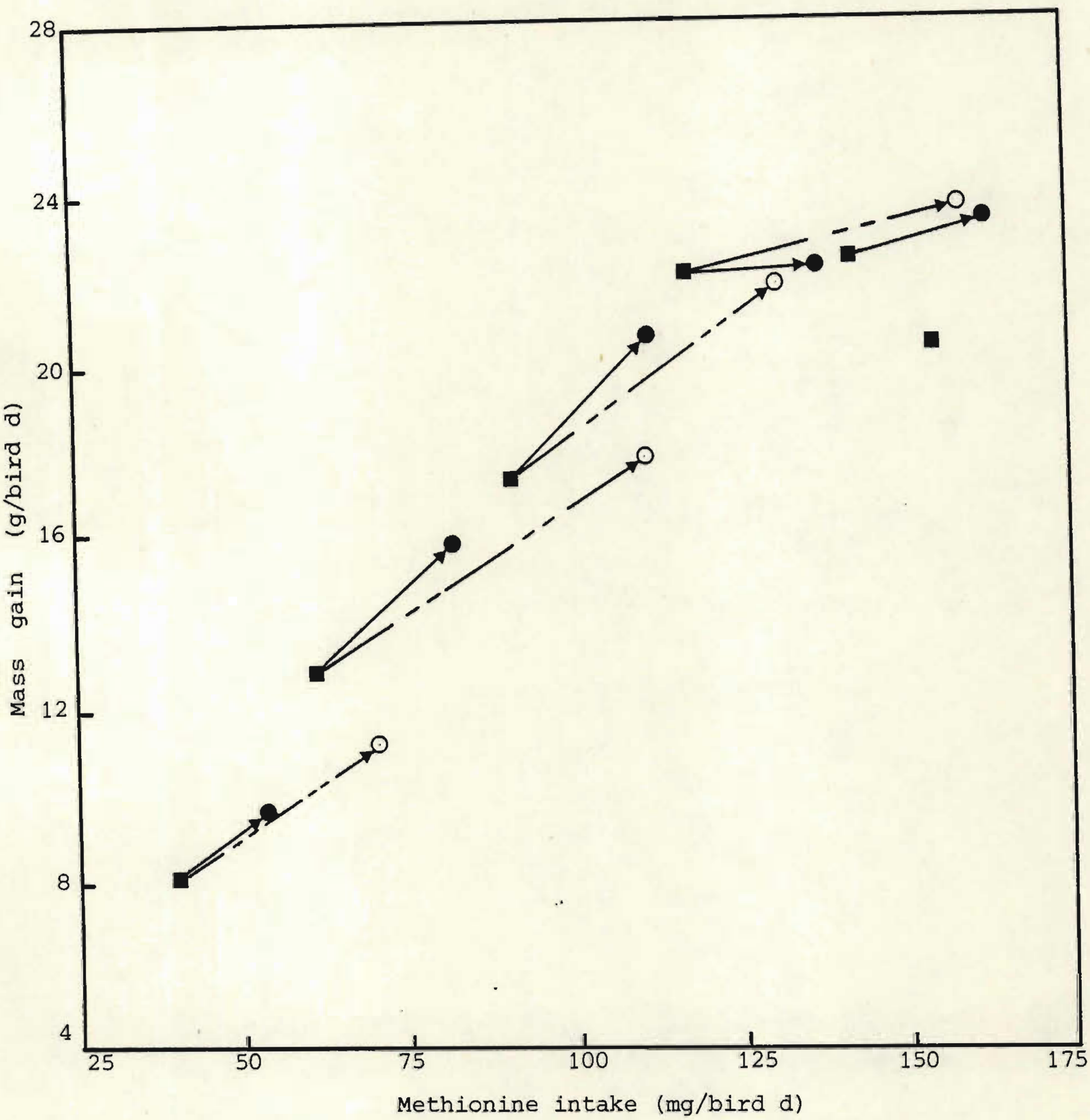

Figure 3.2 Response of male chickens to increasing methionine concentrations from 0 - 21 days of age

■ First dilution series (initial dilution)

● Second dilution series (methionine addition)

○ Third dilution series (methionine addition)

and its squared term (7,029) were highly significant, whereas those for methionine (0,227) and methionine squared (1,228) were non significant. This result is indicative that the birds did not respond fully to the methionine supplementation, even though methionine was first-limiting in the supplemented diets. One of the other essential amino acids presumably became limiting at high levels of methionine supplementation and suppressed the additional response which should have been elicited by the added methionine.

The responses in livemass gain to methionine and protein intakes for the period 0 to 21 days of age are presented in Table 3.5. These results were subjected to the same multiple regression analysis as outlined in the first period. In this period, as in period 1, highly significant t-values were recorded for testing the significance of methionine and protein intake, and their respective squared term, when tested separately (Table 3.6). However, when all four terms were subjected to analysis, as in the first period, the t-value for testing the significance of methionine intake (0,227) and the squared term for methionine intake (1,228) were non significant, whereas the t-value for protein intake (8,076) and the squared term for protein intake (7,029) were highly significant. The regression coefficients and t-values for testing their significance for both periods are presented in Table 3.6.

The mean values for growth rate and methionine intake were used in fitting the Reading model to the data in order to describe the relationship between mass gain and methionine intake. Only those treatments where it was certain that methionine was first-limiting were used. This applies to diets 2 to 6 in the initial dilution series, diets 7 to 11 in the second series and diet 12 in the third series. Although methionine was shown to be first-limiting in this experiment, the addition of large doses of synthetic methionine did not elicit the full response to methionine indicating an effect of a second-limiting amino acid. The results of fitting a Reading model to this data is shown in Table 3.7, together with the parameters used

TABLE 3.6 Regression coefficients describing response of broiler chickens to increasing dietary concentrations of methionine and protein

Methionine		Protein	
linear coefficient	quadratic coefficient	linear coefficient	quadratic coefficient
0 - 14 days			
0,2694	-0,0009256	---	---
[1](7,036)**	(3,753)**	---	---
---	---	5,3164	-0,3552
---	---	(12,930)**	(8,835)**
0,2287	-0,0009848	0,7433	---
(6,258)**	(4,384)**	(3,614)**	---
0,009330	-0,0003024	4,6438	-0,3597
(0,227)^{NS}	(1,228)^{NS}	(8,076)**	(7,029)**
0 - 21 days			
0,3056	-0,0008748	---	---
(11,149)**	(6,691)**	---	---
---	---	5,3845	-0,2731
---	---	(13,921)**	(9,848)**
0,2890	-0,0009084	0,3485	---
(10,432)**	(7,105)**	(2,129)*	---
0,1101	-0,0001320	3,4179	-0,2074
(3,629)**	(0,977)^{NS}	(8,217)**	(7,678)**

[1]t - value for testing significance of regression coefficients is shown in brackets. ^{NS}denotes non-significant, *P < 0,05; **P < 0,01

TABLE 3.7 Parameters used in determining the relationship between mass gain (g/bird d) and methionine intake (mg/bird d) by means of the Reading model using male chickens from 0 - 21 days of age

Parameter	0 - 14 days	0 - 21 days
$\bar{W}$	320,00	320,00
$\Delta\bar{W}$	17,96	22,63
a	4,57	5,00
b	0,004	0,010
$\sigma\bar{W}$	53,00	53,00
$\sigma\Delta\bar{W}$	2,90	3,68
r	0,60	0,60

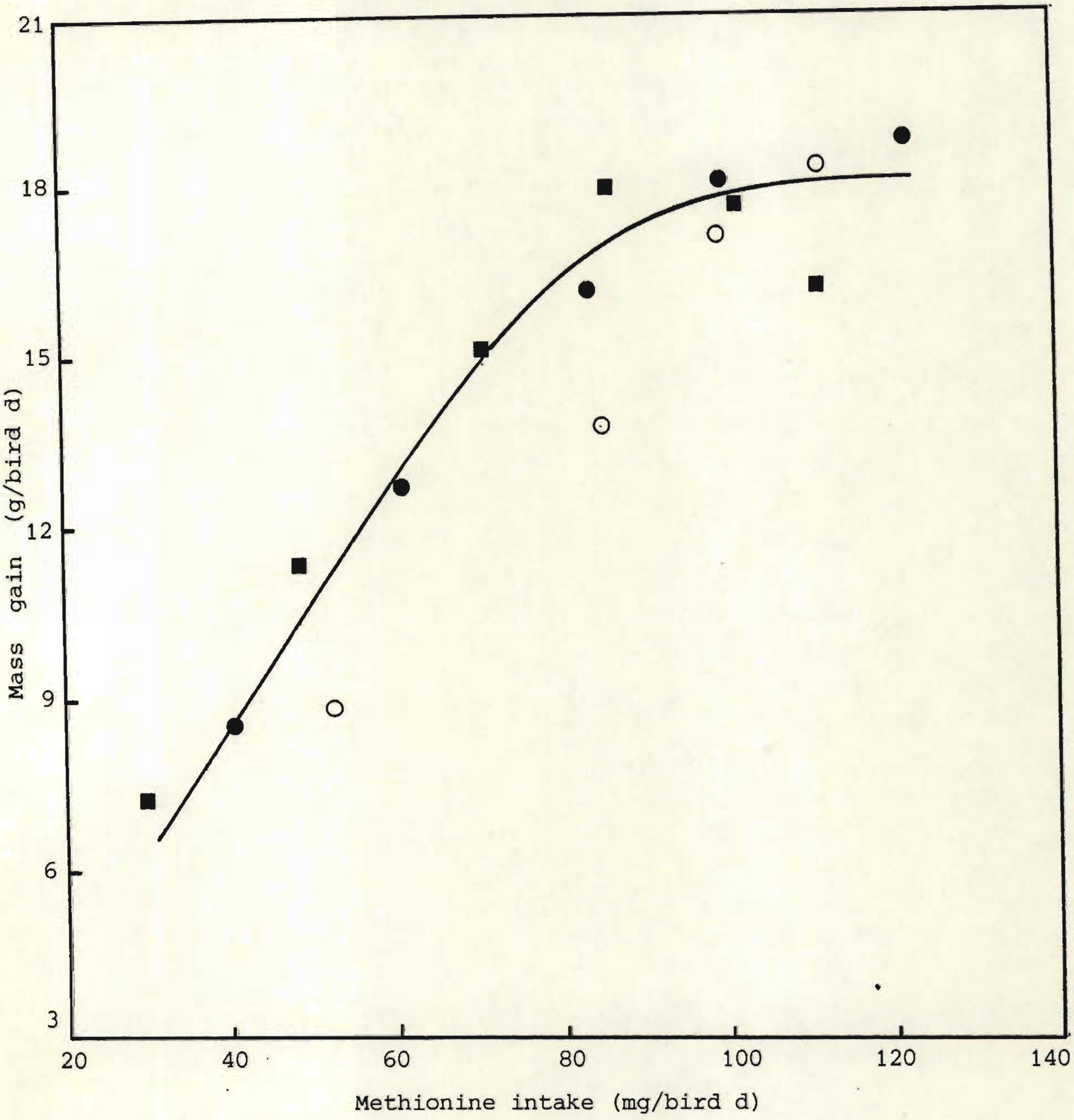

Figure 3.3 Response of male chickens to methionine intake and estimated response using Reading model (Table 3.7) from 0 - 14 days of age

■ First dilution series (initial dilution)

● Second dilution series (methionine addition)

○ Third dilution series (methionine addition)

In fitting the model, not all data points shown above were included. For more details see text

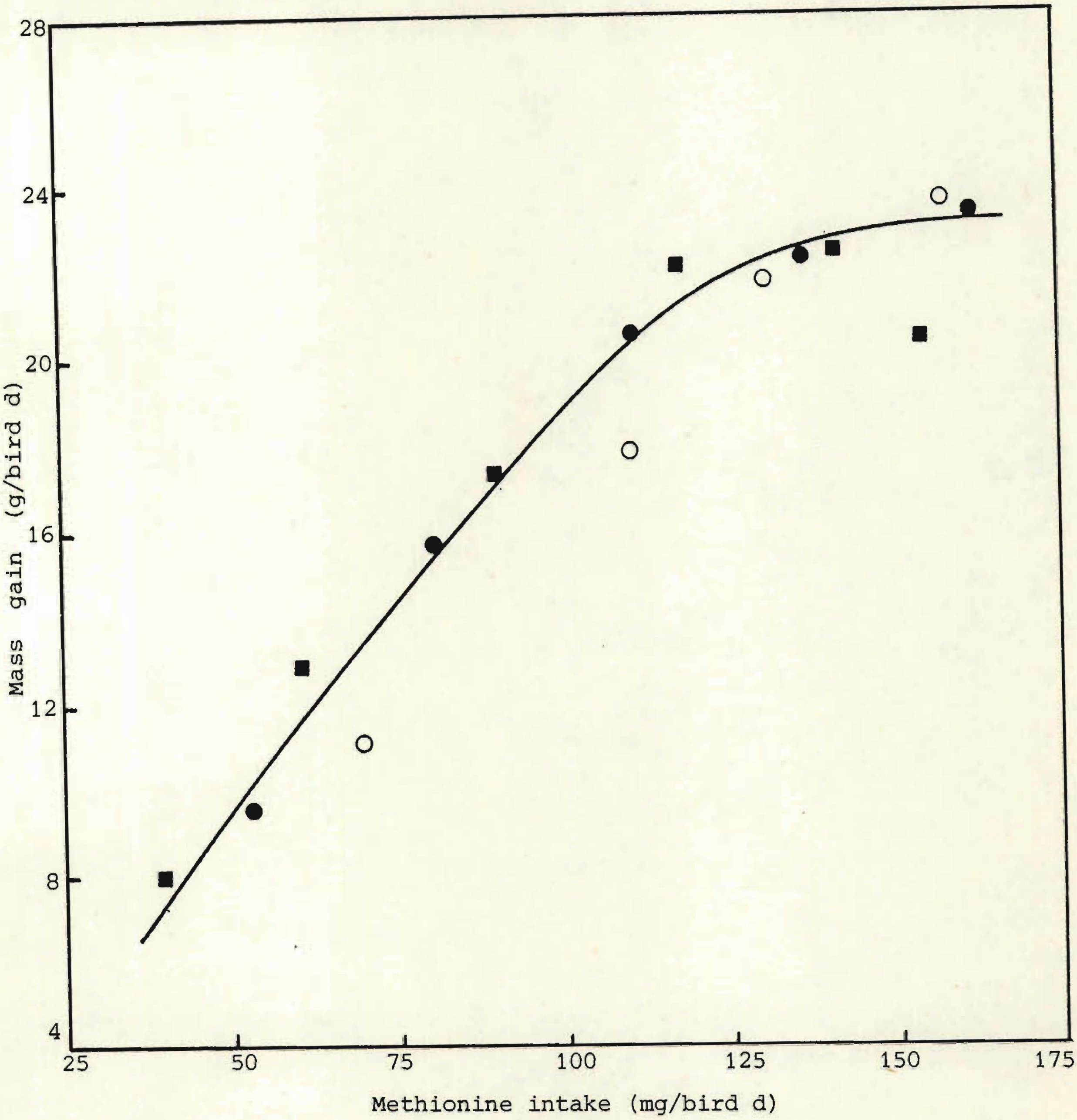

Figure 3.4 Response of male chickens to methionine intake and estimated response using Reading model (Table 3.7) from 0 - 21 days of age

■ First dilution series (initial dilution)

● Second dilution series (methionine addition)

○ Third dilution series (methionine addition)

In fitting the model, not all data points shown above were included. For details see text

TABLE 3.8 Response in crude protein gain (ΔCP) and carcass fat gain (ΔFT) to dietary methionine and metabolisable energy intakes of male chickens from 0 - 21 days of age

Diet code	Methionine intake (mg/bird d)	Energy intake (MJ/bird d)	Protein gain (g/bird d)	Fat gain (g/bird d)
Series 1				
1	153,5	0,38	3,54	1,46
2	138,9	0,40	3,78	1,84
3	118,0	0,41	3,74	2,29
4	91,2	0,39	2,77	1,83
5	62,7	0,35	2,03	1,71
6	40,8	0,33	1,26	1,21
Series 2				
7	161,2	0,42	4,08	2,06
8	134,7	0,41	3,70	2,18
9	109,5	0,41	3,51	2,27
10	81,7	0,38	2,60	2,24
11	52,9	0,33	1,45	1,62
Series 3				
12	156,9	0,42	4,05	2,08
13	129,5	0,41	3,54	2,47
14	112,1	0,40	2,85	2,29
15	69,2	0,35	1,77	1,83

TABLE 3.9 Parameters used in determining the relationship between crude protein gain (g/bird d) and methionine intake (mg/bird d) by means of the Reading model using male chickens from 0 - 21 days of age

Parameter	0 - 21 days
$\bar{W}$	34,00
$\Delta\bar{W}$	3,76
a	30,34
b	0,043
$\sigma\bar{W}$	6,00
$\sigma\Delta\bar{W}$	0,63
r	0,60

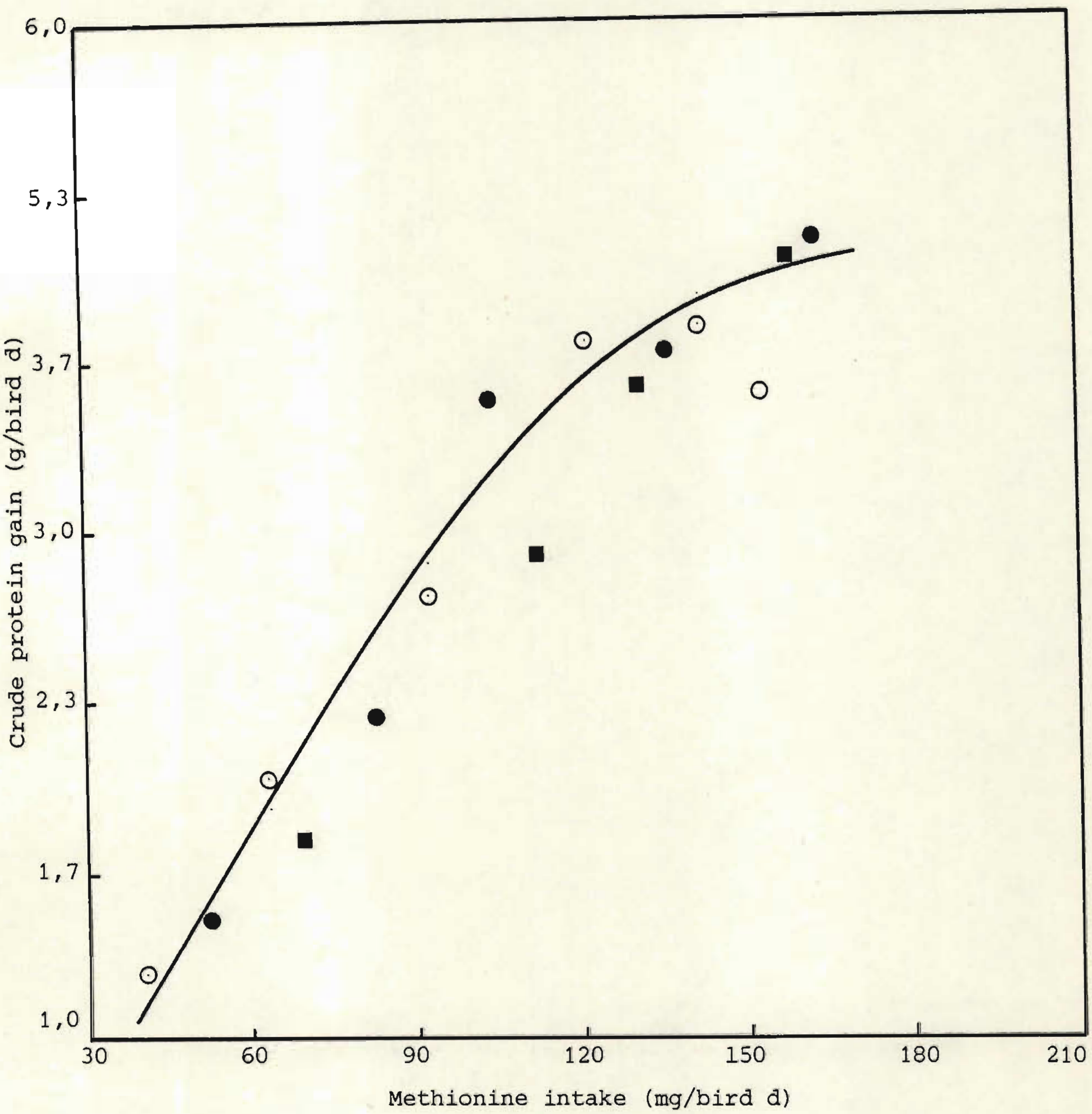

Fig 3.5 Response of male chickens in protein gain to methionine intake, and estimated response using Reading model (Table 3.9) from 0 - 21 days of age

■ First dilution series (initial dilution)

● Second dilution series (methionine addition)

○ Third dilution series (methionine addition)

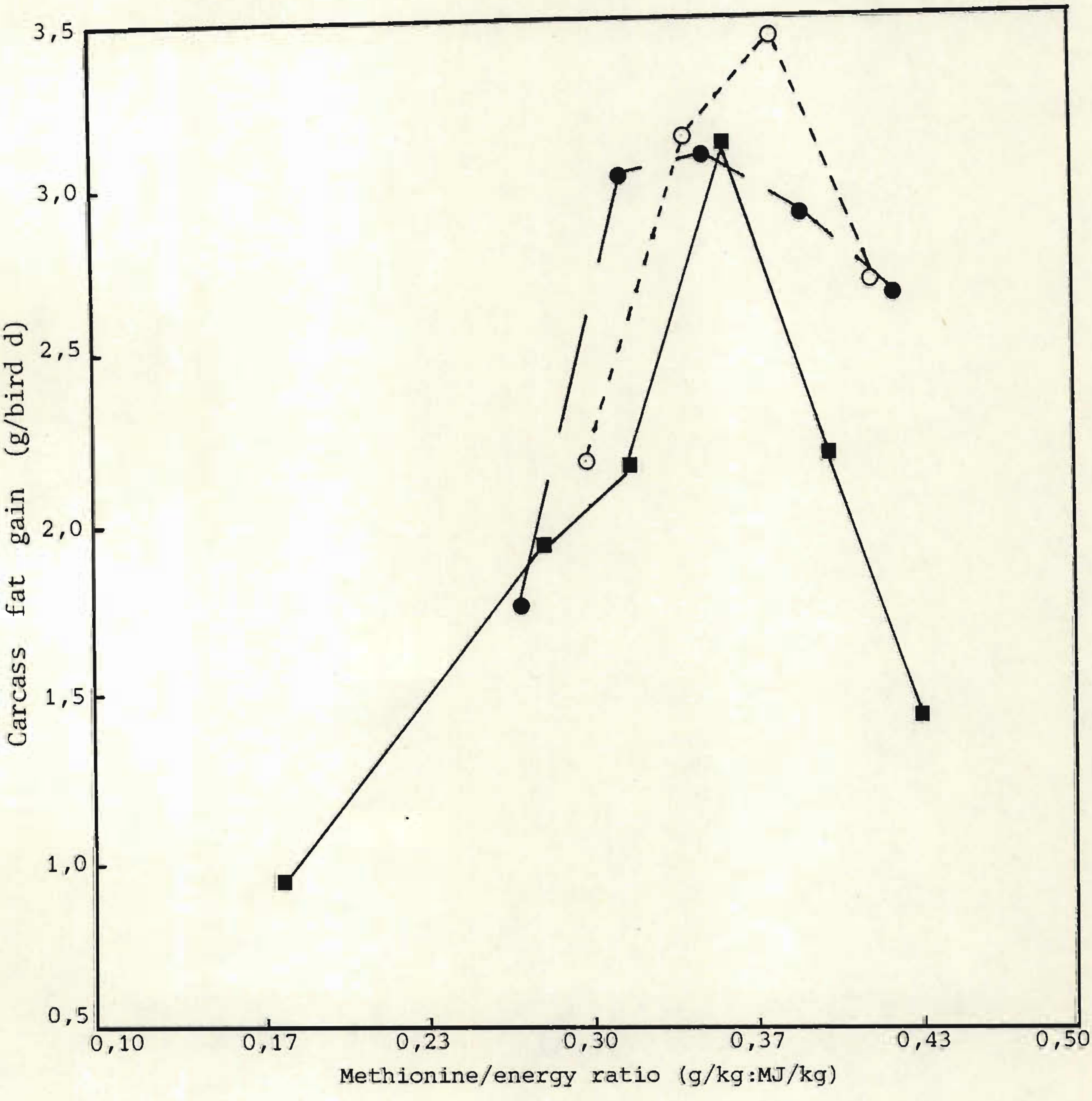

Figure 3.6 Response of male chickens in carcass fat gain to methionine/energy ratio from 0 - 21 days of age

■ First dilution series (initial dilution)

● Second dilution series (methionine addition)

○ Third dilution series (methionine addition)

in fitting the model. The data for both periods are illustrated in Figures 3.3 and 3.4 respectively.

The Reading model was used to describe the effect of methionine intake on protein gain, using the carcass analysis data from treatments 2 to 12 inclusive. The results are presented in Table 3.9 and Figure 3.5 respectively. Figure 3.6 illustrates the effect of the methionine/DME ratio on the carcass fat gain of the broilers during the starter period.

DISCUSSION

The major objective of this experiment was to evaluate a diet dilution technique for measuring the response of broiler chickens to increasing methionine concentrations.

The concept of using a dilution technique for determining the response of chickens to amino acid intakes has been presented in relation to the nutrition of laying hens, and more recently for the determination of the available tryptophan requirements of broilers. The major advantages of this techniqe over prior methods of determining amino acid requirements have been adequately discussed in Chapters 1 and 2 respectively.

The results in Table 3.6 show that when methionine and protein together with their respective squared terms were tested separately high t-values were recorded, indicating that they fit the data accurately. However, when all four terms were tested, the t-values for testing the regression coefficients of methionine and its squared term were non significant, indicating that methionine and its squared term are not contributing significantly to the model.

The most reasonable explanation for this is that the full response to methionine supplementation was not obtained in series 3 diets.

Some nutrient other than methionine presumably became limiting at the highest levels of methionine supplementation indicating either that the nutrient composition of the ingredients used in the diets was not sufficiently accurate or that the requirements of broilers for such nutrients are not adequately defined. Under such circumstances the broilers would not be expected to respond to the additional methionine supplementation, thereby reducing the correlation between growth rate and methionine intake.

The Reading model (Fisher *et al.*, 1973) shown in Table 3.7 adequately describes the response in body mass gain to methionine intake, as illustrated in Figure 3.4. Because diets in series 3 did not respond fully to the methionine concentration in the diet, these values were not included in the model. This applies also to the fitting of the model to the protein gains shown in Figure 3.5. These results confirm that the dilution technique of Fisher and Morris (1970) is an adequate method of determining the response of broiler chickens to increasing concentrations of an amino acid.

The effect of the methionine/DME ratio on carcass fat content is shown in Table 3.8 and Figure 3.6. It is evident that carcass fat content increases as this ratio widens. The methionine content of the diets decreases as the ratio widens, thus creating a marginal amino acid deficiency which is responsible for an increased food intake (Solberg *et al.*, 1971). The excess energy which is ingested is then laid down as adipose tissue. Once the methionine deficiency becomes severe the food intake is suppressed resulting in little body mass gain and fat deposition. The graphs accurately depict the curvilinear nature of this response to a change in methionine/DME ratio.

CHAPTER 4

EVALUATION OF A DIET DILUTION TECHNIQUE FOR MEASURING THE RESPONSE OF BROILER CHICKENS TO METHIONINE INTAKE DURING THE FINISHER PERIOD

INTRODUCTION

In the previous chapters, experiments were reported which were designed to evaluate a diet dilution technique for measuring the response of broilers to amino acid intakes. To complete this series of experiments the present trial was conducted in order to evaluate this dilution technique on broilers between 21 and 53 days of age using methionine as the test amino acid.

MATERIALS AND METHODS

One-day-old chicks of the Ross broiler strain were allocated at random to 36 pens, such that each pen contained 90 male chicks, and 90 female chicks. The stocking density was 19,4 birds/m^2. The pens were in an environmentally controlled broiler house of a commercial design and management procedures conformed as closely as possible with commercial practice. Gas canopy brooders were used during the brooding period, and the photo-period was 23 hours/day. The chicks were reared on a commercial broiler starter diet to 21 days of age, after which the numbers were equalised, and the body mass of the birds was determined.

A pair of summit and dilution diets based on the principles of Fisher and Morris (1970) as modified by Pilbrow and Morris (1974) were formulated at a ME level of 13,39 MJ/kg with amino acid levels

TABLE 4.1 Composition (g/kg) of the summit and dilution diets

Ingredient	Summit diet	Dilution diet
Yellow maize meal	490,2	767,7
Wheat bran		24,0
Sunflower meal		38,0
Groundnut meal	101,0	88,0
Soyabean unextracted	200,0	
Fish meal	178,0	
Blood meal	19,0	25,0
Bone meal		42,0
Monocalcium phosphate	1,0	
Limestone flour	7,0	
Salt		4,5
Lysine HCl		7,29
DL - Methionine	0,34	
Vitamins and minerals *	3,0	3,0
Anti-coccidial	0,5	0,5
Calculated analysis		
Arginine	20,00	10,70
Histidine	7,19	3,87
Isoleucine	11,09	4,10
Leucine	24,53	14,53
Lysine	17,90	9,20
Methionine	6,30	2,80
Cystine	6,10	3,10
Phenylalanine	12,79	7,17
Tyrosine	9,82	5,27
Threonine	10,83	5,22
Tryptophan	3,63	1,70
Valine	14,71	7,27
Calcium	9,00	9,90
Phosphorus	6,90	6,90
Crude protein (gN x 6,25/kg)	292,00	153.00
Metabolisable energy (MJ/kg)	13,39	
Net energy (MJ/kg)	9,48	9,48

Supplies per kg of diet: Vit A 7 025 IU, Vit D_3 2 000 IU, Vit E 8,5 IU, Hetrazine 3 ppm, Thiamine hydrochloride 0,969 ppm, Riboflavin 8 ppm, Calcium pantothenate 7,837 ppm, Niacin 24, 42 ppm, Folic Acid 0,95 ppm

Copper 125 ppm, Iron 40 ppm, Zinc 28 ppm, Iodine 2 ppm, Manganese 78 ppm, Selenium 0,1 ppm

TABLE 4.2 Calculated amino acid contents of the summit and dilution diets relative to the requirements of broilers during the finisher period

	Requirements according to Thomas *et al.* (1978) (g/kg)*	Amino acid contents expressed as multiples of requirement	
		Summit diet	Dilution diet
Arginine	12,08	1,66	0,89
Histidine	4,37	1,65	0,89
Isoleucine	8,29	1,34	0,56
Leucine	15,28	1,61	0,95
Lysine	11,64	1,54	0,79
Methionine	5,34	1,18	0,52
Cystine	3,64	1,68	0,85
Phenylalanine	7,71	1,66	0,93
Tyrosine	6,55	1,50	0,80
Threonine	7,71	1,40	0,68
Tryptophan	1,75	2,07	0,97
Valine	9,31	1,58	0,78

* Requirements are expressed in g/kg for a diet containing 13,39 MJ/kg (equivalent to 9,48 MJ NE/kg). Amino acid contents as multiples of requirements are expressed in terms of the requirements at the corresponding energy level of the diet

based on the recommendations of Thomas *et al.* (1978). The wide range of methionine contents required in the experimental diets was obtained by formulating the summit diet calculated to contain 6,3 g/kg methionine, and the dilution diet calculated to contain 2,8 g/kg methionine, and the intermediate methionine contents were obtained by blending these diets in appropriate proportions as shown in Table 4.3.

Specific protein contents were not used in the formulation but the minimum contents of all essential amino acids except methionine were set at 1,6 times for the summit and 0,9 times for the dilution, the requirements proposed by Thomas *et al.* (1978). In some instances it was impossible to reach the higher level set for some amino acids in the summit and dilution diets, with the raw ingredients at our disposal. In these cases the highest feasible levels were then accepted. The extremes of the range of methionine contents used, namely 6,3 g/kg and 2,8 g/kg, were 1,2 and 0,5 times the estimated requirements of Thomas *et al.* (1978).

Five serial dilutions of the summit diet were made as shown in Table 4.3, concentrations of methionine in each diet being 1,2; 1,1; 0,9; 0,8 and 0,6 times the requirement for methionine respectively. A second series of dilution diets was prepared in the same manner as that above. Synthetic methionine was then added to diets 2 to 5, the amount supplemented (0,7 g/kg) being equal to 1,0 times the difference in methionine content between two adjacent diets in the first series. The methionine contents of the second series were then 1,2; 1,1; 0,9 and 0,8 times the requirement respectively. A third series was prepared in which the level of methionine was increased by the addition of methionine, the amount being added amounting to 2,0 times the difference in methionine content between two adjacent diets in the first series. The level added (1,4 g/kg) to diets 3 to 5 in this series increased the methionine to 1,2; 1,1 and 0,9 times the requirement. The net result of this diet blending and supplementation technique is that

TABLE 4.3 Summary of dilution technique and calculated analysis of the experimental diets

Diet code	Blending ratio Summit diet		Dilution diet		Methionine supple-mentation (g/kg)	Calculated dietary methionine (g/kg)	Calculated dietary protein (gN x 6,25/kg)
Series 1							
1	100	+	0			6,30	292,0
2	80	+	20			5,60	264,0
3	60	+	40			4,90	236,0
4	40	+	60			4,20	209,0
5	20	+	80			3,50	181,0
Series 2							
6	80	+	20	+	0,70	6,30	264,0
7	60	+	40	+	0,70	5,60	236,0
8	40	+	60	+	0,70	4,90	209,0
9	20	+	80	+	0,70	4,20	181,0
Series 3							
10	60	+	40	+	1,40	6,30	236,0
11	40	+	60	+	1,40	5,60	209,0
12	20	+	80	+	1,40	4,90	181,0

there were identical dilutions in each series, with similar protein and amino acid balances, except for increasing methionine levels as shown in Table 4.3. The experimental diets were mixed individually, using the technique described in Chapter 2.

The 12 experimental diets were allocated at random, there being three replicates per treatment. Pelleted food, in tube feeders, and water were provided *ad lib*. The body mass of the birds was measured at 21, 46 and 53 days of age and food consumption was recorded for intervals corresponding to the body mass recordings. At 53 days of age a male and a female which were representative of the pen mean were drawn for carcass analysis.

RESULTS

The results of body mass gain, food intake, methionine and protein intake for the first period are presented in Table 4.4. The response to added methionine in the second and third series of diets confirms that methionine was the first-limiting amino acid in the first series of diets.

The failure of the supplemented methionine to elicit a higher response in the third series of diets is indicative that an amino acid other than methionine had become limiting after the addition of the higher level of methionine. The addition of 1,40 g/kg methionine in the third series was excessive in relation to the levels of the next most limiting nutrient(s) in the first series of diets. The composition of the experimental diets presented in Table 4.2 confirms the suspicion that isoleucine and threonine may have been limiting after the supplementation of 1,40 g/kg methionine in the third series.

A multiple regression analysis was performed on this data to test

TABLE 4.4 Response of broiler chickens to dietary methionine and protein intakes from 21 - 46 days of age

Diet code	Calculated dietary methionine (g/kg)	Calculated dietary protein (gN x 6,25/kg)	Gain in mass (g/bird d)	Methionine intake (mg/bird d)	Protein intake (g/bird d)	Food intake (g/bird d)
Series 1						
1	6,30	292,0	46,0	611,0	28,3	97,0
2	5,60	264,0	46,1	554,0	26,1	99,0
3	4,90	236,0	44,8	495,0	23,8	101,0
4	4,20	209,0	43,1	416,0	20,7	99,0
5	3,50	181,0	39,1	350,0	18,1	100,0
Series 2						
6	6,30	264,0	46,1	624,0	26,1	99,0
7	5,60	236,0	45,0	543,0	22,9	97,0
8	4,90	209,0	43,9	490,0	20,9	100,0
9	4,20	181,0	41,8	412,0	17,7	98,0
Series 3						
10	6,30	236,0	45,1	636,0	23,8	101,0
11	5,60	209,0	43,6	560,0	20,9	100,0
12	4,90	181,0	41,7	495,0	18,3	101,0

TABLE 4.5 Response of broiler chickens to dietary methionine and protein intakes from 21 - 53 days of age

Diet code	Calculated dietary methionine (g/kg)	Calculated dietary protein (gN x 6,25/kg)	Gain in mass (g/bird d)	Methionine intake (mg/bird d)	Protein intake (g/bird d)	Food intake (g/bird d)
Series 1						
1	6,30	292,0	43,4	605,0	28,0	96,0
2	5,60	264,0	43,5	549,0	25,9	98,0
3	4,90	236,0	43,0	505,0	24,3	103,0
4	4,20	209,0	41,9	424,0	21,1	101,0
5	3,50	181,0	39,4	357,0	18,5	102,0
Series 2						
6	6,30	264,0	44,3	617,0	25,9	98,0
7	5,60	236,0	43,1	543,0	22,9	97,0
8	4,90	209,0	41,9	490,0	20,9	100,0
9	4,20	181,0	41,0	412,0	17,7	98,0
Series 3						
10	6,30	236,0	42,9	643,0	24,1	102,0
11	5,60	209,0	41,6	577,0	21,5	103,0
12	4,90	181,0	40,5	510,0	18,8	104,0

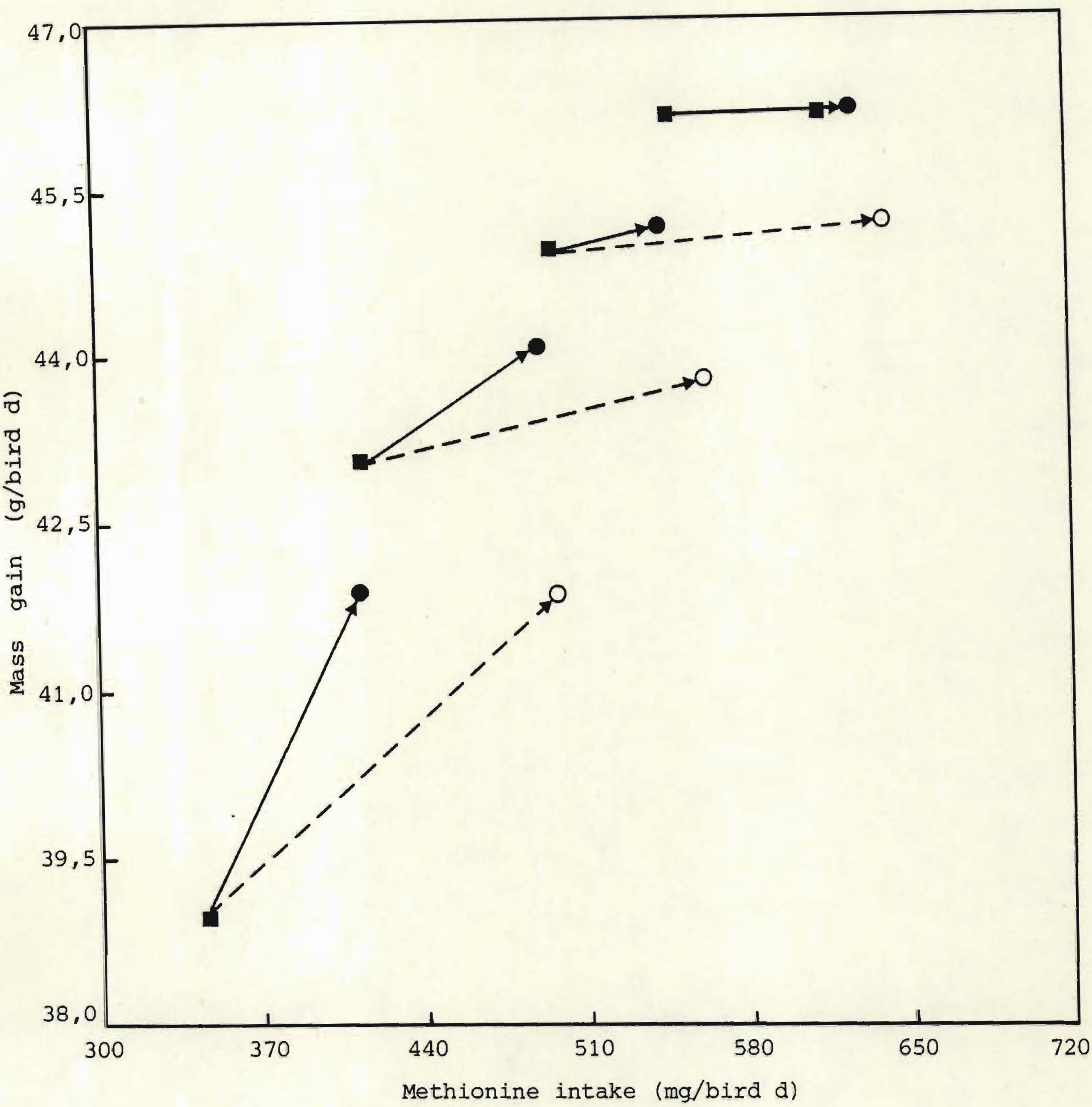

Figure 4.1 Response of male and female chickens to increasing methionine concentrations from 21 - 46 days of age

■ First dilution series (initial dilution)

● Second dilution series (methionine addition)

○ Third dilution series (methionine addition)

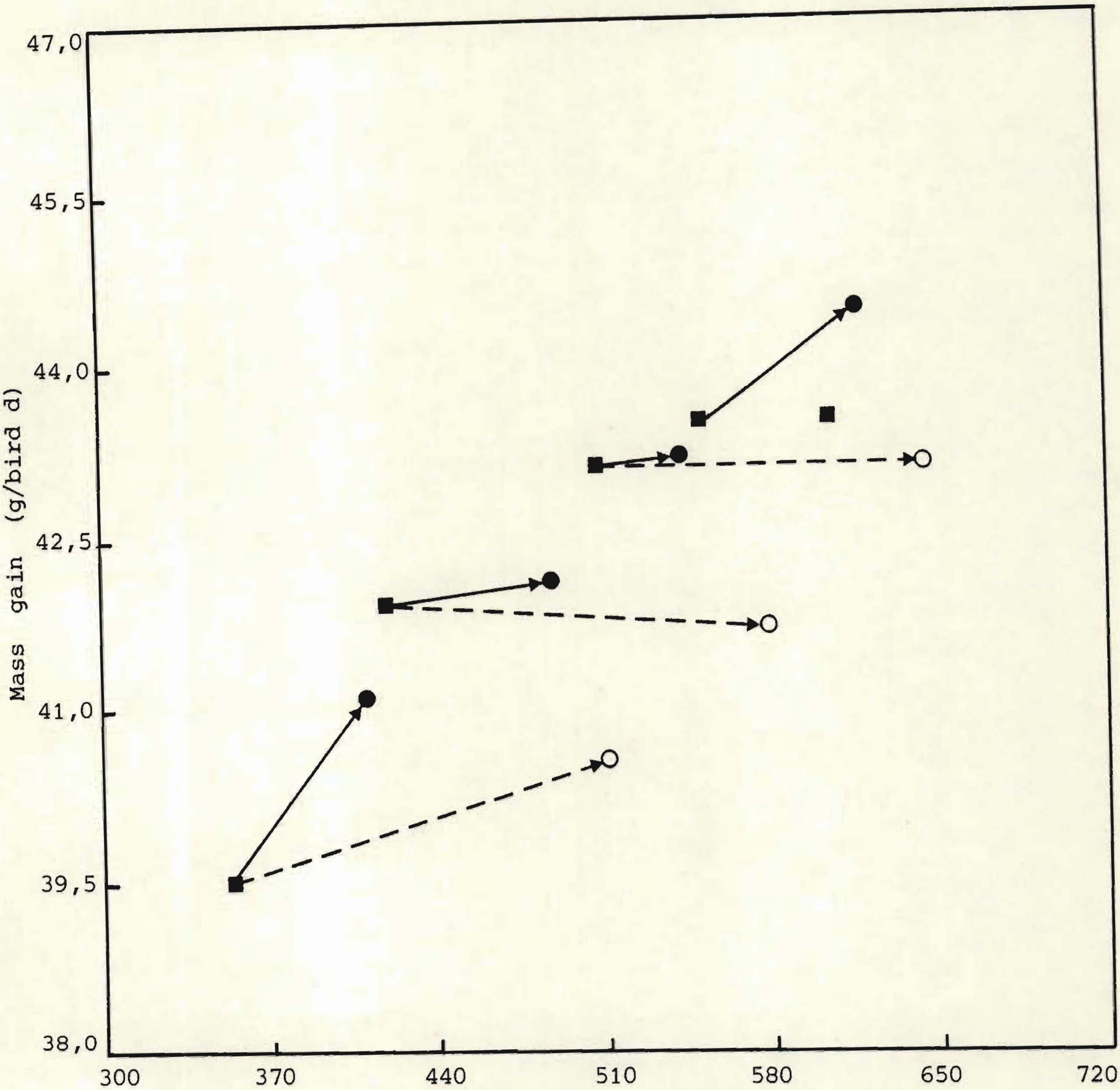

Figure 4.2 Response of male and female chickens to increasing methionine concentrations from 21 - 53 days of age

■ First dilution series (initial dilution)

● Second dilution series (methionine addition)

○ Third dilution series (methionine addition)

the relative importance of methionine intake and protein intake on livemass gain. The terms tested in the analysis covering the two periods were the same as in Chapter 3.

The regression analysis of the results of the first period indicate that both methionine intake and protein intake were important in determining body mass gain. When all four terms were tested simultaneously the t-values for testing the significance of methionine intake (3,475) and the squared terms for methionine intake (3,160) were highly significant (t-value for significance at $P < 0,01$ is 2,747), whereas the t-value for protein intake (2,790) and the squared term for protein intake (2,125) were only just significant at the higher and lower level respectively (t-value for significance at $P < 0,05$ is 2,040). The variance inflation factor for protein intake (250) and the squared term for protein intake (240) were rather high, indicating that this result is very unreliable and should be viewed with caution.

The responses in livemass gain to methionine and protein intakes for the period 21 to 53 days are presented in Table 4.5. These results were subjected to the same multiple regression analysis as used for the results of the first period. In this period, unlike period 1, non significant t-values were recorded for testing the significance of methionine and protein intake, and their respective squared terms, when tested together (Table 4.6). These results indicate that methionine is no longer first-limiting in the third series of diets, which then accounts for the absence of a full response in body mass gain to the high methionine intakes on diets supplemented at the higher level. The regression coefficients and t-values for testing their significance for both periods are presented in Table 4.6.

The Reading model (equation 1.1) was used to describe the relationship between mass gain and methionine intake for the two experimental periods. Only those treatments where it was certain that methionine

TABLE 4.6 Regression coefficients describing response of broiler chickens to increasing dietary concentrations of methionine and protein

Methionine		Protein	
linear coefficient	quadratic coefficient	linear coefficient	quadratic coefficient
21 - 46 days			
0,0825	-0,0000618	--	--
[1](3,065)**	(2,305)*	--	--
--	--	2,925	-0,0521
--	--	(4,430)**	(3,574)**
0,0818	-0,0000739	0,421	--
(4,447)**	(4,011)**	(6,216)**	--
0,0660	-0,0000594	1,738	-0,0286
(3,475)**	(3,160)**	(2,790)**	(2,125)*
21 - 53 days			
0,0446	-0,0000318	--	--
(1,805)^{NS}	(1,303)^{NS}	--	--
--	--	1,673	-0,0283
--	--	(2,552)*	(1,958)^{NS}
0,0370	-0,0000336	0,329	--
(1,918)^{NS}	(1,766)^{NS}	(4,741)**	--
0,0282	-0,0000256	1,178	-0,0184
(1,363)^{NS}	(1,274)^{NS}	(1,601)^{NS}	(1,160)^{NS}

[1]t-value for testing significance of regression coefficients is shown in brackets. NS denotes non significant, *P < 0,05; ** P < 0,01

was first limiting were used. This applies to diets one to five in the initial dilution series. The result of fitting a Reading model to this data is shown in Table 4.8 together with the parameters used in fitting the model. The results are illustrated graphically in Figures 4.3 and 4.4 respectively.

The Reading model was used also to describe the effect of methionine intake on protein gain (Table 4.7) and the parameters used, together with the coefficients for the average individual are given in Table 4.9 and shown in Figure 4.5. The fat content of the carcass was also determined and these results are presented in Table 4.7 and Figure 4.6 respectively.

TABLE 4.7 Response in crude protein gain (ΔCP) and carcass fat gain (ΔFT) in relation to dietary methionine and metabolisable energy intakes for male and female broilers from 21 - 53 days of age

Diet Code	Methionine intake (mg/bird d)	Energy intake (MJ/bird d)	Protein gain (g/bird d)	Fat gain (g/bird d)
Series 1				
1	605,0	1,29	7,99	9,15
2	549,0	1,31	7,85	8,27
3	505,0	1,38	7,59	7,31
4	424,0	1,35	7,49	8,48
5	357,0	1,37	6,87	8,75
Series 2				
6	617,0	1,31	7,77	8,99
7	543,0	1,30	7,88	8,26
8	490,0	1,34	7,37	8,85
9	412,0	1,31	6,62	8,79
Series 3				
10	643,0	1,37	7,53	8,16
11	577,0	1,38	7,35	8,99
12	510,0	1,39	7,07	9,14

TABLE 4.8 Parameters used in determining the relationship between mass gain and methionine intake using the Reading model using male and female chickens from 21 - 53 days of age

Parameter	21 - 46 days	21 - 53 days
$\bar{W}$	800,00	800,00
$\Delta\bar{W}$	45,51	43,24
a	5,36	5,13
b	0,02	0,03
$\sigma\bar{W}$	80,00	80,00
$\sigma\Delta\bar{W}$	23,05	21,75
r	0,60	0,60

TABLE 4.9 Parameters used in determining the relationship between protein gain (ΔCP) and methionine intake using the Reading model using male and female chickens from 21 - 53 days of age

Parameter	21 - 53 days
$\bar{W}$	150,00
$\Delta\bar{W}$	6,94
a	16,10
b	0,04
$\sigma\bar{W}$	12,00
$\sigma\Delta\bar{W}$	8,98
r	0,60

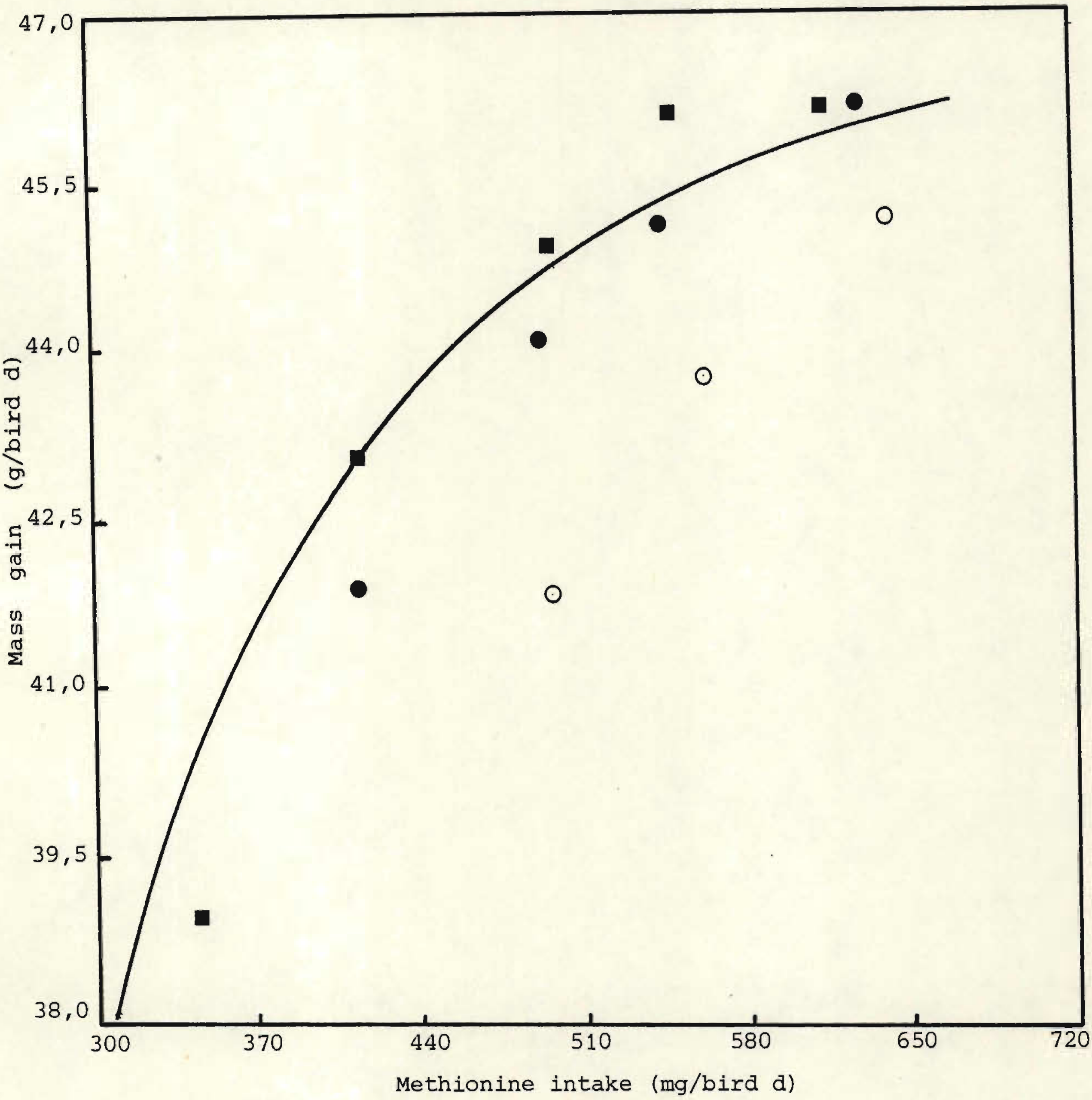

Figure 4.3 Response of male and female chickens to methionine intake and estimated response using Reading model (Table 4.8) from 21 - 46 days of age

■ First dilution series (initial dilution)

● Second dilution series (methionine addition)

○ Third dilution series (methionine addition)

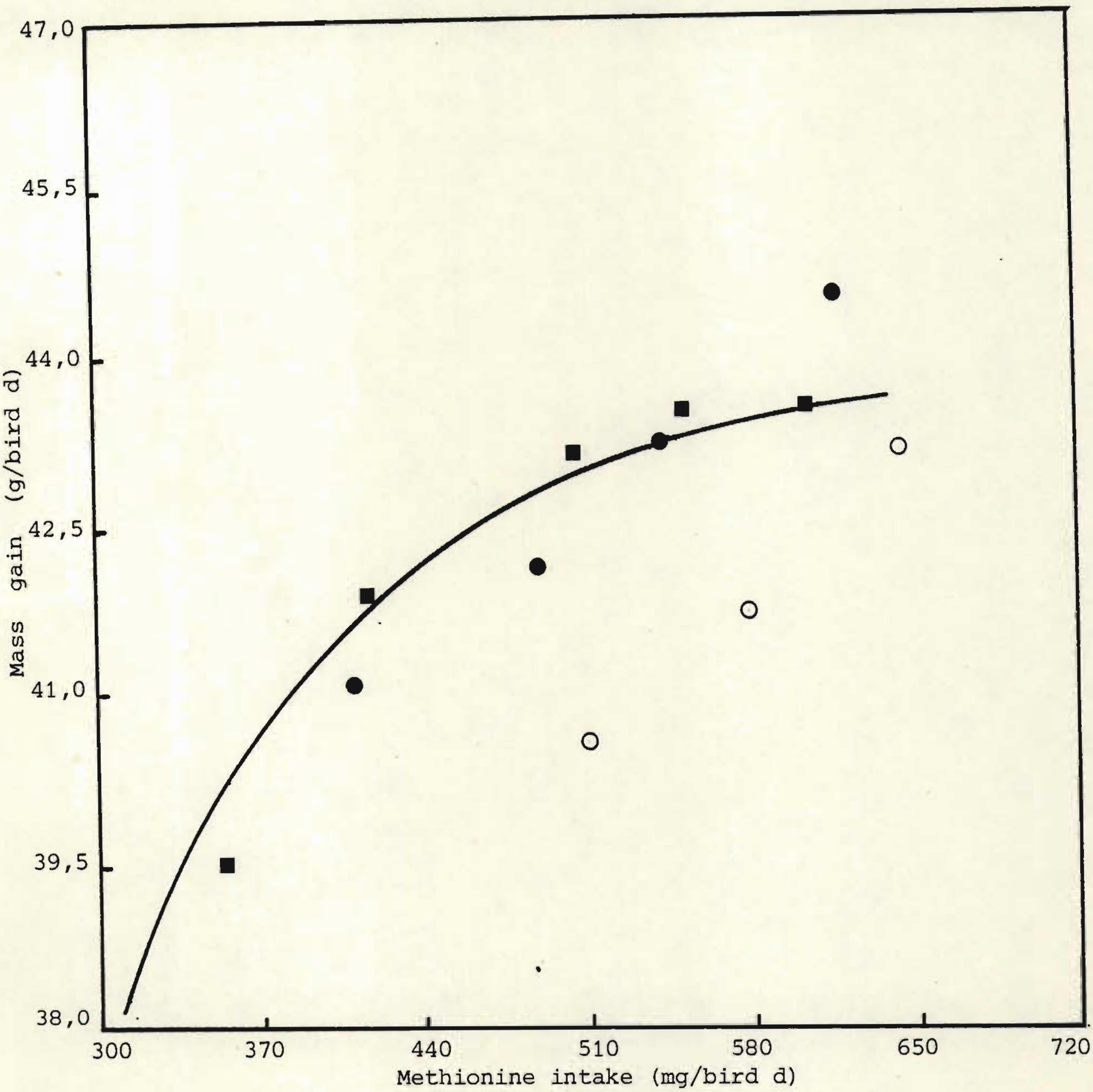

Figure 4.4 Response of male and female chickens to methionine intake and estimated response using Reading model (Table 4.8) from 21 - 53 days of age

■ First dilution series (initial dilution)

● Second dilution series (methionine addition)

○ Third dilution series (methionine addition)

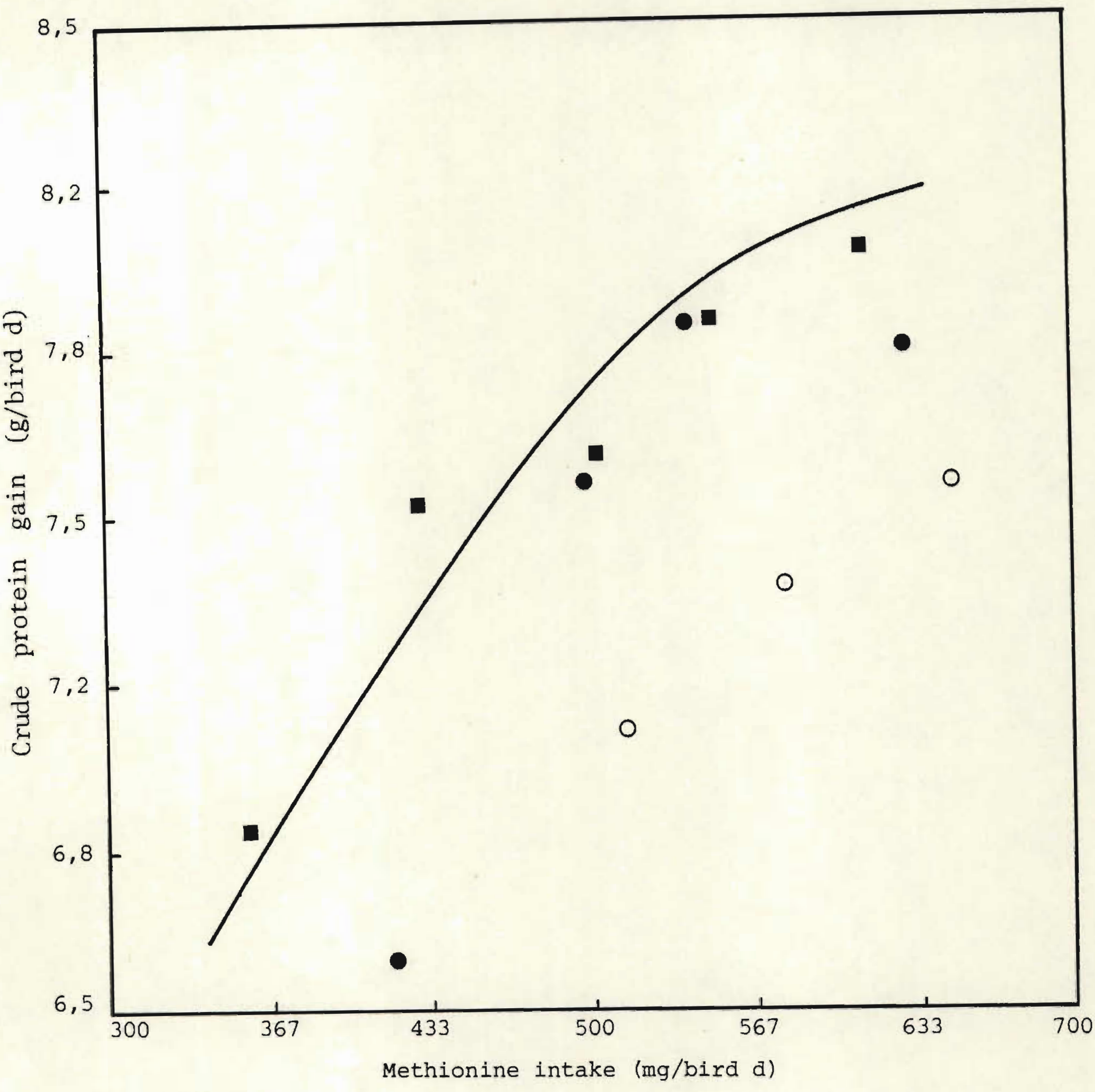

Figure 4.5 Relationship between methionine intake and crude protein gain, and estimated response using Reading model (Table 4.9) in broilers from 21 - 53 days of age

■ First dilution series (initial dilution)

● Second dilution series (methionine addition)

○ Third dilution series (methionine addition)

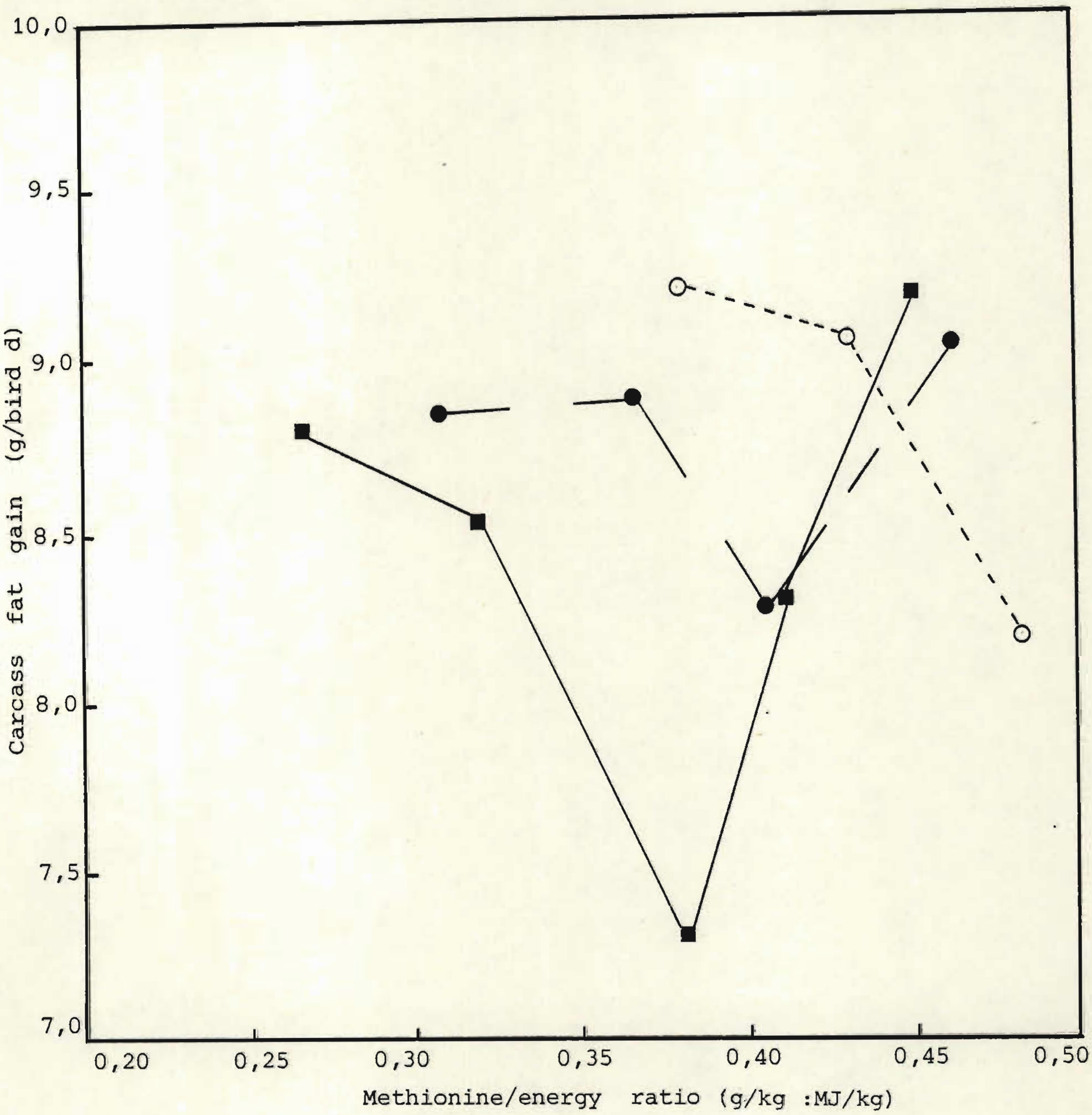

Figure 4.6 Relationship between carcass fat gain and methionine/energy ratio in broilers from 21 - 53 days of age (g/kg:MJ/kg)

■ First dilution series (initial dilution)

● Second dilution series (methionine addition)

○ Third dilution series (methionine addition)

DISCUSSION

The major objective of this experiment was to evaluate a diet dilution technique for measuring the response of broiler chickens to increasing dietary methionine intake, by establishing whether broilers will respond to supplemental methionine when a diet deficient in methionine is fed to the birds, even if no further supplemental protein is fed. Although it is generally conceded that intake of the first-limiting nutrient governs body mass gain, nevertheless, in using a technique such as this, where the amino acid and protein concentrations are varying simultaneously, it is possible to separate out the various effects by supplementing the diets in the initial dilution series with the first-limiting amino acid and to then measure the response to these supplemented diets.

In this experiment, as in that described in Chapter 3, the response to additional methionine was lower than expected again probably due to some nutrient other than methionine becoming limiting at the highest levels of supplementation. This adds further credence to the probability that either the methionine content of the ingredients used was not accurately known, or that the requirements for certain nutrient(s) other than methionine are higher than expected, leading to a deficiency of that nutrient at a lower level than was catered for when formulating the diets.

In fitting the Reading model to the data, on account of the lower than expected responses, only the results of feeding the diets in the initial dilution series were used. The graphical representation of the model (Figures 4.3 and 4.4) indicate a very good agreement between the data and the fitted model. Again, this model is not discussed further at this point, as a more complete coverage of the response to methionine intake, using data from this experiment will be considered later.

The main objective of studying the gain in carcass protein was to ascertain whether the gain would be similar for equivalent methionine intakes on the three dilution series used in this experiment. Because the full response to methionine was not obtained in all dilution series only the protein gain resulting from the first dilution series was considered when fitting a model to the data. This data will be used in the later chapter when measuring responses to methionine. From the results of Chapter 2 it would not be unreasonable to assume that if the full response to methionine had been obtained by feeding diets in dilution series 2 and 3 in the experiments reported in Chapters 3 and 4, the gain in protein would have been very similar on diets containing equivalent concentrations of methionine in all three series.

GENERAL DISCUSSION ON CHAPTERS ONE TO FOUR

The dilution technique of Fisher and Morris (1970) has been evaluated using both lysine and methionine as the limiting amino acids, and by feeding chickens both in the starter (0 - 21 days of age) and in the finisher (21 to 52 days of age) period. The method used to evaluate the technique was to feed the initial dilution series, which consists of graded levels of both the amino acid under study and protein, then to supplement these diets with the amino acid that was designed to be first-limiting. This had the effect, first, of confirming that the dilution series was first-limiting in the amino acid under study, and secondly, of measuring the relative contributions of the first-limiting amino acid and of protein to mass gain. This was possible because the diets supplemented with the test amino acid had identical protein levels to the corresponding diets in the initial dilution series, but the limiting amino acid concentrations differed. By means of a multiple regression analysis it was possible to determine the relative contribution of each of these nutrients to body mass gain.

Where the full response to supplementation of the test amino acid was obtained the regression analysis confirmed that the amino acid intake and not protein intake was the most important factor determining growth rate. Due to inaccuracies either in the feed composition matrix used, or in the assumed requirements for certain nutrients (including the requirement for the test amino acid), in certain instances, particularly in the experiments involving methionine as the test amino acid, the full response to supplementation of the test amino acid was not obtained. This meant that higher levels of the test amino acid were being consumed with no additional apparent response, thereby reducing the correlation between intake of the amino acid and body mass gain.

The general impression from the four experiments reported in this section was that excellent response curves can be obtained by the use of this dilution technique; that the Reading model describes the data most adequately; and that provided the full response to supplementation of the test amino acid is achieved, body mass gain will be dependent primarily on the intake of that amino acid. For these reasons a further series of experiments was conducted in order to obtain more data relating intake of amino acids to body mass gain in broilers.

Protein gain data was collected primarily to further evaluate the dilution technique, the argument being that if protein gain was similar when birds were fed diets with similar limiting amino acid concentrations but which differed in protein content, then this technique could be expected to adequately measure the response to dietary amino acid concentrations. Only in experiment 2 did the diets in two of the dilution series elicit a full response to the test amino acid. In the case of this data protein gains were virtually identical on diets where intake of the limiting amino acid was similar. In all other experiments where protein gains were calculated the response in body mass gain (and hence protein gain) was lower than expected, so little importance can be attached to this information.

The protein gain and fat gain data collected in this series of experiments will be discussed collectively in a later section dealing specifically with these matters.

CHAPTER 5

REVIEW OF LITERATURE RELATING TO THE LYSINE, METHIONINE AND METABOLISABLE ENERGY REQUIREMENTS OF BROILER CHICKENS

INTRODUCTION

The extent to which the amino acid pattern of a diet of an animal may be altered without influencing amino acid requirements has not been adequately investigated. It is generally accepted, however, that even a small surplus of certain amino acids can sometimes increase the need for others. This recognition is based upon observations that dietary additions of incomplete mixtures of amino acids cause severe growth depressions which are prevented by supplementation of the diet with the amino acid that is most limiting (Harper, 1958). Apart from these observations, quantitative data on the extent to which amino acid requirements can be expected to vary as a result of changes in the dietary amino acid pattern is severely lacking.

Since there is essentially no storage of amino acids in the body, it has been frequently assumed that any surplus ingested and not subsequently utilised in protein synthesis is disposed of without impairing growth. It is now acknowledged that in most instances a surplus of an essential amino acid will impose a limitation upon the efficiency of nutrient utilisation commensurate with the magnitude of the deviation from a perfect balance. There are also recognised to be certain occasions when a dietary excess of an amino acid or of a mixture of amino acids will precipitate an ill-effect that is totally disproportionate to the degree of imbalance. Harper (1958, 1964) grouped these effects into three categories: imbalances, toxisities and antagonisms without examining in detail the aetiological basis for this separation. Lewis (1965) suggested that this classification could not be justified and proposed that adverse effects of amino acids could best be considered as specific interactions between pairs of amino acids.

The results of D'Mello and Lewis (1970 a) indicate that under conditions of optimum dietary amino acid balance, the requirements of individual essential amino acids are minimal. As the balance deviates from this perfect pattern, the need for some amino acids is increased proportionately. This interdependence in amino acid requirements has been demonstrated for several categories of interaction. Numerous research workers have demonstrated the occurrence of the lysine - arginine interaction in chick nutrition (O'Dell, Laerdal, Jeffay and Savage, 1958; Jones, 1961, 1964; D'Mello and Lewis, 1971). Excess lysine depresses growth severely when the arginine content of the diet is marginally adequate. A concomitant supply of arginine in the diet precludes the onset of this phenomenon.

Information regarding the specificity of the interaction between lysine and arginine is virtually absent due to the assumption that arginine is a general non-specific detoxifying amino acid and as such is not involved in a specific interaction (Snetsinger and Scott, 1961). Boorman and Fisher (1966) arrived at the conclusion that the lysine - arginine interrelationship was not unique in spite of some contradictory evidence. These authors showed that lysine was singularly potent as an agent of interaction when compared with other amino acids in excess in the diet. They further demonstrated that the ill-effects of excess lysine were reversed by arginine supplementation in the diet, but the adverse effects of large quantities of methionine or phenylalanine were not similarly alleviated.

Since massive doses of any amino acid might be expected to be toxic

(Almquist, 1952) mere demonstration of a growth depression on addition of a surplus to a diet need not necessarily constitute a basis for establishing or disputing the existence of a particular interaction. Evidence of complete reversal of the adverse effects of the agent by the target amino acid should also be taken into account. It is therefore only possible to decide upon the specificity of an interaction when the above criteria have been satisfied.

It is evident from the observations of Jones, Petersburg and Burnett (1967) that the mechanism whereby the ill-effects of excess dietary lysine are brought about is via an alteration of the metabolic fate of arginine. Surplus lysine precipitates a deficiency of arginine in spite of the impending lack in the basal diets of adequate supply of methionine, tryptophan, histidine or threonine. This accounts for the partial reversal of the ill-effects of excess lysine by arginine supplementation in some of the results of D'Mello and Lewis (1970 a). Complete reversal would certainly be attained at higher supplementary doses of arginine only (D'Mello, Hewitt and Lewis, 1967).

In the lysine - arginine interaction D'Mello and Lewis (1970 a) found that satisfactory growth rate of chicks is obtained when the dietary lysine concentration is raised to 1,35 percent of the diet, the arginine requirement is 0,92 percent, whilst at a lysine content of 1,60 percent the arginine need is 1,04 percent of the diet. Further increase in the dietary consentration of lysine to 1,85 percent is accompanied by a concomitant increase in the arginine requirement to 1,15 percent. This represents a linear increase in the arginine requirement of the chick as the lysine content of the diet is increased. It follows, therefore, that the requirement of the young chick for arginine cannot be determined accurately, without also considering the levels of lysine in the diet.

Interdependence in amino acid needs is by no means only restricted to lysine and arginine. An analogous situation has been observed to exist in the case of interactions between leucine, isoleucine and valine. The results of D'Mello and Lewis (1970 a) demonstrate that as the dietary concentration of leucine increases there appears to be a linear increase in the requirement of the young chick for isoleucine and valine respectively. At concentrations of 1,4; 2,15 and 2,9 percent leucine, the isoleucine requirement is 0,58; 0,62 and 0,65 percent respectively. At levels of 1,40; 2,40 and 3,40 percent leucine, the valine requirement is 0,77; 0,89 and 1,01 percent respectively. An entirely similar pattern of interdependence has been demonstrated in the case of the fourth interrelationship - that between threonine and tryptophan. Increasing the dietary concentration of threonine from 0,8 percent to 2,3 percent results in a linear increase in the tryptophan requirement from 0,17 to 0,20 percent of the diet.

In studies of the amino acid requirements of the growing chick mass gain, efficiency of food utilisation and nitrogen retention are the parameters commonly used. Recently, plasma amino acid levels have received attention as indices of amino acid nutrition (Hewitt and Lewis (1972 a). The approach has been to determine for each amino acid the plasma concentration when a test diet is given and express it as a function of the level when the animal is starved or given a reference diet (Smith and Scott, 1965 a, b; Dean and Scott, 1965). The extent of the deficiency of the limiting amino acids in the test diets were predicted from these ratios with some success. This indicates that the levels of amino acids in the plasma are influenced primarily by the supply of amino acids from the diet and confirms the findings of Zimmerman and Scott (1965) and Dean and Scott (1965). Plasma amino acids therefore probably reflect the balance between the dietary supply and the utilisation of amino acids in metabolism. It follows that when amino acid utilisation for protein synthesis is improved by altering the balance between the amino acids in the diet, total plasma amino acid levels should fall. When the level of one amino acid is severely inadequate other amino acids might in the same way accumulate in the blood. Increasing the level of this amino acid to meet requirement would result in increased utilisation of the other amino acids and reductions in their plasma levels. When the requirement of the amino acid is exceeded, its plasma level is likely to rise sharply and total plasma amino acid levels may increase again.

Zimmerman and Scott (1965) proposed that the dietary amino acid level above which the plasma level rises sharply represents the dietary concentration that is just adequate for that amino acid. However, the results of Hewitt and Lewis (1972b) indicate that this relationship is not so precise that requirements can be accurately determined from plasma amino acid levels. In general it appeared that the total plasma amino acid content was diminished when the dietary amino acid level was equal or just less than the requirement. At this point it would be expected that amino acid utilisation would be at a maximum for this series of diets. This suggests that total amino acid levels may be considered as a sensitive index of changes in the amino acid balance of the dietary supply (Hewitt and Lewis, 1972 b).

The demonstration of interdependence in amino acid requirements introduces an additional dimension in the assessment of amino acid requirements of animals. That the "classical" method is inadequate in providing an accurate assessment of amino acid requirements has been conclusively demonstrated by the results of D'Mello and Lewis (1970 b) Lysine and arginine are inextricably engaged in interaction and reciprocally affect the requirement of each other. Leucine, isoleucine and valine; and threonine and tryptophan are similarly involved in interaction. Therefore, D'Mello and Lewis (1970 b) are of the opinion that the requirements for these amino acids can be determined precisely only by adopting the factorial method of amino acid supplementation used in their investigation.

The frequent failure to appreciate the relevance of such instances of interaction accounts for much of the variability in quotations of amino acid requirements for the chick. The benefit of recognising the importance of amino acid interactions in the assessment of amino acid requirements is evident from the results of D'Mello and Lewis (1970 b). When the concentrations of lysine and arginine in the diet were lowered proportionately, 0,94 per cent lysine and 0,81 per cent arginine are sufficient in meeting the requirements of the chick for these amino acids. These figures are lower than the generally recommended values of 1,10 per cent lysine and 1,20 per cent arginine (National Research Council, 1966).

The observations of numerous studies emphasise the point that for some indispensable amino acids at any rate, requirement values are variable entities. Complete reliance, therefore, should not be placed on minimum requirments for these amino acids, and tables of recommended amino acid allowances have value chiefly as guides in attaining a desirable pattern of amino acids in the diet.

Evidence that the requirements for one amino acid may depend on the level of another amino acid may also partly explain why there has been so much variability in amino acid requirement determinations. Other factors may include variations in amino acid availability in the basal ingredients, inadequate levels of nutrients other than amino acids and the use of different types of chicks.

The major factor responsible for the variation in amino acid recommendations is the wide range of amino acid values attributed to raw ingredients utilised in compounding animal feeds. The discrepancy in raw ingredients analyses is due to the variation in equipment and technique used in the analytical laboratories. The effects of hydrolytic technique on amino acid values is comprehensively surveyed in the publication of Davies and Thomas (1973). In brief these are:

1 The protein source. In a purer protein source lysine would appear to be released more slowly than in one containing

impurities such as carbohydrates and minerals. It would appear that the optimum hydrolysis is 60 hours and 20 hours in 6N HCl at 137 °C respectively for the purer protein source casein, and the impure protein source of *Fusarium graminearum*.

2 A particular peptide bond. It was shown that valine, leucine and isoleucine are released much more slowly than other amino acids in the above two protein sources when using 6N HCl. The discrepancy in amino acid values after 50 and 24 hours (standard time) of hydrolysis could be in excess of 30 per cent. This difference is due to the peptide bond involved, particularly the carboxyl group of both leucine and valine.

3 The length of hydrolysis of a particular protein. Although some amino acids need longer hydrolysis times to be fully released, some on the other hand become destroyed if excessive hydrolysis takes place. With 6N HCl at 37 °C and the protein from *Fusarium*, arginine, histidine, cysteine, methionine, threonine and others start being destroyed after 20 hours, or less.

4 The hydrolytic agent used and its concentration. If casein is hydrolysed for 16 hours with 12 N H_2SO_4 a value at least 20 per cent higher for aspartic acid is obtained than any other method tried. If a resin is used with 6N HCl the optimum hydrolysis time for leucine and isoleucine is nearer 20 hours since leucine is destroyed by 60 hours. Tryptophan can only be measured in alkaline medium in the absolute absence of oxidising agents.

In the light of the above discussion it is eveident that the determination of the amino acid levels in a feedstuff is a very difficult task indeed, and it is imperative that standardisation is introduced with regard to the equipment and technique for amino acid analysis. Once this step has been taken there will be a reduction in the wide range of amino acid recommendations for chickens, since raw ingredient matrix values will be relatively more comparable than is the case at present.

An important factor contributing to the variation in amino acid recommendations is the difference in composition of the experimental diets used. Excellent responses have been recorded on pure crystalline amino acid diets, but it would be unwise to relate these results to practical type diets composed mainly of intact proteins supplemented with small quantities of synthetic amino acids. It would appear that pure amino acids are more readily available, giving rise to a better net efficiency of food utilisation. It is therefore imperative that cognisance be taken of the composition of experimental diets when results or amino acid recommendations are equated.

The earlier amino acid recommendations for chickens were often associated with a minimum protein content in the diet. This work was conducted on experimental diets composed of natural ingredients, since pure amino acids were either unavailable or too costly. If we accept that amino acids are a function of protein, then the only method of increasing the amino acid level in a diet would be to raise the protein content. The concept of a minimum protein level in a chick diet has fallen away with the advent of synthetic amino acids. Modern poultry diets are composed of natural ingredients supplemented with pure amino acids to meet the essential amino acid requirements of the diet.

The observation that the lysine requirement for most efficient food utilisation may be slightly higher than that for maximum growth rate has been described by numerous authors (Combs and Nicholson, 1962; Combs, 1968; Boomgaardt and Baker, 1973 a,b; Bornstein and and Lipstein, 1975 a). This phenomenon may be associated with the decrease in voluntary food consumption per unit gain as the level of protein is raised above that needed to supply the minimum levels of essential amino acids (Combs and Nicholson, 1965). The results of Bornstein and Lipstein (1966) indicated that the total sulphur amino acids requirement expressed as a fraction of dietary protein remained constant throughout the growing period (although it was lowered when changing from starter to finisher diets if expressed as percent of diet). Bornstein (1970) demonstrated that this finding could be equally extended to the lysine requirement for the age 0 to 8 weeks. In other words, protein levels can be lowered when changing from starter to finisher diets but the protein composition of these diets should not be changed - at least as far as lysine and sulphur amino acids are concerned. The reports of several workers (Schwartz *et al.*, 1958; Zimmerman and Scott, 1965; Boomgaardt and Baker, 1973 b) have indicated that the amino acid requirement expressed as a percentage of the diet decreases with increasing age.

The amino acid - age relationship has been the subject of numerous studies some of which were partially reviewed by Graber *et al.* (1971). These authors questioned the concept of a constant protein composition for the chick with increasing age since it failed to consider changes in maintenance requirements. The study of Boomgaardt and Baker (1973 b) suggests that the lysine requirement decreases in direct proportion to the decrease in the protein requirement, and this may indicate that the maintenance requirement for lysine plays only a small role in the total requirement for maximal performance. The results of the above mentioned studies would indicate that the requirement for most essential amino acids expressed as a percent of the diet decreases with increasing age of the growing animal.

LYSINE

The lysine requirement of the broiler is of major economic significance even for feeding regimes in which broiler diets based on soyabean meal and fish meal supply an excess of this essential

TABLE 5.1 Summary of lysine requirement values published in the recent literature

Source	Percentage of diet	Percentage of protein	g/MJ
AEC (1978)			
Starter (0-4 w)	1,21	--	0,93
Finisher (4-8 w)	0,95	--	0,73
ARC (1975)			
Starter (1-4 w)	--	--	0,86
Finisher (4-8 w)	--	--	0,61
Bornstein (1970)			
Starter (1-5 w)	--	4,90	--
Finisher (5-8 w)	--	5,00	--
Post finisher (8-10 w)	--	4,80	--
Boomgaardt and Baker (1973 a)			
Starter (2-4 w)	1,06	4.62	--
Finisher (6-8 w)	0,92	4,61	--
Combs (1968)			
Starter	--	--	0,88
Finisher	--	--	0,78
Post finisher	--	--	0,67
NRC (1977)			
Starter (0-3 w)	1,20	5,20	0,90
Finisher (3-6 w)	1,00	4,00	0,75
Post finisher (6-9 w)	0,85	4,70	0,64
Scott *et al.* (1976)			
Starter (1-4 w)	1,16	5,0	0,90
Finisher (4-8 w)	1,02	4,2	0,74
Thomas *et al.* (1978)			
Starter (0-3 w)	--	5,51	0,98
Finisher (3-6 w)	--	5,22	0,84
Post finisher (6-8 w)	--	4,37	0,64

amino acid. In modern broiler diets more emphasis is placed on the amino acid content and balance rather than the protein level in the diet, since mass gain is dependent on the amino acid intake rather than protein consumption *per se*. As the protein level is progressively reduced in practical rations during the broilers' lifetime, by substituting cereal grains for these protein fractions, lysine levels are reduced at a more rapid rate than protein. Lysine therefore, becomes one of the first limiting essential amino acids in commercial broiler diets.

A partial review of the recent literature on the lysine requirement up to 8 weeks of age is presented in Table 5.1 for the sake of comparison. As a whole, the requirements found in the recent publications are higher than the earlier recommendations. This is due to the fact that modern broilers give better performance and higher levels of lysine may be required for birds exhibiting a more rapid growth rate. Moreover, breed or strain effects should not be ignored.

METHIONINE

A large number of reports have been published on the subject of methionine and total sulphur amino acid (SAA) requirements and their supplementaion. Unfortunately, the requirement values published have been quite variable and at times even contradictory. Bornstein and Lipstein (1964a) have mentioned the following factors contributing to such a situation: (1) most authors used calculated and not assayed values of methionine content in the feedstuffs, (2) the great variability of the assumed values employed in these calculations (Wilgus, 1958), (3) the problem of biological availability of SAA in different dietary constituents (Evans, Bandemer and Bauer, 1956), (4) the dependence of the methionine requirement on dietary protein (Grau and Kamei, 1950) and energy levels (Baldini and Rosenberg, 1955), (5) the effect of source and manufacturing methods on the SAA content of processed animal and plant proteins, (6) the existence of genetic differences in respect to methionine requirements (Hess, Edwards and Dembnicki, 1962), and (7) the effect of hot weather on methionine requirement (Camp and Couch, 1959).

Numerous experiments have been carried out in order to study the methionine requirement of the growing chick when fed diets of various protein and energy content. (Almquist, 1952; Rosenberg and Baldini, 1957; Bornstein and Lipstein, 1964 a,b; 1975 a,b). The results obtained with isocaloric diets at different protein levels indicate that the energy content of the diet governs the methionine requirement. When sufficient energy is available from non-protein sources to permit full utilisation of the protein for tissue synthesis and repair. Methionine requirement expressed as percent of diet increases as protein level increases in diets composed of natural ingredients. In the absence of a sufficient amount of energy to permit the bird to make full use of the protein offered for growth purposes, increasing levels of dietary protein were found not to require corresponding amounts of methionine. Rosenberg and Baldini (1957) calculated that within limits, the methionine requirement of isonitrogenous diets, expressed as percent of the diet, increases as the energy content of the diets is increased.

It has been observed that a moderate deficiency of methionine decreases the efficiency of food conversion of chicks more markedly than it decreases body mass gain. It therefore appears that the young chick responds to a moderate deficiency of methionine by increasing its food intake. In that this increase is not reflected in body mass gain, the fate of the extra energy ingested has been of some interest. It is possible that the extra energy ingested results in both increased energy deposition and increased heat production (Davidson, Mathieson, Williams and Boyne, 1964). A feature of the response of chicks to a diet marginally deficient in methionine that does not appear to have been commented upon is the mechanism underlying the increase in food intake. It has been observed frequently that chicks respond to deficiencies of essential amino acids by decreasing their food intake (Khalil, Thomas and Combs, 1968; Sugahara, Baker and Scott, 1969). It would appear that the degree of deficiency is critical in determining the animals response: comparatively severe deficiencies causing a reduction of food intake while less severe deficiencies are not of sufficient magnitude to invoke a mechanism causing reduction in food intake, and under these conditions food intake increases either in response to changes in the energy metabolism or in response to the increased demand for the deficient amino acid. It is evident therefore that observations on the effect of an amino acid deficiency on the utilisation of dietary energy must be considered in the light of the effect of the deficiency on food intake (Solberg *et al.*, 1971).

Bornstein and Lipstein (1975a; 1975b) investigated the extent to which protein concentration could be lowered in chick diets, while ensuring that this reduction did not involve a corresponding decrease in the first two limiting amino acids, methionine and lysine, and to attempt to quantify the replacement value of these amino acids for soyabean meal in practical broiler chick diets. Replacing soyabean meal by grain reduces dietary protein on the one hand but raises the energy content of the diet on the other hand. This causses a relative improvement in food utilisation except when counteracted by a decreased growth rate. This increase in energy is worth remembering when analysing the economic aspects of the replacement of soyabean meal by grain plus amino acids.

The improvement in energy concentrations tended to confound the effects of protein reduction (with or without amino acid supplementation) on food utilisation. Nevertheless, chick performance on the supplemented experimental diets demonstrates that the adverse effects of protein deficiency are due primarily to a deficiency of the first limiting amino acids, and that their supplementation tends to counteract this damaging influence. The fact that supplementations with methionine and lysine were not able to compensate completely for the suboptimal protein concentrations indicates that one or more additional essential amino acids were not less limiting than lysine. According to Warnick and Anderson (1968) the limiting amino acids of soy-protein relative to chick requirements are SAA, threonine, valine and lysine in that order.

Bornstein and Lipstein (1975b) noted that food utilisation appeared to be more affected than growth rate by lowering the protein concentrations and to respond more strongly to amino acid supplementation. This is in line with previous observations that methionine and lysine requirements for most efficient food utilisation are slightly higher than those for maximum growth (Bornstein and Lipstein, 1964a; Bornstein, 1970). The observation that dietary protein can be reduced below the normal requirement by almost one percentage point, as long as SAA concentration (expressed as percentage of diet) is increased, is supported by previous results, that methionine requirements increase with widening metabolisable energy/protein ratios (Twining *et al.*, 1955; Rosenberg and Baldini, 1957; Bornstein and Lipstein, 1964b).

Numerous attempts have been made to overcome the growth depressing effects of a methionine - induced toxicity. The underlying mechanism by which excess methionine exerts its pathogenicity, however, is not well understood, although numerous theories prevail. The work of Katz and Baker (1975a) has established 1,25 percent excess methionine as a level that would result in approximately a 40 percent depression in mass gain.

Katz and Baker (1975b) conducted several assays with young chicks fed crystalline amino acid diets to investigate the effects of supplemental glycine, serine, threonine, arginine or adenine on the growth depression resulting from consumption of excess methionine. Glycine was partially effective in alleviating the growth depression caused by excess methionine. The addition of threonine

TABLE 5.2 Summary of methionine requirement values published in the recent literature

Source	Percentage of diet	Percentage of protein	g/MJ
AEC (1978)			
Starter (0-4 w)	0,55	--	0,42
Finisher (4-8 w)	0,41	--	0,32
Combs (1968)			
Starter	--	--	0,34
Finisher	--	--	0,30
Post finisher	--	--	0,25
NRC (1977)			
Starter (0-3 w)	0,50	2,17	0,39
Finisher (3-6 w)	0,38	1,90	0,29
Post finisher (6-9 w)	0,32	1,78	0,25
Scott *et al.* (1976)			
Starter (0-4 w)	0,47	2,0	0,37
Finisher (0-8 w)	0,41	2,0	0,30
Thomas *et al.* (1978)			
Starter (0-3 w)	0,52	2,26	0,40
Finisher (3-6 w)	0,50	2,38	0,39
Post finisher (6-8 w)	0,39	2,05	0,30

together with glycine improved performance still further. Efficiency of food utilisation for mass gain was greater in birds fed the methionine imbalanced diet supplemented with glycine and threonine than in those fed the control diet. Supplemental glycine, threonine, or adenine, but not arginine, was effective in ameliorating the hypoglycemia resulting from consumption of excess methionine. The rate of oxidation of a tracer dose of threonine was increased markedly by feeding 1,25 percent excess methionine.

This was reflected in a 20 percent depression in threonine utilisation for mass gain as measured by slope ratio. The data of Girand - Globa *et al* (1972) and Katz and Baker (1975 b) suggests that both threonine and glycine are antagonised by consumption of excess methionine.

Hafez *et al.* (1978) found that homocystine also caused a growth depression. Although not as severe as that caused by methionine. Glycine, which is capable of alleviating to a considerable degree the toxicity of methionine and homocystine in the rat (Benevenga and Harper, 1967) was also able to alleviate these toxicities in the poult. Betaine, which is a donor of methyl groups, was effective in reducing the toxicity of homocystine, but was ineffective in alleviating the toxicity of methionine.

The fact that homocystine also depressed growth, and betaine was not growth depressing, suggests that the adverse effect was due to the homocystine moiety of the methionine molecule rather than a toxicity of methyl groups. On the other hand, the supply of methyl groups by betaine, which can methylate homocystine to methionine, alleviated the toxicity of homocystine but not of methionine. Hafez *et al.* (1978) are of the opinion that the toxicity of methionine is not due to its methyl group and suggest the assignment of this role to the homocystine moiety.

DIETARY METABOLISABLE ENERGY

It is well documented that over a wide range of dietary energy concentrations chicks tend to eat in order to satisfy their energy requirements. At very low energy concentrations the chick may not meet its energy requirement and at high energy concentrations may consume more food than is required for maximum growth rate and the excess energy may be deposited as fat (Spring and Wilkinson, 1957;

Scott *et al.*, 1976). The earlier recommended ME concentrations of broiler diets were 11,5 MJ/kg (National Research Council, 1966) and 11,9 MJ/kg (Agricultural Research Council, 1963), although a more recent recommendation is 13,4 MJ/kg (National Research Council, 1971). Adherence to fixed, relatively high-energy diets prevents flexibility in the formulation of broiler diets. For example ingredients such as bran and pollard may be precluded in large amounts from diets computed by least-cost formulation because of their comparatively low energy concentration.

There are numerous reports on the effects of dietary energy concentration on the performance of growing chickens. However, many of these studies failed to recognise the importance of maintaining energy-to-protein relationships, or more generally the energy-to-first-limiting-nutrient ratio constant as suggested by Combs (1968). This important principle should be borne in mind when considering experimental results.

Farrell *et al.*, (1973) found that when broiler chickens were kept under essentially commercial conditions and given diets with a range of energy concentrations there were large differences in their growth rate, food intake, efficiency of food utilisation and carcass composition. Farrell (1974) suggests that there is an optimum energy concentration in the diet beyond which performance of birds does not appear to improve, and in some cases actually deteriorates. Above about 13,81 MJ/kg of diet, food intake, energy intake, joules required per gram of gain, days to reach the specified live-mass, generally increased, while the percentage of food ME that was retained in the carcass, decreased. Payne and Lewis (1963) found that growth rate of broilers did not increase at energy levels above 12,97 MJ/kg and joules consumed per gram of gain were the same for diets above 13,39 MJ/kg.

The gradual improvement in performance of birds fed on diets that increase in dietary energy consentration up to 13,81 MJ/kg may be due to an increasing proportion of energy stored in gain and less used for maintenance as birds grow more rapidly. Farrell (1974) found a marked improvement in the efficiency of utilisation of ME as determined by the percentage retained in tissue, between 11,30 MJ/kg and 13,39 MJ/kg while over this energy range there was no apparent similar accelerated change in growth rate.

The problem of excessive fatness in broilers is of economic importance, since the production of this excess fat is associated with a waste of dietary energy. The degree of fatness is affected by both non-nutritional and nutritional factors. Age and sex seem to be the most important non-nutritional factors, the percentage of fat in the carcass increasing with age (Edwards, 1971; Thomas and Twining, 1971; Kubena *et al.*, 1972).

More recently Yoshida *et al.* (1970), Thomas and Twining (1971), Kubena *et al.* (1972), Bartov *et al.* (1974), Lee and Blair (1974) and Janky, Riley and Harms (1976) have investigated the specific effects of dietary protein, energy, and joule-to-protein (J:P) ratio on the carcass composition of chicks. Throughout these studies it was established that, as the dietary J:P ratio was widened, energy intake and carcass fat deposition increased, while body water content decreased. Yoshida and Morimoto (1970) reported that the effects of dietary protein concentration on carcass fat content is rapid and reversible. Thomas and Twining (1971), too, observed considerable changes in carcass fat content, as early as 10 days after alterations were made in protein concentration.

Bartov *et al.* (1974) maintain that in contrast to the sensitivity of carcass fat concentration to manipulations of dietary protein or J:P ratio, body fat deposition does not appear to be influenced by concentrations of dietary fat supplements as long as the J:P ratio remains unchanged. In other words dietary oil *per se* does not stimulate the accumulation of fat in the carcass, in agreement with Edwards and Hart (1971). On the other hand, Donaldson *et al.*

(1956) reported that increasing fat concentrations in diets containing a constant protein concentration increased carcass fat content. However, this effect might have been the result of widening the dietary J:P ratio rather than a specific effect of the dietary fat supplement.

Fisher and Wilson (1974b) state that the primary response of the chicken to dietary energy level is in food consumption and productive efficiency rather than in production level. Therefore the optimum energy concentration in broiler diets should be determined in economic rather than biological terms. The basis of such a determination is knowledge, in physical and money terms, of the responses in animal performance to changes in the energy concentration of the diet. Fisher and Wilson (1974a) have analysed the information about such responses which have been published since 1950.

An analysis of 160 estmates of the response of growing chickens to dietary ME (DME) is summarised in Figure 5.1 which shows the pooled regressions of livemass gain (ΔW, g/d), food intake (F, g/d), ME intake (MED, k cal/d) and food conversion efficiency (FCE, $\Delta W/F$) on DME. Separate analyses showed that these responses could be justifiably described by linear regressions although there was a slight and significant curvature in the cases of F and FCE. Fisher and Wilson (1974a) found that sex, age, breed and energy-to-protein ratio influenced the rate of response in some characteristics but the effect of diet form and environmental factors could not be properly evaluated from the available data. In the light of these findings a sub-section of the published data, with 31 estimates of response, was selected to provide the best estimates of response for use in commercial broiler production. This sub-section was reanalysed and the resulting regression coefficients are recommended for use in the formulation of practical broiler diets. The responses to the parameters measured were summarised as follows:

Response in livemass gain (ΔW/DME)

The average improvement in ΔW with increasing DME was 4,3 g/d per k cal/g (Figure 5.1). However, the response was significantly larger in males than in females and the difference in response between the periods 0 to 28 and 29 to 56 d was not significant (Table 5.3). Responses in livemass gain can also be expressed as days required to reach a fixed killing mass. Farrell, Cumming and Hardaker (1973) reported their results in this way and found that time to reach a fixed mass varied by 5 d when DME was increased from 12,13 to 15,06 MJ/kg.

Response in food consumption (F/DME)

The responses in food consumption were very variable between experiments and the overall regression coefficient of -5,39 g/d per k cal/g shown in Figure 5.3 although highly significant, accounts for only 28 per cent of the variation in F (r = 0,532). This variability of response may be associated partly with non-linearity since the quadratic regression was significant in this case.

Response in energy consumption (MED/DME)

Although food consumption declines with increasing DME the rate of this response is not great enough to prevent an increase in energy intake (Figure 5.1). MED altered by 23,53 k cal/d per k cal/g, the pooled regression accounting for 41,6 per cent of the variation (r = 0,645). The responses in energy and food consumption are not affected by sex but age has a highly significant effect (Table 5.3).

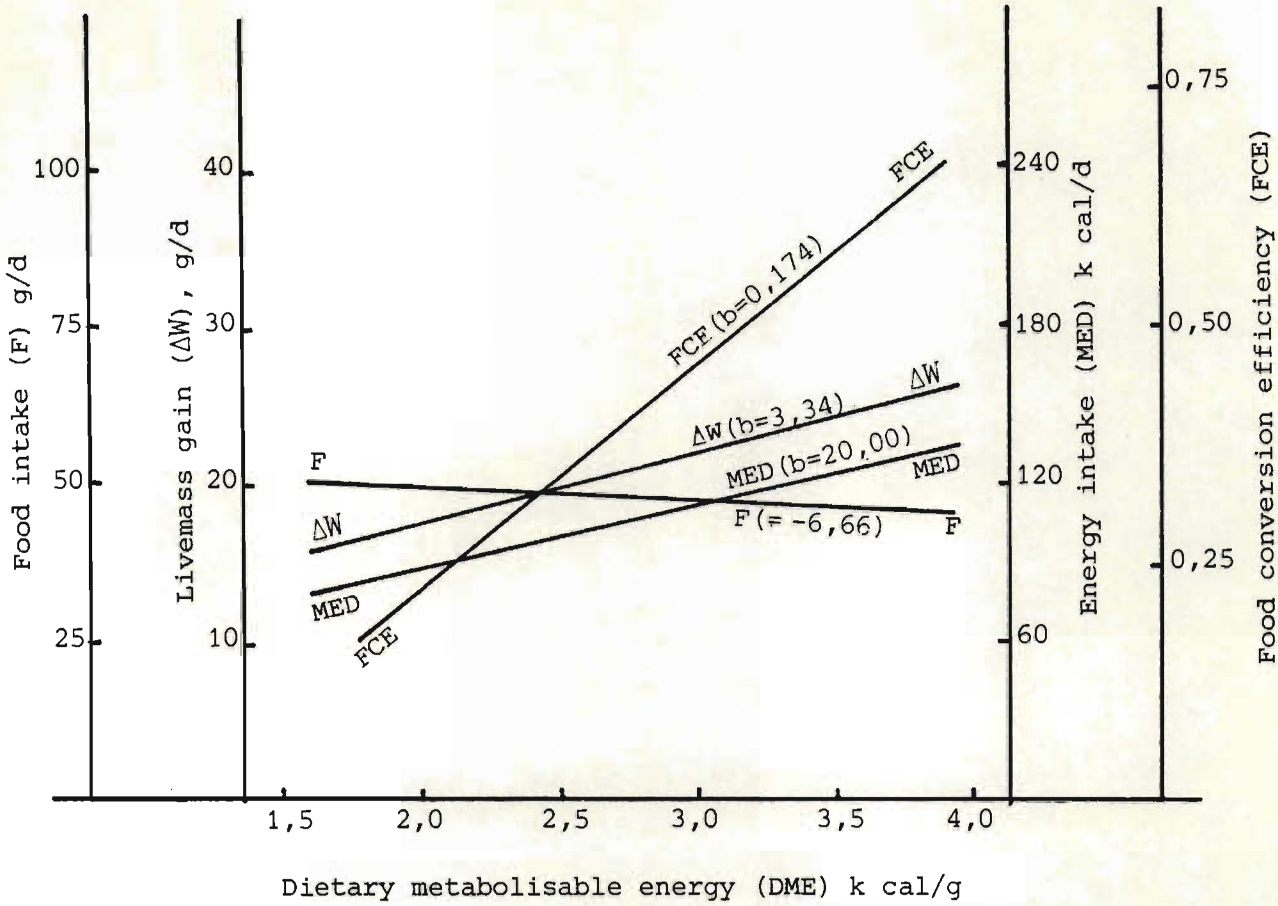

Figure 5.1 Pooled linear regressions of broiler performance characteristics on dietary metabolisable energy level. Derived from 160 estimates of response in published experiments (Fisher and Wilson, 1974 b)

TABLE 5.3 Regression coefficients recommended for use in the formulation of practical broiler diets. The coefficients correspond to metabolisable energy changes of 1 k cal/g (Fisher and Wilson, 1974b)

Response	Sex	Starter phase 1 - 28 d	Finisher phase 29 - 56 d
Livemass gain	M	5,11	6,06
(ΔW, g/d)	F	3,29	4,24
	M/F	4,20	5,15
Food intake	M	-1,64	-15,35
(F, g/d)	F	-3,02	-16,73
	M/F	-2,33	-16,04
Food conversion	M	0,21	0,15
efficiency (FCE, ΔW/F)	F	0,17	0,12
	M/F	0,19	0,13

Response in food conversion efficiency FCE/DME

In spite of the variable response in food consumption an improvement in food conversion efficiency is an extremely consistent observation as dietary energy level is raised. Out of 160 estimates of this response only 2 were negative and the pooled regression coefficient of 0,174 accounts for 83,3 per cent of the variation in FCE (r = 0,913) (Figure 5.1). In spite of this excellent fit the differences between the coefficients for each sex and for each stage of growth were significant (Table 5.3).

Effect of dietary metabolisable energy level on body composition

There is little information on the effect of DME on body composition when energy-to-protein ratios are constant. Fisher and Wilson (1974a) found 15 estimates of this response in four publications, but the majority of these data referred to birds aged 28 d or less. The results of the published experiments were very similar and the pooled regression coefficients are shown in Table 5.4. As DME rises the dry matter content of the carcass increases; the protein and fat content of the dry matter decrease and increase respectively. The overall result, is that body protein content is little affected by DME while fat content increases by 28 g/kg per k cal/g increase in energy level. Over a practical range of energy levels this is a relatively small effect.

Farrell *et al.* (1973) found that killing-out percentage increased by about 2 percent as DME increased. Although non-significant in this case, such an effect would be of considerable economic importance. Farrell (1974) reported the relationship between DME and body composition of male and female broilers killed at a fixed mass. The results are in very close agreement with the general analysis summarised in Table 5.4. Although the sexes differ in body composition the effect of changing DME is similar in males and females.

TABLE 5.4 Pooled regression coefficients of body composition on dietary ME level (DME) (Fisher and Wilson, 1974b)

Character regressed on DME	Regression coefficient (b) (g/kg per k cal/g)	Significance	r
Dry matter (DM)	23,77	* * *	0,758
Crude protein in DM	-11,64	* * *	0,714
Fat in DM	16,69	* * *	0,780
Crude protein	-1,20	NS	0,144
Fat	28,20	* * *	0,763

* * * P < 0,001

P A R T T W O

RESPONSE TO DIETARY LYSINE AND METABOLISABLE ENERGY CONCENTRATIONS

I N T R O D U C T I O N

The dilution technique, developed by Fisher and Morris (1970) for measuring the response of laying birds to increasing concentrations of an amino acid, was successfully evaluated for use with broilers by Gous (1980) and by the author in the preceding chapters. In order to further evaluate the technique and to collect more data relating amino acid input to broiler growth a series of experiments was conducted using both lysine and methionine as test amino acids. In all these experiments more than one level of energy was used when evaluating the response of broilers to increasing concentrations of the test amino acids. This had a two-fold purpose: to determine the effects of energy on the production parameters, body mass gain, food intake and feed conversion efficiency; and to investigate whether an interaction exists between those factors that determine food intake (as exemplified by differing energy concentrations) and the utilisation of essential nutrients.

These subsequent studies, as with those reported previously, were conducted both in the starter and in the finisher periods in order to cover the full range of feed intakes that might be encountered in a commercial broiler operation. Also, in all cases, males and females were tested separately in order to enable the determination of the effect of sex on the utilisation of amino acids and energy. Although few commercial broiler operations at present rear male and female chickens separately, when economic conditions change and if the sexing of broilers at day-old can become less costly with for example, the production of broilers that can be feather-sexed at day-old, response curves such as those presented in this thesis will be a considerable aid in decision-making.

CHAPTER 6

RESPONSE OF MALE BROILER CHICKENS TO DIETARY LYSINE AND METABOLISABLE ENERGY CONCENTRATIONS DURING THE PERIOD 7 TO 21 DAYS OF AGE

INTRODUCTION

Although a large number of studies have been made to determine the requirement of broilers for lysine, in only one (Gous, 1980) has the dilution technique of Fisher and Morris (1970) been applied. The aim of this experiment was, therefore, to increase the data pertaining to the response of broilers to lysine using the dilution technique, and to compare the results obtained with those of Gous (1980) and with other reports where the classical technique was used. In this experiment the response to lysine and energy of male broilers was tested, in order to determine the optimum intake of dietary lysine and metabolisable energy in the first three weeks of life.

MATERIALS AND METHODS

Ross male broiler chickens were used in the experiment. They were reared to one week of age in wire-floored brooders on a commercial starter diet. Management of the chickens to seven days of age and the handling of chickens in order to obtain equal body mass groups per replication, have been outlined previously (Chapter 1). The same procedure was used in this experiment.

The required range of dietary lysine concentrations was obtained by formulating a summit diet, calculated to contain 1,20 times the requirement, and a dilution diet calculated to contain 0,36 times the requirement, the intermediate lysine contents being obtained by blending the basal diets in appropriate proportions as shown in Table 6.3. The net result of the dilution technique was the formation of five experimental diets with lysine contents of 1,20; 0,99; 0,78; 0,57 and 0,36 times the lysine requirement of broilers recommended by Thomas *et al.* (1978) in which the available amino acid figures were converted to total requirements based on an amino acid availability of 90 percent.

In order to determine the effect of metabolisable energy on broiler performance four energy levels were included in the design. Summit and dilution diets were formulated at 12,13; 12,55; 12,97 and 13,39 MJ/kg and blended proportionally to form the 20 experimental diets presented in Table 6.3. The ratio between lysine and metabolisable energy was kept constant in the summit and dilution diets.

From studies carried out with young chicks (Carew and Hill, 1964, 1967; Carew *et al.*, 1964; De Groote, 1968a, b; De Groote *et al.*, 1971), sufficient evidence can be presented to show that growing chicks utilise the metabolisable energy of fats and proteins respectively more and less efficiently compared with carbohydrates. De Groote (1974) argued that a system based on metabolisable energy values considerably underestimates fats and fat-rich feedingstuffs and over-estimates protein-rich feedingstuffs in comparison with carbohydrates. It is evident that considerable differences in net energy would occur if the summit and dilution diets were formulated using metabolisable energy values of feed ingredients. The summit and dilution diets were thus formulated to contain the same net energy concentration. The procedure followed was to formulate the summit diet at the required metabolisable energy level, to determine its net energy content, and then to formulate the corresponding dilution diet to the same net energy concentration.

The composition of the summit and dilution diets is shown in

TABLE 6.1 Composition (g/kg) of the summit and dilution diets

	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet
Diet code	1	5	6	10	11	15	16	20
ME (MJ/kg)	12,13		12,55		12,97		13,39	
NE (MJ/kg)	8,44	8,44	8,80	8,80	9,16	9,16	9,61	9,61
Ingredients								
Yellow maize meal	488,9	663,0	487,3	707,0	479,6	758,0	419,2	769,2
Wheat bran		250,7		171,8		85,0		58,5
Lucerne meal	10,0		11,0		10,0		19,0	
Soyabean unextracted	12,0		72,0		133,0		150,0	
Groundnut meal	99,0	35,0	120,0	33,0	120,0	10,0	120,0	87,0
Sunflower meal	199,0	2,0	102,0	39,0	3,0	98,0	9,0	35,0
Fish meal	179,0		180,0		180,0		180,0	
Blood meal		37,0	1,0	38,0	1,0	39,0	1,0	
Bone meal			2,0		1,0		10,0	40,0
Limestone flour	6,0	4,0	5,0	3,0	6,0	2,0		2,0
Salt		4,5		4,5		4,5		4,5
Sunflower oil							24,0	
Feather meal			13,0		60,0		61,0	
DL - Methionine	3,1	0,8	3,7	0,7	3,4	0,5	3,8	0,8
Vitamins and trace minerals *	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0

TABLE 6.1 continued

	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet
Diet code	1	5	6	10	11	15	16	20
ME (MJ/kg)	12,13		12,55		12,97		13,39	
NE (MJ/kg)	8,44	8,44	8,80	8,80	9,16	9,16	9,61	9,61
Calculated analysis								
Arginine	20,19	7,54	19,99	8,79	19,99	8,41	20,67	9,38
Histidine	6,73	3,98	6,59	4,23	6,21	2,85	6,37	2,89
Isoleucine	10,62	3,42	10,58	3,96	11,78	3,98	12,08	4,02
Leucine	22,39	10,58	22,34	15,12	26,15	11,84	26,29	12,18
Lysine	14,40	4,27	14,90	4,42	15,40	4,57	15,90	4,71
Methionine	9,11	2,86	9,32	3,16	8,62	3,09	9,06	3,15
Cystine	4,82	2,90	4,80	3,38	5,27	3,12	5,32	3,02
Phenylalanine	12,19	4,82	12,38	7,48	12,92	5,47	13,18	5,78
Tyrosine	8,60	4,26	9,02	5,03	9,05	4,25	9,09	4,93
Threonine	10,21	3,96	10,42	5,21	11,25	4,52	11,56	4,16
Tryptophan	3,44	1,36	3,45	1,63	3,45	1,48	3,57	1,48
Valine	13,86	5,29	16,07	8,01	15,55	5,81	15,82	5,84
Calcium	10,00	10,00	10,00	10,00	10,00	10,00	10,00	10,00
Phosphorus	8,30	7,60	8,20	7,30	7,70	7,10	8,60	7,00
Crude protein (gN x 6,25/kg)	288,0	119,0	296,0	124,0	318,0	127,0	327,0	133,0

* Supplies per kg of diet: Vit A 7 027 IU, Vit D_3 2 000 IU, Vit E 20 IU, Hetrazine 3 ppm, Thiamine hydrochloride 0,985 ppm, Riboflavin 8 ppm, Calcium pantothenate 7,846 ppm, Niacin 29,823 ppm, Folic acid 0,95 ppm, Biotin 0,08 ppm, Choline Chloride 300 ppm

Copper 125 ppm, Iron 40 ppm, Zinc 28 ppm, Iodine 2 ppm, Manganese 78 ppm, Selenium 0,1 ppm

TABLE 6.2 Calculated amino acid contents of the summit and dilution diets relative to the requirements of broilers during the starter period

	Requirements according to Thomas *et al.* (1978) (g/kg)	Amino acid contents expressed as multiples of requirement	
		Summit diet	Dilution diet
Diet code		1	5
Arginine	11,59	1,74	0,65
Histidine	4,21	1,60	0,95
Isoleucine	7,91	1,34	0,43
Leucine	14,76	1,52	0,72
Lysine	11,86	1,20	0,36
Methionine	4,83	1,89	0,59
Cystine	3,56	1,35	0,81
Phenylalanine	7,38	1,65	0,65
Tyrosine	6,33	1,36	0,67
Threonine	6,99	1,46	0,57
Tryptophan	2,11	1,63	0,64
Valine	8,96	1,55	0,59
Diet code		6	10
Arginine	12,00	1,67	0,73
Histidine	4,36	1,51	0,97
Isoleucine	8,18	1,29	0,48
Leucine	15,28	1,46	0,99
Lysine	12,28	1,20	0,36
Methionine	5,00	1,86	0,63
Cystine	3,68	1,30	0,92
Phenylalanine	7,64	1,62	0,98
Tyrosine	6,55	1,38	0,77
Threonine	7,23	1,44	0,72
Tryptophan	2,18	1,58	0,75
Valine	9,28	1,73	0,86

TABLE 6.2 continued

	Requirements according to Thomas *et al.* (1978) (g/kg)	Amino acid contents expressed as multiples of requirement	
		Summit diet	Dilution diet
Diet code		11	15
Arginine	12,40	1,61	0,68
Histidine	4,51	1,38	0,63
Isoleucine	8,45	1,39	0,47
Leucine	15,78	1,66	0,75
Lysine	12,68	1,20	0,36
Methionine	5,17	1,67	0,60
Cystine	3,80	1,39	0,82
Phenylalanine	7,89	1,64	0,69
Tyrosine	6,76	1,34	0,63
Threonine	7,47	1,51	0,61
Tryptophan	2,25	1,53	0,66
Valine	9,58	1,57	0,61
Diet code		16	20
Arginine	12,80	1,61	0,73
Histidine	4,66	1,37	0,62
Isoleucine	8,73	1,38	0,46
Leucine	16,30	1,61	0,75
Lysine	13,10	1,20	0,36
Methionine	5,34	1,70	0,59
Cystine	3,93	1,35	0,77
Phenylalanine	8,15	1,62	0,71
Tyrosine	6,98	1,30	0,71
Threonine	7,71	1,50	0,54
Tryptophan	2,33	1,53	0,64
Valine	9,89	1,60	0,59

TABLE 6.3 Summary of dilution technique and calculated analysis of the experimental diets

Diet code	Blending ratio Summit diet		Dilution diet	Calculated dietary lysine (g/kg)	Calculated dietary protein (g/kg)
12,13 MJ/kg					
1	100	+	0	14,40	288,0
2	75	+	25	11,90	246,0
3	50	+	50	9,30	204,0
4	25	+	75	6,80	161,0
5	0	+	100	4,27	119,0
12,55 MJ/kg					
6	100	+	0	14,90	296,0
7	75	+	25	12,30	253.0
8	50	+	50	9,70	210,0
9	25	+	75	7,00	167,0
10	0	+	100	4,42	124,0
12,97 MJ/kg					
11	100	+	0	15,40	318,0
12	75	+	25	12,70	270,0
13	50	+	50	10,00	223,0
14	25	+	75	7,30	175,0
15	0	+	100	4,57	127,0
13,39 MJ/kg					
16	100	+	0	15,90	327,0
17	75	+	25	13,10	278,0
18	50	+	50	10,30	230,0
19	25	+	75	7,50	182,0
20	0	+	100	4,71	133,0

Table 6.1. Specific protein contents were not used in formulation but the minimum contents of all essential amino acids except lysine were set at 1,75 times for the summits and 0,7 times for the dilutions, of the requirements proposed by Thomas *et al.* (1978). In a number of instances it was impossible to formulate to the minimum amino acid specifications outlined above with the raw ingredients available, so it was necessary to reduce the levels of these amino acids in order to obtain feasible diets.

The experimental diets were fed from 7 to 21 days of age, and the criteria studied were growth rate and food intake during the two week experimental period. The data on each variate were subjected to statistical analysis, using the method of Rayner (1967). Means, standard errors of the means (SEM) and least significant differences (LSD) at $P < 0,05$ and $P < 0,01$ were calculated.

RESULTS

Means of the twenty treatments for the variates mass gain, lysine intake, food intake, dietary metabolisable energy intake and food conversion efficiency are given in Tables 6.4 to 6.8 respectively. The main effects of DME and the lysine/DME ratio are also shown, together with standard errors of the means; LSD's; and an analysis of variance table for each variate.

Response to dietary lysine concentration

The Reading model, described in Chapter 1, was used to fit an equation to the data relating body mass gain to lysine intake. Equations were fitted to data from each energy level, as well as to the combined data. The parameters used in fitting these equations, and the resulting coefficients, are given in Table 6.9. $\bar{W}$ was calculated as the arithmetic mean of the initial and final body mass of the birds; $\sigma_{\bar{W}}$, $\sigma_{\Delta\bar{W}}$ and $r_{\bar{W}.\Delta\bar{W}}$ were calculated from

TABLE 6.4 Mass gain (g/bird d) of male broilers from 7 - 21 days of age

Mass Gain

	DME MJ/kg	Lysine/DME Ratio 1,19	0,98	0,77	0,56	0,35	Mean
Males	12,13	21,1	21,3	20,0	13,1	5,6	16,3
	12,55	23,3	22,2	20,5	11,0	5,7	16,6
	12,97	24,2	22,1	19,7	14,8	4,8	17,1
	13,39	25,0	24,0	20,7	14,3	5,3	17,8
Mean		23,4	22,4	20,3	13,3	5,4	16,9

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,235	0,67	0,90
Mean of 5 entries	0,263	0,75	1,01
Mean of 20 entries	0,525	1,50	2,01
	CV	5,37%	

Analysis of variance

Source	DF	SS	MS	F
Replications	2	0,623	0,311	0,376 NS
Lysine	4	2757,036	689,259	832,800 **
Energy	3	21,576	7,192	8,693 **
Lysine x energy	12	43,137	3,595	4,345 **
Error	38	31,438	0,827	
Total	59	2853,810		

SEM denotes standard error of means
CV denotes coefficient of variation (percent)
LSD denotes least significant difference between two treatment means
NS denotes non significant
* P < 0,05
** P < 0,01

TABLE 6.5 Lysine intake (mg/bird d) of male broilers from 7 - 21 days of age

Lysine Intake

	DME MJ/kg	Lysine/DME Ratio 1,19	0,98	0,77	0,56	0,35	Mean
Males	12,13	461	411	328	206	90	299
	12,55	499	439	347	213	85	317
	12,97	514	442	337	218	106	323
	13,39	525	459	360	227	91	333
Mean		500	438	343	216	93	318

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	3,135	8,98	12,03
Mean of 5 entries	3,505	10,04	13,45
Mean of 20 entries	7,010	20,08	26,90
	CV	3,82%	

Analysis of variance

Source	DF	SS	MS	F
Replications	2	610,652	305,326	$2,071^{NS}$
Lysine	4	1308197,000	327049,000	2218,000**
Energy	3	8896,615	2965,385	20,112**
Lysine × energy	12	4753,393	396,116	2,686**
Error	38	5602,797	147,442	
Total	59	1328060,000		

SEM, CV etc see footnote Table 6.4

TABLE 6.6 Food intake (g/bird d) of male broilers from 7 - 21 days of age

Food Intake

	DME MJ/kg	Lysine/DME Ratio 1,19	0,98	0,77	0,56	0,35	Mean
Males	12,13	32,0	34,7	35,1	30,4	21,0	30,6
	12,55	33,5	35,7	35,9	30,2	19,3	30,9
	12,97	33,4	34,8	33,8	29,9	23,2	31,0
	13,39	33,0	35,0	34,9	30,2	19,4	30,5
Mean		33,0	35,1	34,9	30,2	20,7	30,8

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,463	1,33	1,78
Mean of 5 entries	0,518	1,48	1,99
Mean of 20 entries	1,036	2,97	3,97
	CV	5,83%	

Analysis of variance

Source	DF	SS	MS	F
Replications	2	11,131	5,565	1,729 NS
Lysine	4	1698,702	424,675	132,016 **
Energy	3	2,430	0,810	0,252 NS
Lysine x energy	12	41,310	3,442	1,070 NS
Error	38	122,240	3,217	
Total	59	1875,812		

SEM, CV etc see footnote Table 6.4

TABLE 6.7 Dietary metabolisable energy intake (MJ/bird d) of male broilers from 7 - 21 days of age

MED Intake

	DME MJ/kg	Lysine/DME Ratio 1.19	0,98	0,77	0,56	0,35	Mean
Males	12,13	0,39	0,42	0,43	0,37	0,25	0,37
	12,55	0,42	0,45	0,45	0,38	0,24	0,39
	12,97	0,43	0,45	0,44	0,39	0,30	0,40
	13,39	0,44	0,47	0,46	0,40	0,26	0,41
Mean		0,42	0,45	0,44	0,38	0,26	0,39

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,006	0,02	0,02
Mean of 5 entries	0,007	0,02	0,03
Mean of 20 entries	0,013	0,04	0,05
	CV	5,91%	

Analysis of variance

Source	DF	SS	MS	F	
Replications	2	0,002	0,0010	1,708	NS
Lysine	4	0,274	0,0680	128,064	**
Energy	3	0,011	0,0040	6,867	**
Lysine x energy	12	0,007	0,0006	1,132	NS
Error	38	0,020	0,0005		
Total	59	0,314			

SEM, CV etc see footnote Table 6.4

TABLE 6.8 Food conversion efficiency of male broilers from 7 - 21 days of age

F C E

	DME MJ/kg	Lysine/DME Ratio 1,19	0,98	0,77	0,50	0,35	Mean
Males	12,13	0,66	0,61	0,57	0,43	0,27	0,51
	12,55	0,70	0,62	0,57	0,36	0,30	0,51
	12,97	0,73	0,64	0,58	0,50	0,22	0,53
	13,39	0,76	0,68	0,59	0,47	0,27	0,56
Mean		0,71	0,64	0,58	0,44	0,26	0,53

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,007	0,02	0,03
Mean of 5 entries	0,008	0,02	0,03
Mean of 20 entries	0,016	0,05	0,06
	CV	5,23%	

Analysis of variance

Source	DF	SS	MS	F
Replications	2	0,004	0,002	2,593 NS
Lysine	4	1,501	0,375	494,100 **
Energy	3	0,021	0,007	9,215 **
Lysine x energy	12	0,043	0,004	4,721 **
Error	38	0,029	0,008	
Total	59	1,598		

SEM, CV etc see footnote Table 6.4

pooled data then rounded off; the value of $\Delta\bar{W}$ (maximum gain in body mass) was obtained from the iterative procedure adopted by the computer programme used (Curnow, 1973), as were the values of *a* and *b*. The combined equation, together with the means for each energy level, are illustrated in Figure 6.1.

Response to dietary energy concentration

Responses to DME are assumed to be linear throughout this paper. A general test of linearity was made by fitting a model of the form shown in the following equation:

$$Y = a \pm bx$$

where Y = variate being regressed
a = constant term
b = regression coefficient
x = dietary energy level.

The data used in the analysis consisted of the results of the first and second dilution series only. There were thus 24 values that were pooled for each of the variates ΔW, F, MED and FCE. The equations are presented in Table 6.10 and illustrated in Figure 6.2 respectively.

TABLE 6.9 Parameters used in determining the relationship between mass gain (g/bird d) and lysine intake (mg/bird d) by means of the Reading model using male chickens from 7 - 21 days of age

Parameter	Energy concentration (MJ/kg)				
	12,13	12,55	12,97	13,39	Combined
$\bar{W}$	250,00	250,00	250,00	250,00	250,00
$\Delta\bar{W}$	21,34	22,98	23,34	25,00	23,05
a	14,70	17,08	14,05	16,08	15,95
b	0,04	0,02	0,12	0,03	0,03
$\sigma\Delta\bar{W}$	3,55	2,33	4,00	4,17	2,90
$\sigma\bar{W}$	40,00	40,00	40,00	40,00	40,00
r	0,60	0,60	0,60	0,60	0,60

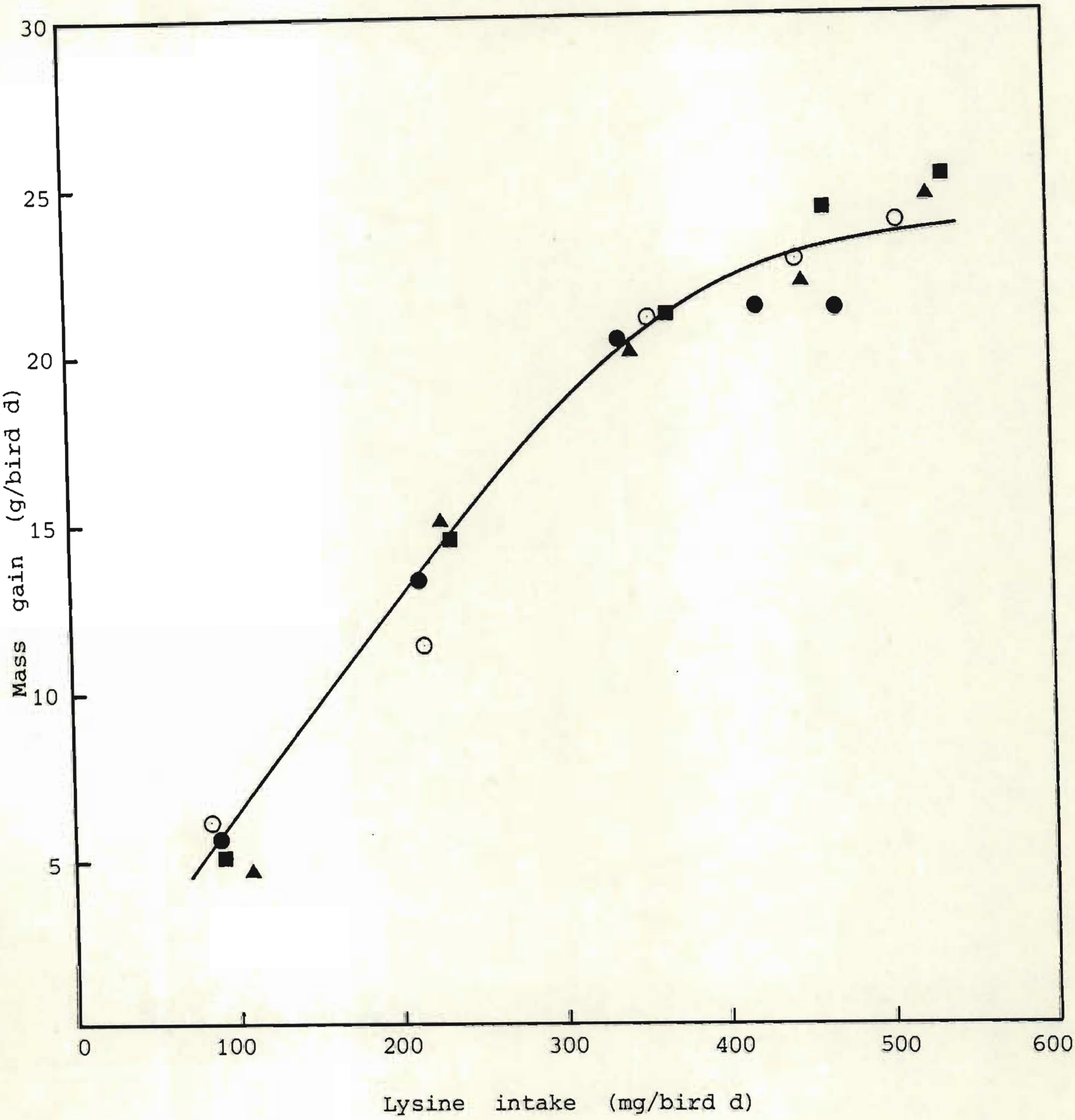

Figure 6.1 Response in mass gain (g/bird d) of male chickens from 7 - 21 days of age to lysine intake (mg/bird d) and estimated response using Reading model (Table 6.9)

● 12,13 MJ/kg ▲ 12,97 MJ/kg

○ 12,55 MJ/kg ■ 13,39 MJ/kg

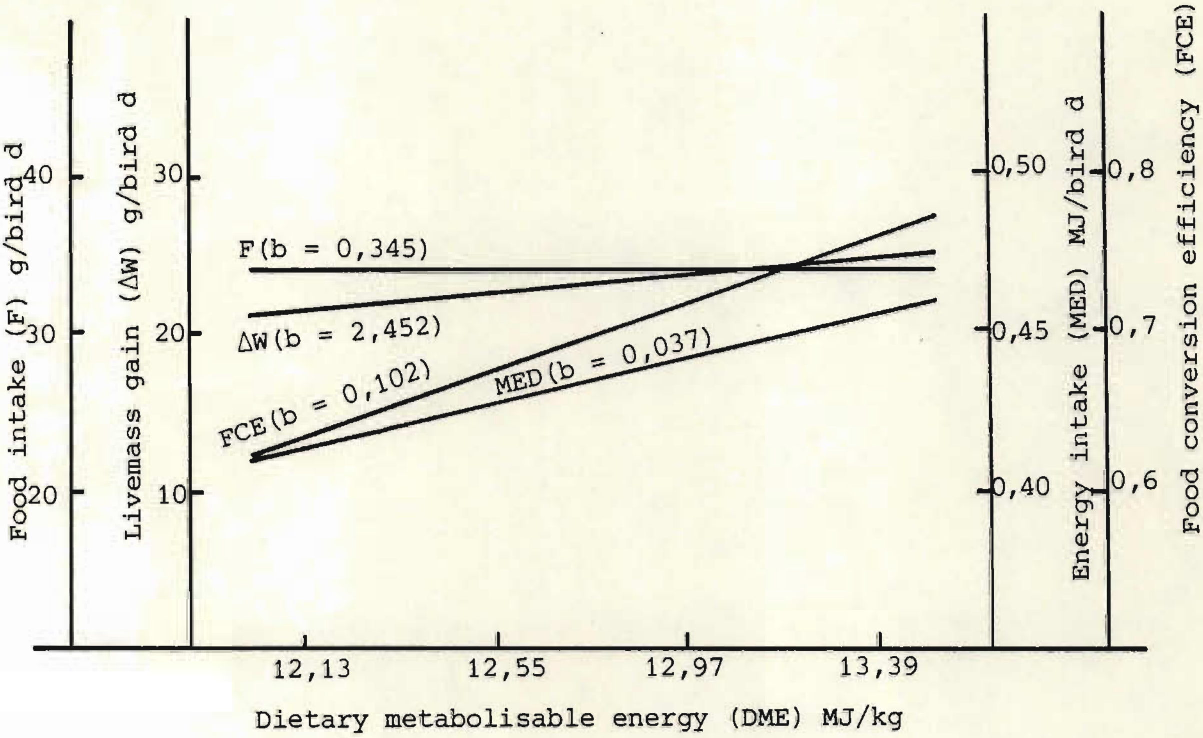

Figure 6.2 Pooled linear regressions of male broiler performance characteristics on dietary metabolisable energy levels for the starter period

TABLE 6.10 Pooled linear regression coefficients of male broiler performance characteristics on dietary metabolisable energy concentration in the starter period (units are per MJ/kg)

Character regressed on DME	Constant term	Regression coefficient (b)	SE (b)
Livemass gain (ΔW)	-8,392	2,452	0,484**
Food intake (F)	29,610	0,345	0,953^{NS}
Energy intake (MED)	-0,037	0,037	0,012**
Food conversion efficiency (FCE)	-0,619	0,102	0,022**

^{NS}, **, see footnote Table 6.4

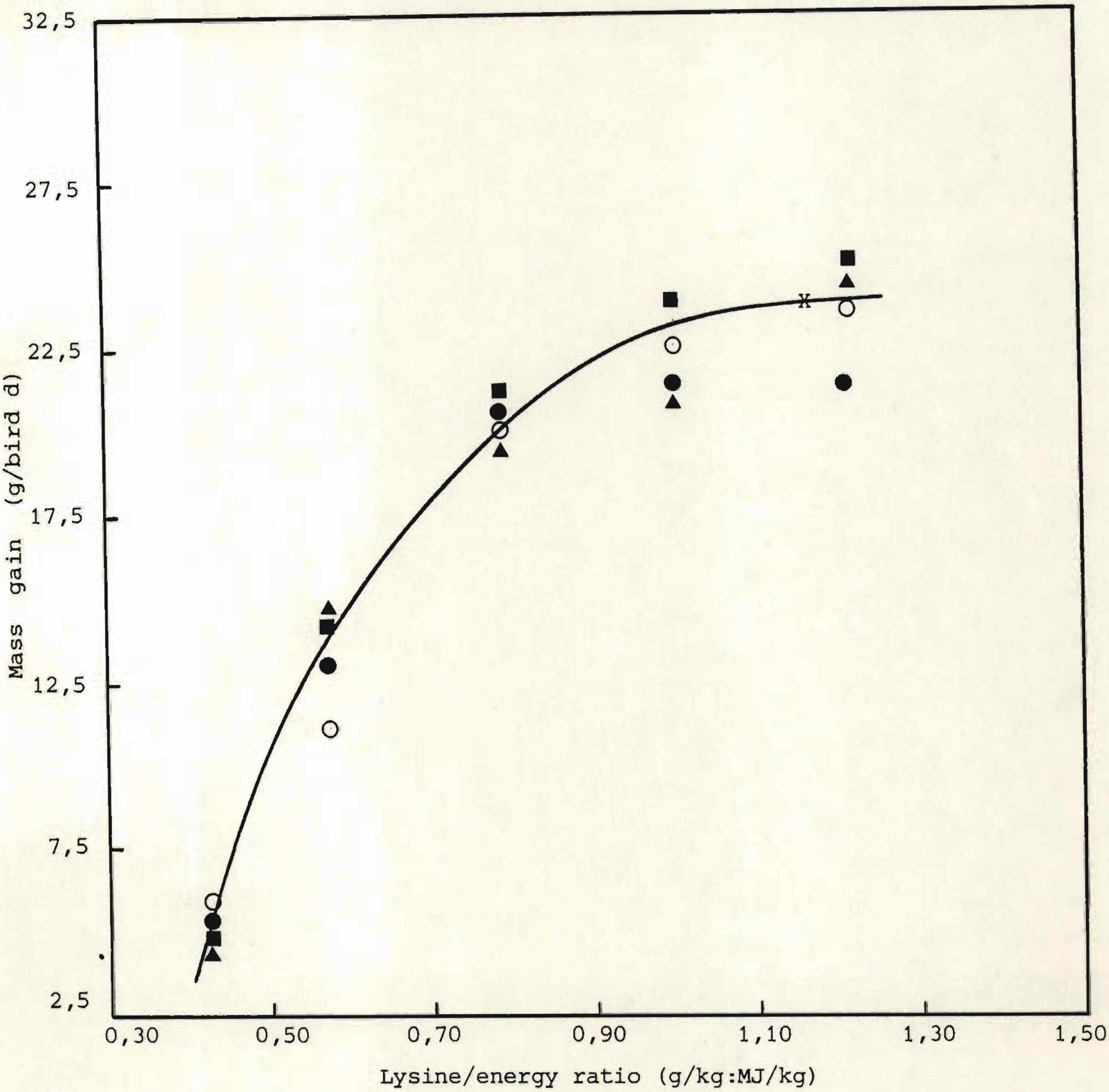

Figure 6.3 Response in mass gain (g/bird d) of male chickens from 7 - 21 days of age to lysine/energy ratio and estimated optimum lysine/energy ratio (X)

● 12,13 MJ/kg ▲ 12,97 MJ/kg

○ 12,55 MJ/kg ■ 13,39 MJ/kg

TABLE 6.11 The regression of mass gain on lysine/energy ratio and optimum lysine/energy ratio for male chickens from 7 - 21 days of age

DME (MJ/kg)	Constant term	Linear term (b_1)	Quadratic term ($\times 10^{-2}$) (b_2)	SE (b)	Multiple correlation coefficient (R)	Optimum lysine/energy ratio (g/kg:MJ/kg)
12,13	-15,58	0,716	-0,343	0,76*	0,997	1,043
12,55	-13,95	0,630	-0,265	$1,86^{NS}$	0,984	1,189
12,97	-14,99	0,681	-0,299	1,03*	0,995	1,139
13,39	-16,02	0,716	-0,312	0,12**	0,999	1,146
Combined	-15,14	0,686	-0,305	0,15**	0,998	1,125

^{NS}, * etc see footnote Table 6.4

TABLE 6.12 The regression of food conversion efficiency on lysine/energy ratio and optimum lysine/energy ratio for male chickens from 7 - 21 days of age

DME (MJ/kg)	Constant term	Linear term (b_1)	Quadratic term ($\times 10^{-2}$) (b_2)	SE (b)	Multiple correlation coefficient (R)	Optimum lysine/energy ratio (g/kg:MJ/kg)
12,13	-0,123	0,013	-0,546	0,02*	0,997	1,190
12,55	0,004	0,009	-0,234	$0,05^{NS}$	0,979	1,846
12,97	-0,233	0,016	-0,674	$0,05^{NS}$	0,983	1,179
13,39	-0,119	0,013	-0,473	0,01**	0,999	1,367
Combined	-0,118	0,013	-0,482	0,06*	0,998	1,310

^{NS}, * etc see footnote Table 6.4

Response to lysine/DME ratio

Curvilinear regressions of mass gain and FCE on lysine/DME ratio were calculated using a multiple regression analysis. The coefficients at each energy level and for the combined data over all energy concentrations are given in Tables 6.11 and 6.12 and illustrated in Figures 6.3 and 6.4 respectively.

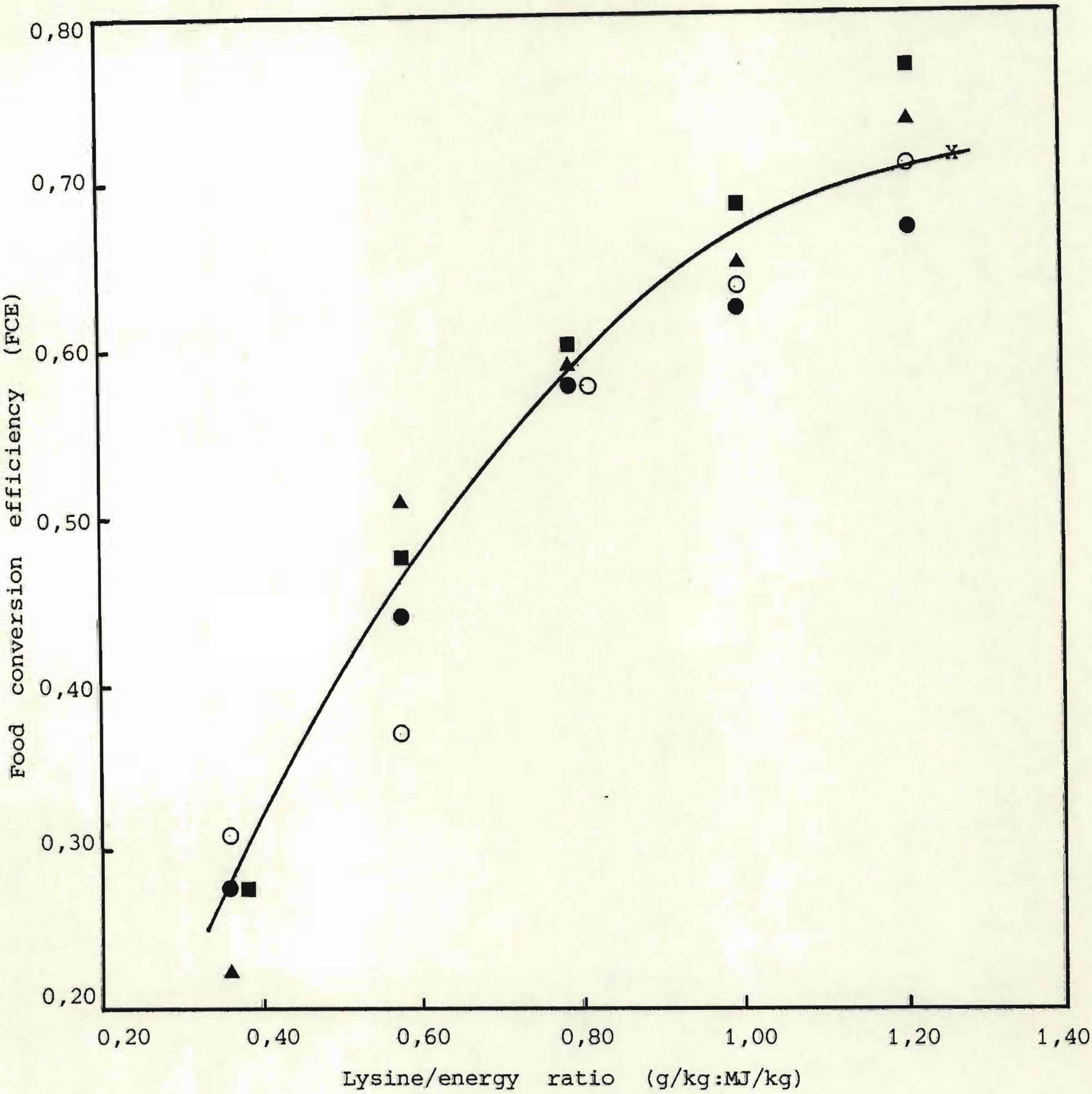

Figure 6.4 Response in food conversion efficiency of male chickens from 7 - 21 days of age to lysine/energy ratio and estimated optimum lysine/energy ratio (X)

● 12,13 MJ/kg ▲ 12,97 MJ/kg

○ 12,55 MJ/kg ■ 13,39 MJ/kg

DISCUSSION

The major objective of this trial was to determine the response of growing chickens to lysine intake using the dilution technique and to ascertain the optimum lysine intake in practical diets for male chickens during the starter period.

Response to dietary lysine concentration

The results of this study indicate that lysine intake plays a major role in controlling body mass gain. In commercial broiler diets based on maize and fishmeal lysine is the first-limiting amino acid, thus it is essential, from an economic standpoint to have an accurate assessment of the response of broilers to lysine intake.

Use was made of the Reading model to describe the relationship between mass gain and lysine intake. Equations fitted to the data for each energy concentration and to the combined data over all energy concentrations (Table 6.9) indicate that at marginal cost of 350c/kg for lysine, and 150c/kg for broiler mass, the optimum intake of lysine at each energy level for the males would be 494, 606, 540 and 625 mg/bird d. From the combined data (illustrated in Figure 6.1) the optimum intake was calculated to be 574 mg/bird d. The subject of optimum intakes of lysine will be discussed when the Reading model will be used to describe the combined response of broiler chickens of different ages and sexes to dietary lysine concentrations.

The concentration of the first-limiting amino acid in a diet has a significant effect on food intake. It has been observed that chickens respond to a moderate deficiency of essential amino acids by increasing their food intake (Khalil *et al.*, 1968). It would appear that the degree of deficiency is critical in determining the chicks response: comparatively severe deficiencies cause a reduction of food intake, while less severe deficiencies are not of sufficient magnitude to invoke a mechanism causing reduction in food intake, and under these conditions food intake increases either in response to changes in the energy metabolism or in response to the increased demand for the deficient amino acid (Solberg *et al.*,1971). At high levels of amino acid concentration, food intake declines so that actual intake of amino acid does not increase as much as could be expected if food intake remained constant. The above observations are shown in Table 6.6 where the effect of lysine on food intake is recorded. The highest intake was recorded on diets in the second dilution series, with intakes declining at an increasing rate as the lysine concentration was diluted further.

The effect of the lysine content of the diets on FCE is well defined in Table 6.8. The best FCE was recorded in the first dilution series diets which had the highest lysine contents, the response in FCE then declined progressively as the lysine level decreased in the remaining dilution series diets.

Although the FCE was highest on the first dilution series (summit) diets, it is not economical to feed such high levels of amino acids, as will be discussed later.

Response to dietary energy concentration

The effect of dietary metabolisable energy on ΔW, F, MED and FCE was examined by means of linear regression analyses and the pooled results are presented in Table 6.10 and Fig 6.2 respectively. The data used in the analyses was restricted to the results recorded for diets of the first and second dilution series, since it was felt that the lysine deficiency in the third, fourth and fifth dilution series diets would have had an unpredictable effect on performance, resulting in a distorted analysis.

DME had a highly significant effect on broiler performance as indicated in Table 6.7. Livemass gain, energy intake and food conversion efficiency increased appreciably as the energy level of the diet was raised. Energy utilisation for growth improves with increasing DME due to the fact that since growth increases with DME a smaller proportion of the ingested energy will be used for maintenance (Fisher and Wilson, 1974 a). Notwithstanding the fact that DME had a significant effect on energy intake, the energy intake was not constant for all energy concentrations, and food intake did not differ significantly between treatments.

Response to lysine/DME ratio

Chickens tend to adjust their daily food intake so as to maintain an appropriate level of energy intake, with the result that the mass of feed consumed generally decreases when balanced diets of increasingly higher energy concentrations are offered. However, the adjustment made by the young chick does not usually compensate entirely for the change in energy content, leading to an overconsumption of energy. More concentrated diets therefore result in higher nutrient intakes with correspondingly higher growth rate. This phenomenon is borne out in the results shown in Table 6.5 and Figure 6.3 where it is evident that the response in ΔW increases at the higher energy levels notwithstanding the fact that the lysine/DME ratio remains constant at each DME level.

If the energy content of a broiler diet is increased without making adjustments to the level of other nutrients, then growth rate may be limited by an inadequate intake of amino acids or other essential nutrients. For this reason it is important that an appropriate balance should be maintained between energy content and the levels of other nutrients both in practical diets and in experiments designed to investigate the effects of dietary energy on growth rate of broilers. The results presented in Table 6.4 show that the maximum response in ΔW to lysine/DME ratio was recorded in the diets of the first dilution series. The response in ΔW decreased progressively as the lysine/DME ratio narrowed in the lower dilutions. For the combined data, illustrated in Figure 6.3, the lysine/DME ratio which yielded maximum body mass gain was 1,125 g lysine/MJ.

The effect of varying the lysine/energy ratio, can be predicted from the response surface relating performance to both dietary variables. This relationship was examined by means of a multiple regression analysis, the results of which are presented in Tables 6.11 and 6.12 respectively. It is evident that FCE responds significantly to DME and to lysine/energy ratio as shown in Figure 6.4. The optimum lysine/energy ratios for the four test diets were calculated to be 1,190; 1,846; 1,179 and 1,367 g lysine/MJ respectively. For the combined data illustrated in Figure 6.4 the ratio which yielded maximum FCE was 1,310 g lysine/MJ. The results of this experiment confirm the opinion of numerous workers (Bornstein and Lipstein, 1964 a; Combs, 1968; Boomgaardt and Baker, 1973 a) that higher dietary concentrations of lysine are required for optimum FCE than for optimum body mass gain.

Because the range of energy concentrations studied was rather narrow (but nevertheless within practical limits) and because the number of observations per energy level were relatively small, a more complete discussion on the effects of dietary lysine and metabolisable energy on factors of economic importance will be reserved to a later chapter (Chapter 8), where the responses obtained in all the preceding experiments will be summarised and discussed in relation to their economic importance in broiler feed formulation.

CHAPTER 7

RESPONSE OF FEMALE BROILER CHICKENS TO DIETARY LYSINE AND METABOLISABLE ENERGY CONCENTRATIONS DURING THE PERIOD 7 TO 21 DAYS OF AGE

INTRODUCTION

The experiment described in this chapter is similar to the study conducted in the previous chapter, the difference being that females were used in place of the male broiler chickens used previously.

The objective of the study was to determine the response of female broilers to dietary lysine and metabolisable energy from 7 to 21 days of age using the dilution technique. This data would then be used to determine the optimum intake of these nutrients for broiler females during the starter period.

MATERIALS AND METHODS

Ross female broiler chicks were subjected to the housing management and experimental procedures described in Chapter 6. The experimental diets were the same for both trials, but were mixed separately for each trial. The composition of the experimental diets were shown in Tables 6.1, 6.2 and 6.3 respectively.

The experimental diets were fed from 7 to 21 days of age, and the parameters determined were growth rate and food intake during the two week experimental period. The data on each variate were subjected to statistical analysis. Means, standard errors of means (SEM) and least significant differences (LSD) at $P < 0,05$ and $P < 0,01$ were calculated.

RESULTS

Means of the twenty treatments for the variates mass gain, lysine intake, food intake, dietary metabolisable energy intake and food conversion efficiency are given in Tables 7.1 to 7.5 respectively. The main effects of DME and the lysine/DME ratio are also shown, together with standard errors of the means; LSD's; and an analysis of variance table for each variate.

Response to dietary lysine concentration

Use was made of the Reading model to fit an equation to the data relating body mass gain to lysine intake. The methods employed to calculate the parameters used in fitting the model and to obtain values of $\Delta\bar{W}$, a and b are described in Chapter 6. Equations were fitted to the data from each energy level, as well as to the combined data. The parameters used in fitting these equations, and the resulting coefficients, are given in Table 7.6. The combined equation, together with the means for each energy level, are illustrated in Fig 7.1.

Response to dietary energy concentration

Data used to measure the response to DME consisted of the results of the first and second dilution series only. There were thus 24 values that were pooled for each of the variates ΔW, F, MED and FCE. Linear responses were calculated and the equations are presented in Table 7.7 and illustrated in Fig 7.2 respectively.

TABLE 7.1 Mass gain (g/bird d) of broiler females from 7 - 21 days of age

Mass Gain

	DME MJ/kg	Lysine/DME Ratio 1,19	0,98	0,77	0,56	0,35	Mean
Females	12,13	21,2	20,6	19,2	18,1	12,0	18,3
	12,55	22,2	22,3	19,6	17,0	11,1	18,4
	12,97	22,3	23,1	19,3	15,7	9,0	17,9
	13,39	22,7	22,2	20,6	18,0	10,4	18,8
Mean		22,1	22,1	19,7	17,2	10,6	18,3

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,251	0,72	0,96
Mean of 5 entries	0,280	0,80	1,07
Mean of 20 entries	0,561	1,61	2,15
	CV	5,30%	

Analysis of variance

Source	DF	SS	MS	F
Replications	2	4,90	2,45	2,61 NS
Lysine	4	1092,17	273,04	290,47 **
Energy	3	6,13	2,04	2,17 NS
Lysine x energy	12	36,36	3,03	3,22 **
Error	38	35,85	0,94	
Total	59	1175,42		

SEM, CV etc see footnote Table 6.4

TABLE 7.2 Lysine intake (mg/bird d) for female broilers from 7 - 21 days of age

Lysine Intake

	ME MJ/kg	Lysine/DME Ratio 1,19	0,98	0,77	0,56	0,35	Mean
Females	12,13	548	452	360	266	155	356
	12,55	548	454	361	259	140	352
	12,97	566	486	346	263	137	360
	13,39	590	480	394	287	144	379
Mean		563	468	365	269	144	362

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	3,223	9,23	12,37
Mean of 5 entries	3,604	10,32	13,83
Mean of 20 entries	7,208	20,64	27,66
	CV	3,45%	

Analysis of variance

Source	DF	SS	MS	F
Replications	2	64,36	32,18	0,21 NS
Lysine	4	1295104,41	323776,10	2077,35 **
Energy	3	6413,56	2137,85	13,72 **
Lysine x energy	12	5818,14	484,84	3,11 **
Error	38	5922,56	155,86	
Total	59	1313323,23		

SEM, CV etc see footnote Table 6.4

TABLE 7.3 Food intake (g/bird d) for female broilers from 7 - 21 days of age

Food Intake

	DME MJ/kg	Lysine/DME Ratio 1,19	0,98	0,77	0,56	0,35	Mean
Females	12,13	38,1	38,0	38,6	39,1	36,3	38,0
	12,55	36,7	37,0	37,3	36,7	31,6	35,9
	12,97	36,7	38,3	34,6	36,1	30,0	35,1
	13,39	37,1	36,6	38,3	38,2	30,5	36,1
Mean		37,2	37,5	37,2	37,5	32,1	36,3

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,317	0,91	1,22
Mean of 5 entries	0,354	1,01	1,36
Mean of 20 entries	0,708	2,03	2,72
	CV	3,38%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	2	0,33	0,17	0,11 NS
Lysine	4	265,57	66,39	44,26 **
Energy	3	66,94	22,31	14,87 **
Lysine x energy	12	62,64	5,22	3,48 **
Error	38	57,18	1,50	
Total	59	452,67		

SEM, CV etc see footnote Table 6.4

TABLE 7.4 Dietary metabolisable energy intake (MJ/bird d) for female broilers from 7 - 21 days of age

MED Intake

	DME MJ/kg	Lysine/DME Ratio 1,19	0,98	0,77	0,56	0,35	Mean
Females	12,13	0,46	0,46	0,47	0,47	0,44	0,46
	12,55	0,46	0,46	0,47	0,46	0,40	0,45
	12,97	0,47	0,49	0,45	0,47	0,39	0,45
	13,39	0,49	0,49	0,51	0,51	0,41	0,48
Mean		0,47	0,48	0,47	0,48	0,41	0,46

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,004	0,01	0,02
Mean of 5 entries	0,005	0,01	0,02
Mean of 20 entries	0,009	0,03	0,03
	CV	3,42%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	2	0,00005	0,00002	0,08 NS
Lysine	4	0,0438	0,01095	44,03 **
Energy	3	0,0089	0,00298	11,19 **
Lysine x energy	12	0,0110	0,00092	3,70 **
Error	38	0,0094	0,00025	
Total	59	0,0733		

SEM, CV etc see footnote Table 6.4

TABLE 7.5 Food conversion efficiency for female broilers from 7 - 21 days of age

F C E

	DME MJ/kg	Lysine/DME Ratio 1,19	0,98	0,77	0,56	0,35	Mean
Females	12,13	0,56	0,54	0,50	0,46	0,33	0,48
	12,55	0,60	0,60	0,53	0,46	0,35	0,51
	12,97	0,61	0,60	0,56	0,44	0,30	0,50
	13,39	0,61	0,61	0,54	0,47	0,34	0,51
Mean		0,60	0,59	0,53	0,46	0,33	0,50

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,006	0,02	0,02
Mean of 5 entries	0,006	0,02	0,02
Mean of 20 entries	0,013	0,04	0,05
	CV	4,37%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	2	0,0028	0,0014	2,8 NS
Lysine	4	0,5818	0,1455	291,0 **
Energy	3	0,0108	0,0036	7,2 **
Lysine x energy	12	0,0159	0,0013	2,6 *
Error	38	0,0182	0,0005	
Total	59	0,6296		

SEM, CV etc see footnote Table 6.4

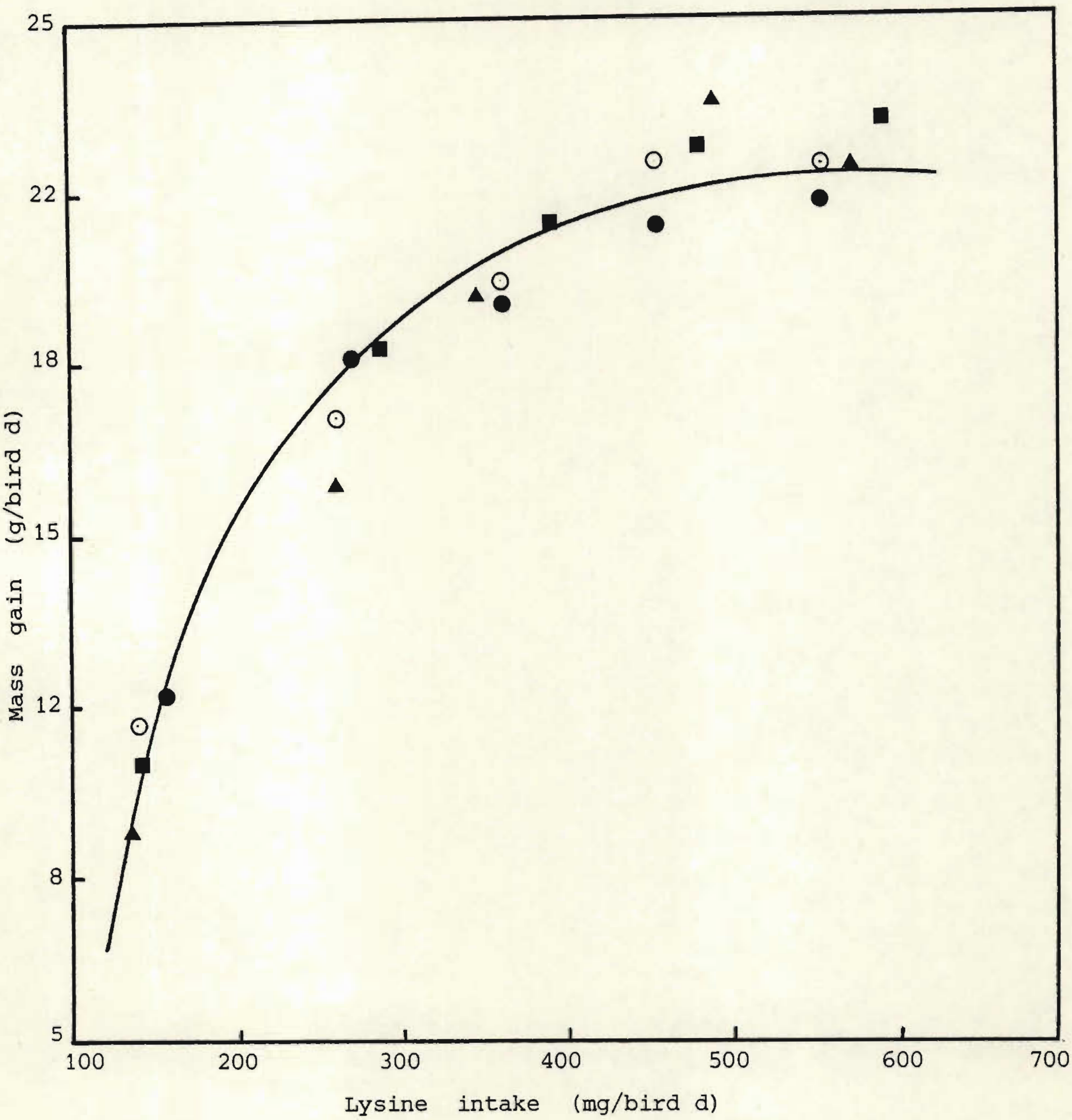

Figure 7.1 Response in mass gain (g/bird d) of female chickens from 7 - 21 days of age to lysine intake (mg/bird d) and estimated response using Reading model (Table 7.6)

● 12,13 MJ/kg ▲ 12,97 MJ/kg

○ 12,55 MJ/kg ■ 13,39 MJ/kg

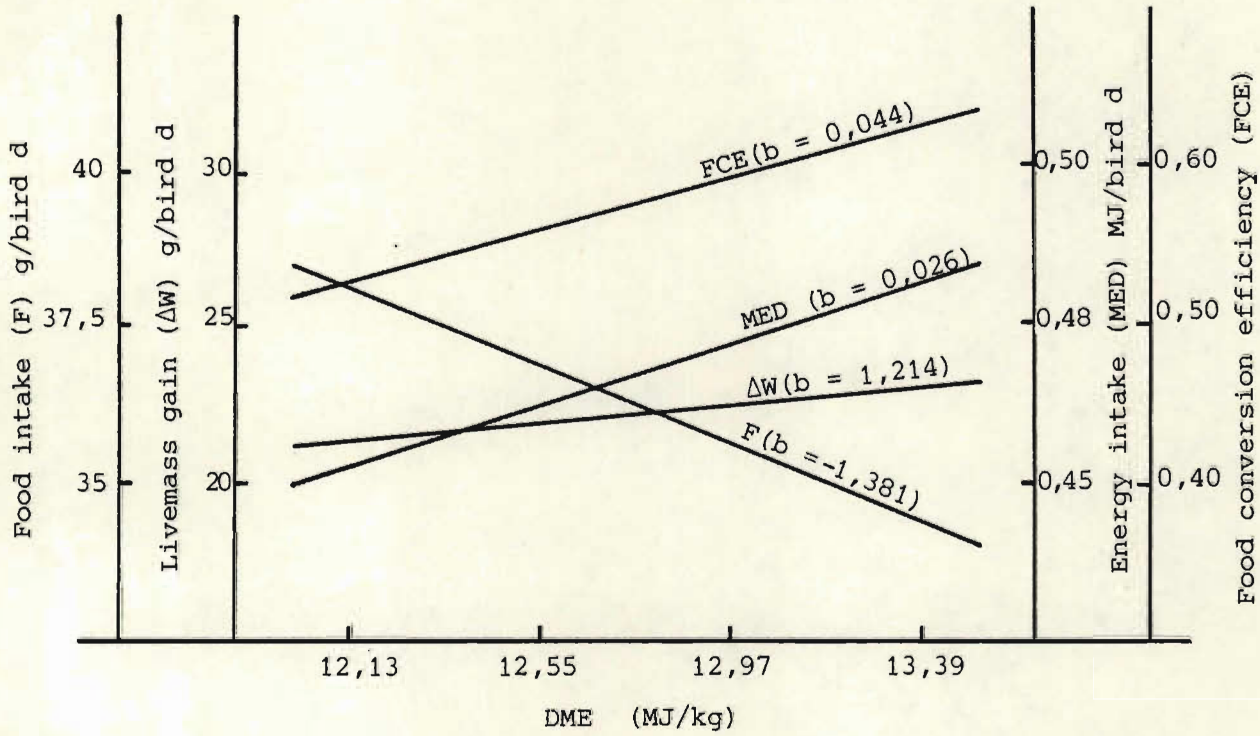

Figure 7.2 Pooled linear regressions of female broiler performance characteristics on dietary metabolisable energy level for the starter period

TABLE 7.7 Pooled linear regression coefficients of female broiler performance characteristics on dietary metabolisable energy concentration in the starter period (units are per MJ/kg)

Character regressed on DME	Constant term	Regression coefficient (b)	SE (b)
Livemass gain (ΔW)	6,581	1,214	0,517*
Food intake (F)	54,900	-1,381	1,285^{NS}
Energy intake (MED)	0,138	0,026	0,008**
Food conversion efficiency (FCE)	0,029	0,044	0,012**

^{NS}, * etc see footnote Table 6.4

TABLE 7.6 Parameters used in determining the relationship between mass gain (g/bird d) and lysine intake (mg/bird d) by means of the Reading model using female chickens from 7 - 21 days of age

Parameter	Energy concentration (MJ/kg)				
	12,13	12,55	12,97	13,39	Combined
$\bar{W}$	250,00	250,00	250,00	250,00	250,00
$\Delta\bar{W}$	20,75	21,92	22,79	22,36	22,10
a	11,23	13,48	16,63	14,19	13,73
b	0,022	0,004	0,004	0,005	0,012
$\sigma\Delta\bar{W}$	9,00	6,44	3,85	6,56	7,36
$\sigma\bar{W}$	40,00	40,00	40,00	40,00	40,00
r	0,60	0,60	0,60	0,60	0,60

TABLE 7.8 The regression of mass gain on lysine/energy ratio and optimum lysine/energy ratio for female chickens from 7 - 21 days of age

DME (MJ/kg)	Constant term	Linear term (b_1)	Quadratic term ($\times 10^{-2}$) (b_2)	SE (b)	Multiple correlation coefficient (R)	Optimum lysine/energy ratio (g/kg:MJ/kg)
12,13	1,67	0,369	-0,175	1,03^{NS}	0,978	1,056
12,55	-1,53	0,431	-0,195	0,52*	0,997	1,108
12,97	-6,88	0,535	-0,242	0,73*	0,996	1,106
13,39	-4,40	0,521	-0,249	0,90*	0,991	1,047
Combined	-2,78	0,464	-0,215	0,63*	0,995	1,079

^{NS}, * etc see footnote Table 6.4

TABLE 7.9 The regression of food conversion efficiency on lysine/energy ratio and optimum lysine/energy ratio for female broilers from 7 - 21 days of age

DME (MJ/kg)	Constant term	Linear term (b_1)	Quadratic term ($\times 10^{-4}$) (b_2)	SE (b)	Multiple correlation coefficient (R)	Optimum lysine/energy ratio (g/kg:MJ/kg)
12,13	0,10	0,008	-0,370	0,02*	0,988	1,114
12,55	0,10	0,008	-0,346	0,01*	0,995	1,219
12,97	-0,07	0,012	-0,564	0,01**	0,998	1,101
13,39	0,06	0,010	-0,408	0,01*	0,998	1,169
Combined	0,04	0,010	-0,420	0,01**	0,999	1,145

^{NS}, * etc see footnote Table 6.4

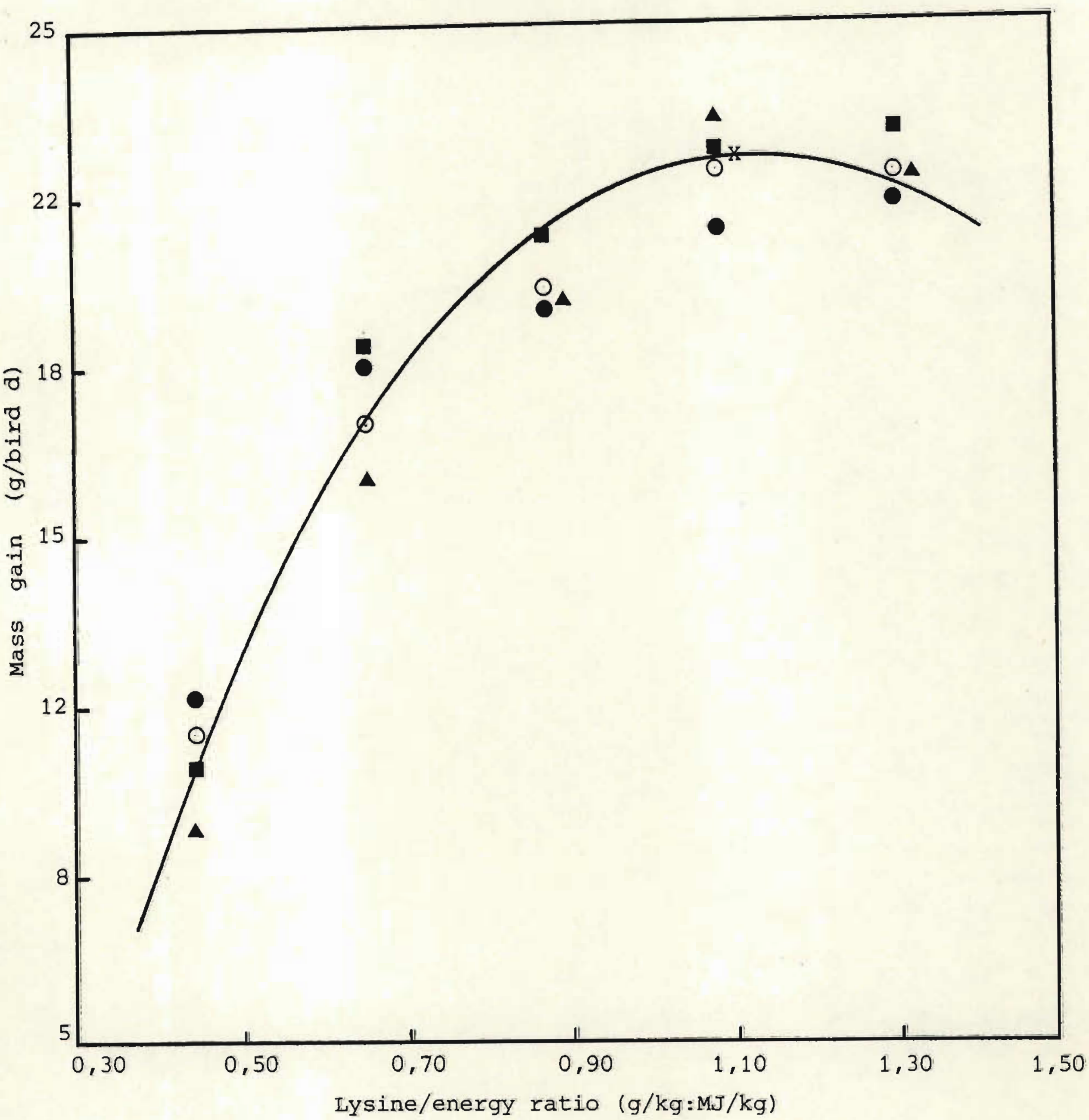

Figure 7.3 Response in mass gain (g/bird d) of female chickens from 7 - 21 days of age to lysine/energy ratio and estimated optimum lysine/energy ratio (X)

● 12,13 MJ/kg ▲ 12,97 MJ/kg

○ 12,55 MJ/kg ■ 13,39 MJ/kg

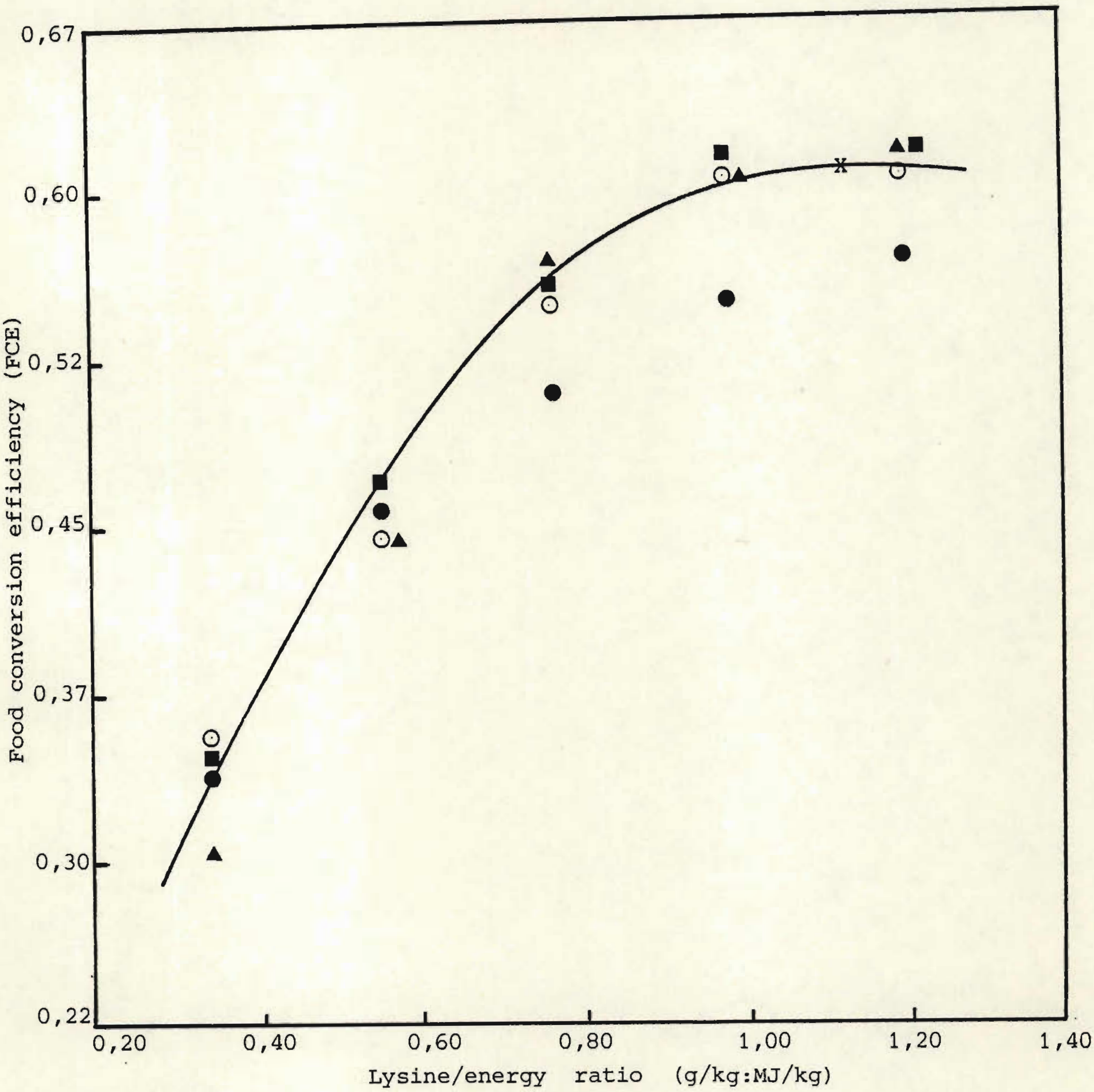

Figure 7.4 Response in food conversion efficiency of female chickens from 7 - 21 days of age to lysine/energy ratio and estimated optimum lysine/energy ratio (X)

● 12,13 MJ/kg ▲ 12,97 MJ/kg

○ 12,55 MJ/kg ■ 13,39 MJ/kg

Response to lysine/DME ratio

Curvilinear regressions of mass gain and FCE on lysine/DME ratio were calculated using a multiple regression analysis. The coefficients at each energy level and for the combined data over all energy concentrations are given in Tables 7.8 and 7.9 and illustrated in Figures 7.3 and 7.4 respectively.

DISCUSSION

The mojor objective of this experiment was to measure the response of growing chickens to lysine intake using the dilution technique and to determine the optimum lysine intake in commercial diets for female chickens during the starter period.

Response to dietary lysine concentration

The first-limiting essential amino acid and dietary metabolisable energy are the major dietary components which exert a profound influence on broiler performance. In practical broiler diets lysine is one of the limiting amino acids, therefore, it is essential that an accurate estimate of the requirement for lysine is made.

The Reading model was used to describe the relationship between mass gain and lysine intake. Equations fitted to the data for each energy concentration and to the combined data over all energy concentrations (Table 7.6) indicate that at marginal costs of 350 c/kg for lysine, and 150 c/kg for broiler mass, the optimum intake of lysine at each energy level for the females would be 374, 462, 582 and 495 mg/bird d. From the combined data (illustrated in Figure 7.1) the optimum intake was calculated to be 474 mg/bird d. This figure is somewhat lower than the optimum of 574 mg/bird d determined for males (Chapter 6).

A comparison between the sexes of the mass gains recorded in the first and second dilution series indicates that the mean gains were 23,4 and 22,4 g/d for the males (Table 6.4) and 22,1 and 22,1 g/d for the females (Table 7.4). However, in the fourth and fifth dilution series the mass gains were 13,3 and 5,4 g/bird d for the males and 17,2 and 10,6 g/bird d for the females respectively. Females have a greater potential to lay down body fat than do males and when the intake of a limiting amino acid is below the level required for maximum growth the composition of the body mass gain may be expected to be different i.e. higher in fat content in females than in males. This would explain the apparent greater net efficiency of amino acid utilisation in females than in males at low intakes of the limiting amino acid (a and b values for females were 13,73 and 0,012 whereas for males these values were 15,95 and 0,03 respectively). Because the experiments on males and females were not conducted concurrently further research on this subject would be needed to draw any more definite conclusions.

The effect of the lysine concentration in the diet on food intake was less pronounced in the females than males. The food intake in the male diets (Table 6.6) followed the classical pattern where the consumption increased in the second and third dilution series due to the marginal lysine deficiency in these diets, and then decreased as the deficiency became severe in the fourth and fifth dilution series. The food intake of the females remained similar in the first four dilution series and only decreased in the fifth dilution series. Nevertheless food intake was significantly affected by the lysine content of the diet (Table 7.3).

Response to dietary metabolisable energy concentration

The effect of DME on ΔW, F, MED and FCE was examined by means of linear regression analyses and the pooled results for the first and second dilution series are presented in Table 7.7 and Figure 7.2 respectively.

The livemass response to DME in the female broiler (Table 7.1) was non significant, whereas male birds responded significantly to DME. Food consumption recorded in Table 7.3 shows that females regulated their energy intake more accurately than did males during the starter period, since DME had a highly significant effect on energy intake in the females. Daily metabolisable energy intake increased with DME in both sexes, the slope being greater among males than among females (0,037 vs 0,026 MJ MED/ MJ DME).

Food conversion efficiency of both males and females improved as the DME was increased. Because males adjusted intake of DME less efficiently than females and hence showed a greater improvement in growth rate as DME was increased, FCE should be expected also to improve more rapidly in males than in females with increasing DME.

Response to lysine/DME ratio

Protein and energy are the most expensive items in a chick diet, and the delicate balance between these two components will determine broiler performance. A surplus of essential amino acids will be costly, whilst a deficiency, albeit marginal, will have a detrimental effect on performance. It is axiomatic, therefore, that there is an optimum balance between dietary energy and essential amino acids which will ensure optimum performance and maximum profit.

The results presented in Table 7.1 show that the maximum response in ΔW to lysine/DME ratio was recorded in the diets of the first and second dilution series. The response in ΔW decreased progressively as the lysine/DME ratio narrowed in the lower dilutions. For the combined data illustrated in Figure 7.3 the optimum ratio was 1,079 g lysine/MJ. This figure is slightly lower than the optimum ratio determined for males (1,125 g lysine/MJ) and supports the view that the sexes could economically be reared separately since the males require diets with greater amino acid concentrations per MJ for maximum performance. These optimum ratios are higher than that of 0,98 g lysine/MJ for the combined sexes recommended by Thomas *et al.* (1978).

The effect of varying the lysine/energy ratio on food conversion efficiency was examined by means of a multiple regression analysis, the results of which are presented in Table 7.9 and illustrated in Figure 7.4. The optimum lysine/energy ratio for the combined data was 1,145 g lysine/MJ, which is lower than that determined for males (1,310 g lysine/MJ). The optimum ratios determined for both males and females confirm the opinion that higher dietary concentrations of lysine are required for optimum FCE than for optimum body mass gain, but it is doubtful whether the higher dietary lysine concentrations would be justified economically.

Because the range of energy concentrations studied was rather narrow, and because the number of observations per energy level were relatively small, a more comprehensive discussion on the effects of dietary lysine and DME on broiler performance will be included in a later chapter (Chapter 8) where the responses recorded in all the experiments will be summarised and discussed in relation to their economic importance in broiler feed formulation.

CHAPTER 8

THE RESPONSE OF MALE AND FEMALE BROILERS TO DIETARY LYSINE AND METABOLISABLE ENERGY CONCENTRATIONS DURING THE PERIOD 28 TO 45 DAYS OF AGE

INTRODUCTION

The lysine requirement of the broiler chicken has been the subject of numerous studies, the majority of which were reviewed in Chapter 5. Because the broiler consumes more than 75 percent of its food during the finisher period, it is reasonable to expect that the finisher diet will have the greatest impact on broiler performance and cost of production. In this respect, changing the nutrient density of the diet has been shown to readily alter mass gain and carcass composition. Although there are many ways to effect this type of alteration, a reduction of protein, or the first-limiting amino acid, from the normal plane of nutrition is the most feasible. Any deviation from the optimum nutrient balance precipitates a progressively poorer performance, with a concomitant decrease in carcass protein and an increase in carcass fat content.

The traditional feeding programme for broilers consisted of feeding a starter diet for four weeks, followed by a finisher diet to slaughter at approximately eight weeks of age. The modern broiler industry has witnessed dramatic improvements in genetics and nutrition, and this has led to a more sophisticated feeding programme consisting of a starter diet (0 - 21 days), a finisher diet (22 - 42 days), and finally a withdrawal (or post-finisher) diet (43 - 49 days) which is fed during the week prior to slaughter. To achieve the optimum live performance it is essential to ensure that the nutrient density of the diet is tailored to the requirements of the bird at each stage of growth.

The major objective of this experiment was to measure the response of broiler chickens to dietary lysine and metabolisable energy concentrations during the period 28 to 45 days of age thereby allowing for the determination of the optimum intake of these nutrients during the finisher period.

MATERIALS AND METHODS

One-day-old chicks of the Ross broiler strain were allocated at random to 80 pens, such that 40 pens each contained 180 male chicks, and 40 pens contained 180 female chicks. The stocking density was 19,4 birds/m^2. The pens were in an environmentally-controlled broiler house of a commercial design and management procedures that conformed as closely as possible with commercial practice were adopted. Gas canopy brooders were used during the brooding period, and the photo-period was 23 hours per day. The chicks were reared on a commercial broiler starter diet to 28 days of age, at which time the numbers in each pen were equalised, and the initial mass of the birds in each pen determined.

A summit and a dilution diet, based on the principles of Pilbrow and Morris (1974) were formulated at a DME level of 12,55 MJ/kg with amino acid levels based on the recommendations of Thomas *et al.* (1978) for finishing broilers. The available lysine and methionine figures quoted were converted to total requirements based on an availability of 90 percent. The dilution diet was formulated at the same net energy level as the summit diets for reasons previously discussed (Chapter 6). The summit diet was calculated to contain 1,18 times, and the dilution diet 0,35 times the recommended concentration of lysine, and at least 1,75 and 0,70 times the recommended concentration of all other amino acids.

TABLE 8.1 Composition (g/kg) of the summit and dilution diets

	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet
Diet code	1	5	6	10	11	15	16	20
ME (MJ/kg)	12,55		12,97		13,39		13,81	
NE (MJ/kg)	9,45	9,45	9,88	9,88	10,31	10,31	10,73	10,73
Ingredients								
Yellow maize meal	563,5	759,6	517,2	814,8	470,5	869,5	439,7	840,4
Wheat bran		165,0		78,0				
Groundnut meal	120,0	24,0	120,0	56,0	120,0	64,0	120,0	68,0
Sunflower meal	61,0		79,0		97,0		100,0	
Soyabean unextracted	100,0		100,0		100,0	8,0	100,0	14,0
Fish meal	104,0		110,0		118,0		129,0	
Blood meal	10,0		10,0	42,0	10,0	1,0	10,0	
Bone meal	23,0	40,0	22,0		20,0	47,0	18,0	47,0
Limestone flour		2,0						
Salt	1,5	5,0	1,5	4,8	1,0	5,0	0,5	5,0
Sunflower oil	9,0		32,0		55,0	1,0	74,0	21,0
DL - Methionine	4,5	0,9	4,8	0,9	5,0	1,0	5,3	1,1
Vitamins and trace minerals *	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0
Anti-coccidial	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5

TABLE 8.1 continued

	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet
Diet code	1	5	6	10	11	15	16	20
ME (MJ/kg)	12,55		12,97		13,39		13,81	
NE (MJ/kg)	9,45	9,45	9,88	9,88	10,31	10,31	10,73	10,73
Calculated analysis								
Arginine	17,0	6,5	17,7	8,0	18,4	7,3	18,7	7,5
Histidine	5,8	2,2	6,0	4,2	6,2	2,5	6,3	2,5
Isoleucine	9,3	3,1	9,6	3,6	9,9	3,4	1,0	3,4
Leucine	20,0	10,5	20,2	15,4	20,5	11,6	20,8	11,5
Lysine	12,9	3,8	13,3	4,0	13,7	4,1	14,2	4,2
Methionine	8,9	2,9	9,3	3,1	9,7	3,1	10,1	3,2
Cystine	4,1	2,7	4,2	3,1	4,4	2,7	4,5	2,6
Phenylalanine	10,7	4,5	11,0	7,4	11,4	5,0	11,6	5,0
Tyrosine	8,0	4,1	8,0	5,3	8,1	4,6	8,3	4,6
Threonine	8,7	3,7	8,9	5,0	9,2	4,0	9,5	4,0
Tryptophan	2,9	1,2	3,0	1,5	3,1	1,2	3,2	1,2
Valine	11,6	4,8	11,9	7,7	12,4	5,0	12,7	5,0
Calcium	10,0	10,0	10,0	10,0	10,0	10,0	10,0	10,0
Phosphorus	8,2	8,2	8,2	8,2	8,2	8,2	8,2	8,2
Crude protein (gN x 6,25/kg)	255,2	111,0	262,1	113,9	269,2	115,1	275,6	116,8

* Supplies per kg of diet: Vit A 7 025 IU, Vit D_3 2 000 IU, Vit E 8,5 IU, Hetrazine 3 ppm, Thiamine hydrochloride 0,969 ppm, Riboflavin 8 ppm, Calcium pantothenate 7,837 ppm, Niacin 24, 42 ppm, Folic acid 0,95 ppm

Copper 125 ppm, Iron 40 ppm, Zinc 28 ppm, Iodine 2 ppm, Manganese 78 ppm, Selenium 0,1 ppm

TABLE 8.2 Calculated amino acid contents of the summit and dilution diets relative to the requirements of broilers during the finisher period

	Requirements according to Thomas *et al.* (1978) (g/kg)	Amino acid contents expressed as multiples of requirement	
		Summit diet	Dilution diet
Diet code		1	5
Arginine	11,32	1,50	0,57
Histidine	4,09	1,42	0,54
Isoleucine	7,77	1,20	0,40
Leucine	14,32	1,40	0,73
Lysine	10,91	1,18	0,35
Methionine	5,00	1,78	0,58
Cystine	3,41	1,20	0,79
Phenylalanine	7,23	1,48	0,62
Tyrosine	6,14	1,30	0,67
Threonine	7,23	1,20	0,51
Tryptophan	1,64	1,77	0,73
Valine	8,73	1,33	0,55
Diet code		6	10
Arginine	11,69	1,51	0,68
Histidine	4,23	1,42	0,99
Isoleucine	8,03	1,20	0,45
Leucine	14,79	1,37	1,04
Lysine	11,27	1,18	0,35
Methionine	5,17	1,80	0,60
Cystine	3,52	1,19	0,88
Phenylalanine	7,47	1,47	0,99
Tyrosine	6,34	1,26	0,84
Threonine	7,47	1,19	0,67
Tryptophan	1,69	1,78	0,89
Valine	9,02	1,32	0,85

TABLE 8.2 continued

	Requirements according to Thomas *et al.* (1978) (g/kg)	Amino acid contents expressed as multiples of requirement	
		Summit diet	Dilution diet
Diet code		11	15
Arginine	12,08	1,52	0,60
Histidine	4,37	1,42	0,57
Isoleucine	8,29	1,20	0,41
Leucine	15,28	1,34	0,76
Lysine	11,64	1,18	0,35
Methionine	5,34	1,82	0,58
Cystine	3,64	1,21	0,74
Phenylalanine	7,71	1,48	0,65
Tyrosine	6,55	1,24	0,70
Threonine	7,71	1,19	0,52
Tryptophan	1,75	1,77	0,69
Valine	9,31	1,33	0,54
Diet code		16	20
Arginine	12,45	1,50	0,60
Histidine	4,50	1,40	0,56
Isoleucine	8,55	1,20	0,40
Leucine	15,75	1,32	0,73
Lysine	12,00	1,18	0,35
Methionine	5,50	1,84	0,58
Cystine	3,75	1,21	0,69
Phenylalanine	7,95	1,46	0,63
Tyrosine	6,75	1,23	0,68
Threonine	7,95	1,20	0,50
Tryptophan	1,80	1,78	0,67
Valine	9,60	1,32	0,52

TABLE 8.3 Summary of dilution technique and calculated analysis of the experimental diets

Diet code	Blending ratio			Calculated dietary lysine (g/kg)	Calculated dietary protein (g/kg)
	Summit diet		Dilution diet		
12,55 MJ/kg					
1	100	+	0	12,89	255,0
2	75	+	25	10,62	219,0
3	50	+	50	8,36	183,0
4	25	+	75	6,09	147,0
5	0	+	100	3,82	111,0
12,97 MJ/kg					
6	100	+	0	13,31	262,0
7	75	+	25	10,97	225,0
8	50	+	50	8,63	188,0
9	25	+	75	6,29	151,0
10	0	+	100	3,95	114,0
13,39 MJ/kg					
11	100	+	0	13,74	269,0
12	75	+	25	11,33	231,0
13	50	+	50	8,92	192,0
14	25	+	75	6,51	154,0
15	0	+	100	4,10	115,0
13,81 MJ/kg					
16	100	+	0	14,17	276,0
17	75	+	25	11,68	236,0
18	50	+	50	9,19	197,0
19	25	+	75	6,69	157,0
20	0	+	100	4,20	117,0

Intermediate lysine contents were obtained by blending these diets in appropriate proportions as shown in Table 8.3. The above formulation procedure was repeated at DME levels of 12,97; 13,39 and 13,81 MJ/kg as shown in Table 8.2. The composition and analysis of the summit and dilution diets is presented in Table 8.1 and their blended intermediate diets are shown in Table 8.3.

The ideal method of manufacturing the experimental diets when using the dilution technique is to prepare the total summit and dilution requirements, and then to blend the intermediate diets proportionately. Unfortunately, because of the large volume of food required (40 ton) this procedure could not be adopted in the manufacture of the experimental diets. The 20 diets were mixed individually with great care being taken to minimise any raw ingredient variation by blending the total requirements of each of the major ingredients prior to compounding the experimental diets.

The 20 experimental diets were allocated such that there were two pens of each sex receiving each diet. Pelleted food in tube feeders and water were provided *ad lib*. Body mass of the birds was measured at 28 and 45 days of age, and food consumption was recorded during this period. At 45 days of age two birds which were representative of the pen mean were drawn from each replicate for carcass analysis. The birds from each pen were minced thoroughly and homogenous five gram samples were taken and freeze-dried to determine the body moisture content. These dried samples were then used to determine the carcass protein content, using the Technicon auto analyser, and the carcass fat content using a Soxhlett fat extraction apparatus after 16 hours of extraction using petroleum ether.

The data on each variate were subjected to statistical analysis using the method of Rayner (1967). Means, standard errors of the means (SEM) and least significant differences (LSD) at $P < 0,05$

and $P < 0,01$ were calculated.

RESULTS

Means of the twenty treatments for the variates mass gain, lysine intake, food intake, dietary metabolisable energy intake and food conversion efficiency for the females, males and combined sexes are given in Tables 8.4 to 8.8 respectively. The main effects of DME and the lysine/DME ratio are also shown, together with standard errors of the means; L.S.D.'s; and analysis of variance table for each variate.

Response to dietary lysine concentration

The Reading model, described in Chapter 1, was used to fit an equation to the data relating body mass gain to lysine intake. Equations for the females, males and combined sexes were fitted to data from each energy level, as well as to the combined data. The parameters used in fitting these equations, and the resulting coefficients are given in Table 8.9. The combined equations, together with the means for each energy level, are illustrated in Figures 8.1, 8.2 and 8.3 respectively. The methods employed to calculate the parameters used in fitting the model and to obtain values for $\Delta\bar{W}$, a and b are described in Chapter 6.

Response to dietary energy concentration

Data used to measure the response to DME consisted of the results of the first and second dilution series only. There were thus 24 values that were pooled for each of the variates ΔW, F, MED and FCE. Linear responses to DME were calculated separately for each sex and for both sexes combined, the equations being presented in

TABLE 8.4 Mass gain (g/bird d) of male and female broilers from 28 - 45 days of age

Mass Gain

	ME	Lysine/DME Ratio					Mean
	MJ/kg	1,03	0,85	0,67	0,49	0,30	
Females	12,55	40,6	39,7	38,3	35,8	20,1	34,9
	12,97	45,3	40,5	40,5	38,0	17,2	36,3
	13,39	41,8	41,8	42,2	37,2	27,7	38,2
	13,81	42,6	41,9	42,3	36,6	28,6	38,4
Mean		42,6	41,0	40,8	36,9	23,4	37,0
Males	12,55	50,3	47,0	46,3	31,5	23,4	39,7
	12,97	46,7	47,8	44,1	45,4	24,9	41,8
	13,39	50,3	52,0	50,5	43,6	28,1	44,9
	13,81	52,7	52,9	50,6	45,1	27,2	45,7
Mean		50,0	49,9	47,9	41,4	25,9	43,0
Mean of Females and Males Combined	12,55	45,4	43,3	42,3	33,7	21,7	37,3
	12,97	46,0	44,2	42,3	41,7	21,0	39,0
	13,39	46,0	46,9	46,4	40,4	27,9	41,5
	13,81	47,7	47,4	46,4	40,8	27,9	42,0
		46,3	45,5	44,3	39,2	24,6	40,0

TABLE 8.4 continued

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 2 entries	0,473	1,35	1,81
Mean of 4 entries	0,669	1,91	2,56
Mean of 5 entries	0,747	2,14	2,86
Mean of 20 entries	1,495	4,28	5,75
	CV	7,48%	

Analysis of variance

Source	DF	SS	MS	F
Replications	1	1,46	1,46	0,16 NS
Lysine	4	5199,00	1299,77	145,38 **
Energy	3	296,22	98,74	11,04 **
Sex	1	739,80	739,80	82,75 **
Lysine x energy	12	166,10	13,84	1,54 NS
Lysine x sex	4	105,20	26,29	2,94 *
Energy x sex	3	19,90	6,66	0,74 NS
Lysine x energy x sex	12	209,20	17,43	1,95 NS
Error	39	348,60	8,94	
Total	79	7085,18		

SEM, CV etc see footnote Table 6.4

TABLE 8.5 Lysine intake (mg/bird d) for male and female broilers from 28 - 45 days of age

Lysine Intake

	ME	Lysine/DME Ratio					Mean
	MJ/kg	1,03	0,85	0,67	0,49	0,30	
Females	12,55	1328	1073	865	642	381	858
	12,97	1415	1091	888	647	409	890
	13,39	1363	1140	904	668	397	895
	13,81	1414	1145	920	665	411	911
Mean		1380	1112	894	656	400	888
Males	12,55	1421	1171	938	653	395	916
	12,97	1419	1182	932	674	420	925
	13,39	1532	1236	954	685	419	965
	13,81	1462	1252	961	724	431	966
Mean		1459	1210	946	684	416	943
Mean of	12,55	1375	1122	901	648	388	887
Females	12,97	1417	1136	910	660	414	908
and Males	13,39	1447	1188	929	676	408	930
Combined	13,81	1438	1199	940	694	421	938
Mean		1419	1161	920	670	408	916

TABLE 8.5 continued

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 2 entries	4,972	14,22	19,05
Mean of 4 entries	7,032	20,12	26,94
Mean of 5 entries	7,861	22,49	30,12
Mean of 20 entries	15,723	44,98	60,24
	CV	3,43%	

Analysis of variance

Source	DF	SS	MS	F
Replications	1	936,19	936,19	0,95 NS
Lysine	4	10120472,00	2530118,06	2558,65 **
Energy	3	32454,99	10818,33	10,94 **
Sex	1	59803,19	59803,19	60,48 **
Lysine x energy	12	8410,39	700,87	0,71 NS
Lysine x sex	4	18569,94	4642,49	4,69 **
Energy x sex	3	3147,73	1049,24	1,06 NS
Lysine x energy x sex	12	13849,02	1154,08	1,17 NS
Error	39	38565,00	988,85	
Total	79	10296208,00		

SEM, CV etc see footnote Table 6.4

TABLE 8.6 Food intake (g/bird d) for male and female broilers from 28 - 45 days of age

Food Intake

	ME	Lysine/DME Ratio					Mean
	MJ/kg	1,03	0,85	0,67	0,49	0,30	
Females	12,55	103	101	103	105	100	103
	12,97	106	99	103	103	104	103
	13,39	99	101	101	103	98	100
	13,81	100	98	100	99	98	99
Mean		102	100	102	103	100	101
Males	12,55	110	110	112	107	103	109
	12,97	107	108	108	107	107	107
	13,39	111	109	107	105	103	107
	13,81	103	107	105	108	103	105
Mean		108	109	108	107	104	107
Mean of Females and Males Combined	12,55	107	106	108	106	102	106
	12,97	106	104	105	105	105	105
	13,39	105	105	104	104	100	104
	13,81	101	103	102	104	100	102
Mean		105	104	105	105	102	104

TABLE 8.6 continued

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 2 entries	0,466	1,33	1,79
Mean of 4 entries	0,659	1,89	2,52
Mean of 5 entries	0,737	2,11	2,83
Mean of 20 entries	1,473	4,21	5,64
	CV	2,80%	

Analysis of variance

Source	DF	SS	MS	F
Replications	1	10,76	10,76	1,24 NS
Lysine	4	119,78	29,94	3,45 *
Energy	3	150,55	50,18	5,79 **
Sex	1	672,67	672,67	77,55 **
Lysine x energy	12	86,36	7,20	0,83 NS
Lysine x sex	4	56,70	14,18	1,63 NS
Energy x sex	3	19,68	6,56	0,76 NS
Lysine x energy x sex	12	104,13	8,68	1,00 NS
Error	39	338,31	8,68	
Total	79	1548,19		

SEM, CV etc see footnote Table 6.4

TABLE 8.7 Dietary metabolisable energy intake (MJ/bird d) for male and female broilers from 28 - 45 days of age

MED Intake

	ME MJ/kg	Lysine/DME Ratio 1,03	0,85	0,67	0,49	0,30	Mean
Females	12,55	1,29	1,26	1,29	1,32	1,25	1,28
	12,97	1,37	1,28	1,33	1,33	1,34	1,33
	13,39	1,32	1,34	1,35	1,37	1,30	1,34
	13,81	1,38	1,35	1,38	1,37	1,35	1,37
Mean		1,34	1,31	1,34	1,35	1,31	1,33
Males	12,55	1,38	1,38	1,40	1,34	1,29	1,36
	12,97	1,38	1,39	1,39	1,38	1,38	1,38
	13,39	1,48	1,45	1,42	1,40	1,37	1,43
	13,81	1,43	1,48	1,44	1,49	1,42	1,45
Mean		1,42	1,43	1,42	1,41	1,36	1,40
Mean of Females and Males Combined	12,55	1,33	1,32	1,35	1,33	1,27	1,32
	12,97	1,37	1,34	1,36	1,36	1,36	1,36
	13,39	1,40	1,40	1,39	1,39	1,33	1,38
	13,81	1,40	1,42	1,41	1,43	1,38	1,41
Mean		1,38	1,37	1,38	1,38	1,34	1,37

TABLE 8.7 continued

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 2 entries	0,006	0,02	0,02
Mean of 4 entries	0,009	0,03	0,03
Mean of 5 entries	0,010	0,03	0,04
Mean of 20 entries	0,019	0,05	0,07
	CV	2,82%	

Analysis of variance

Source	DF	SS	MS	F
Replications	1	0,002	0,002	1,345^{NS}
Lysine	4	0,020	0,005	3,362*
Energy	3	0,086	0,029	19,502**
Sex	1	0,117	0,117	78,682**
Lysine x energy	12	0,014	0,001	0,672^{NS}
Lysine x sex	4	0,009	0,002	1,345^{NS}
Energy x sex	3	0,004	0,001	0,672^{NS}
Lysine x energy x sex	12	0,018	0,002	1,345^{NS}
Error	39	0,058	0,002	
Total	79	0,329		

SEM, CV etc see footnote Table 6.4

TABLE 8.8 Food conversion efficiency for male and female broilers from 28 - 45 days of age

F C E

	ME MJ/kg	Lysine/DME Ratio 1,03	0,85	0,67	0,49	0,30	Mean
Females	12,55	0,39	0,39	0,37	0,34	0,20	0,34
	12,97	0,43	0,41	0,39	0,37	0,17	0,35
	13,39	0,42	0,42	0,42	0,36	0,28	0,38
	13,81	0,43	0,43	0,42	0,37	0,29	0,39
Mean		0,42	0,41	0,40	0,36	0,24	0,36
Males	12,55	0,46	0,43	0,41	0,29	0,23	0,36
	12,97	0,44	0,44	0,41	0,42	0,23	0,39
	13,39	0,45	0,48	0,47	0,41	0,27	0,42
	13,81	0,51	0,50	0,48	0,42	0,26	0,43
Mean		0,46	0,46	0,44	0,39	0,25	0,40
Mean of	12,55	0,42	0,41	0,39	0,32	0,21	0,35
Females	12,97	0,43	0,43	0,40	0,40	0,20	0,37
and Males	13,39	0,44	0,45	0,44	0,39	0,28	0,40
Combined	13,81	0,47	0,46	0,45	0,39	0,28	0,41
Mean		0,44	0,44	0,42	0,37	0,24	0,38

TABLE 8.8 continued

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 2 entries	0,004	0,01	0,02
Mean of 4 entries	0,006	0,02	0,02
Mean of 5 entries	0,007	0,02	0,03
Mean of 20 entries	0,013	0,04	0,05
	CV	6,91%	

Analysis of variance

Source	DF	SS	MS	F
Replications	1	9,58	9,58	0,25 NS
Lysine	4	2040,20	510,05	13,03 **
Energy	3	303,42	101,14	2,59 **
Sex	1	2468,53	2468,53	63,08 **
Lysine x energy	12	308,34	25,70	0,66 NS
Lysine x sex	4	13,72	3,43	0,09 NS
Energy x sex	3	49,34	16,45	0,42 NS
Lysine x energy x sex	12	639,15	53,26	1,36 NS
Error	39	1526,12	39,13	
Total	79	7358,41		

SEM, CV etc see footnote Table 6.4

TABLE 8.9 Parameters used in determining the relationship between mass gain (g/bird d) and lysine intake (mg/bird d) by means of the Reading model using male and female chickens from 28 - 45 days of age

Parameter	Energy concentration (MJ/kg)				
	12,55	12,97	13,39	13,81	Combined
Females					
$\bar{W}$	800,00	800,00	800,00	800,00	800,00
$\Delta\bar{W}$	40,73	44,68	42,02	42,67	42,80
a	12,05	14,52	11,40	11,50	13,10
b	0,11	0,08	0,04	0,01	0,05
$\sigma\Delta\bar{W}$	20,20	19,65	20,90	26,62	21,30
$\sigma\bar{W}$	80,00	80,00	80,00	80,00	80,00
r	0,60	0,60	0,60	0,60	0,60
Males					
$\bar{W}$	800,00	800,00	800,00	800,00	800,00
$\Delta\bar{W}$	49,38	47,99	52,30	53,28	50,50
a	16,54	11,17	11,67	12,18	13,25
b	0,02	0,09	0,05	0,07	0,05
$\sigma\Delta\bar{W}$	23,00	23,90	26,00	23,00	19,66
$\sigma\bar{W}$	80,00	80,00	80,00	80,00	80,00
r	0,60	0,60	0,60	0,60	0,60
Males and females					
$\bar{W}$	800,00	800,00	800,00	800,00	800,00
$\Delta\bar{W}$	45,34	46,21	47,37	47,70	46,25
a	14,88	13,26	12,15	12,79	12,27
b	0,04	0,06	0,02	0,02	0,09
$\sigma\Delta\bar{W}$	23,00	23,00	23,45	23,00	23,15
$\sigma\bar{W}$	80,00	80,00	80,00	80,00	80,00
r	0,60	0,60	0,60	0,60	0,60

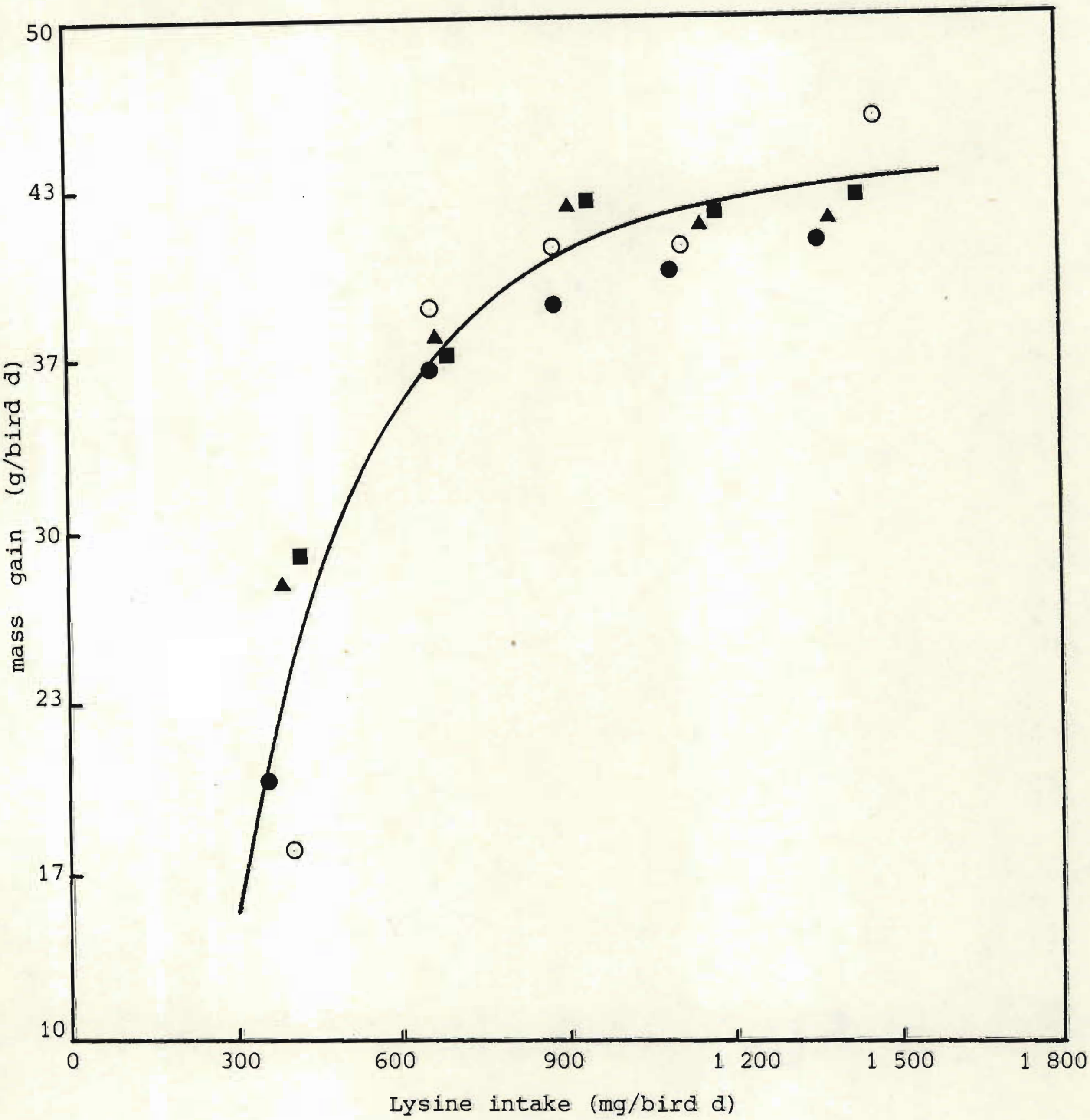

Figure 8.1 Response in mass gain (g/bird d) of female chickens from 28 - 45 days of age to lysine intake (mg/bird d) and estimated response using Reading model (Table 8.9)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

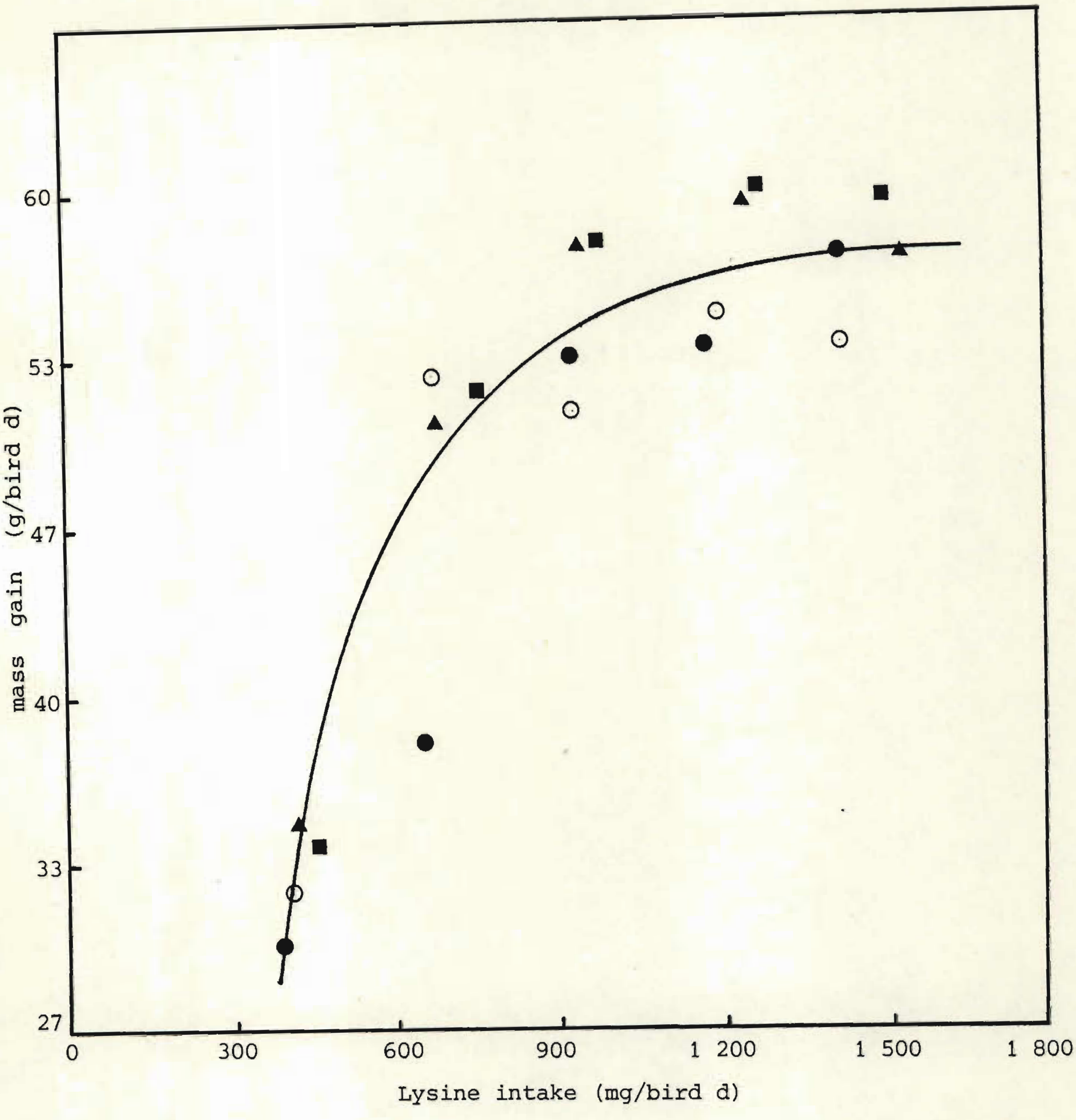

Figure 8.2 Response in mass gain (g/bird d) of male chickens from 28 - 45 days of age to lysine intake (mg/bird d) and estimated response using Reading model (Table 8.9)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

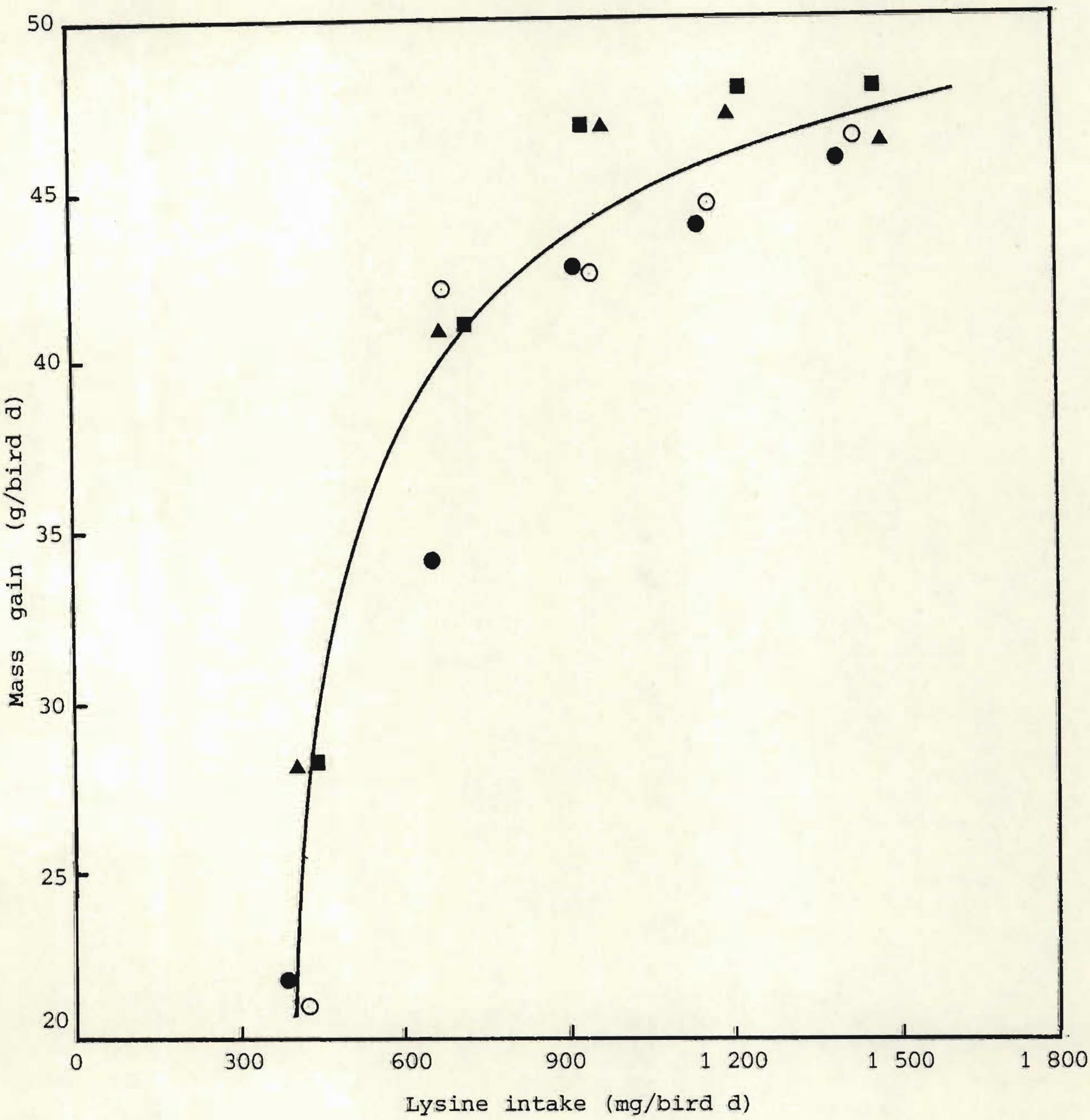

Figure 8.3 Response in mass gain (g/bird d) of male and female chickens from 28 - 45 days of age to lysine intake (mg/bird d) and estimated response using Reading model (Table 8.9)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

TABLE 8.10 Pooled responses of performance characteristics on dietary metabolisable energy concentration for males, females and for the sexes combined in the finisher period (units are per MJ/kg)

Character regressed on DME	Constant term	Regression coefficient (b)	SE (b)
Livemass gain (ΔW,g/bird d)			
males	-1,345	3,893	1,592*
females	25,457	1,238	1,592^{NS}
combined	11,500	2,607	5,033^{NS}
Food intake (F,g/bird d)			
males	147,350	-2,976	3,136^{NS}
females	136,960	-2,738	1,568^{NS}
combined	146,989	-3,214	1,568*
Energy intake (MED,MJ/bird d)			
males	0,464	0,073	0,021**
females	0,630	0,054	0,017**
combined	0,422	0,072	0,021**
Food conversion efficiency (FCE,ΔW,g/F,g)			
males	-0,180	0,049	0,046^{NS}
females	0,038	0,029	0,046^{NS}
combined	-0,079	0,039	0,045^{NS}

^{NS}, * etc see footnote Table 6.4

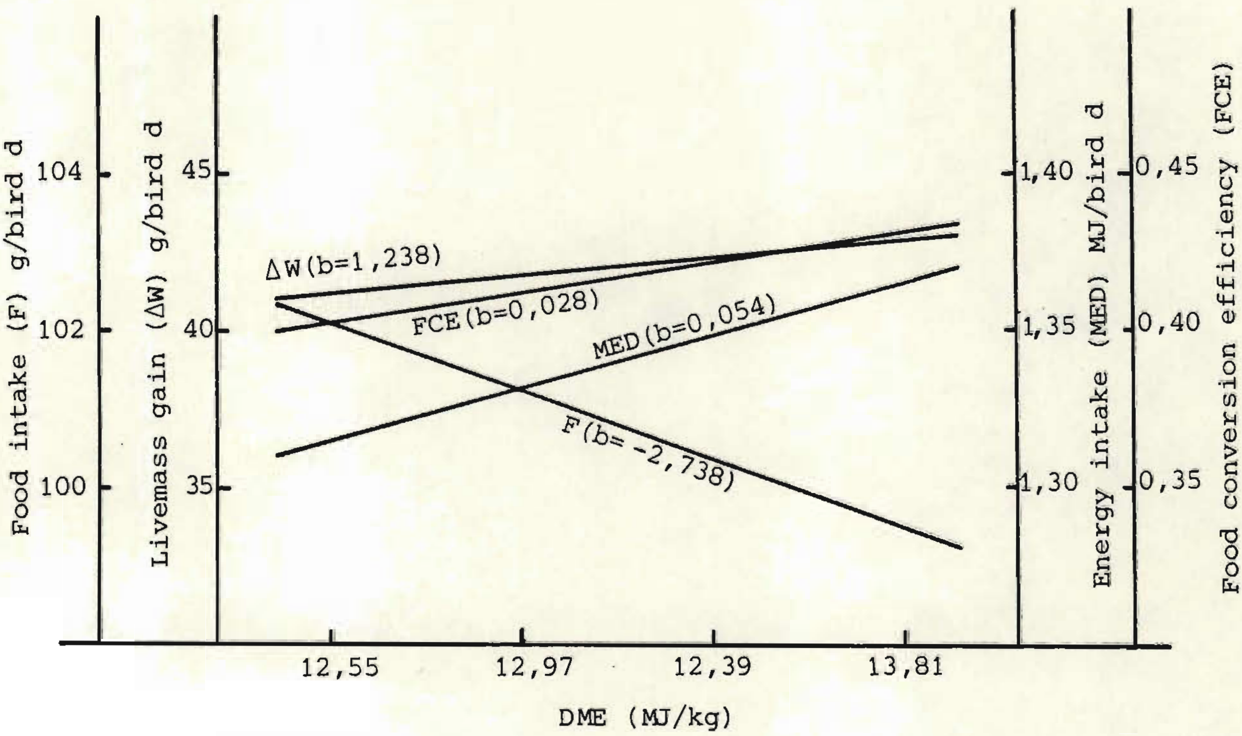

Figure 8.4 Pooled linear regressions of female broiler performance characteristics on dietary metabolisable energy level for the finisher period

Table 8.10 and illustrated in Figures 8.4, 8.5 and 8.6 respectively.

Response to lysine/DME ratio

Curvilinear regressions of mass gain and FCE on lysine/DME ratio were calculated using a multiple regression analysis. Male and female data were analysed separately and combined, the results of these analyses being given in Tables 8.11 and 8.12 and illustrated in Figures 8.7 to 8.12.

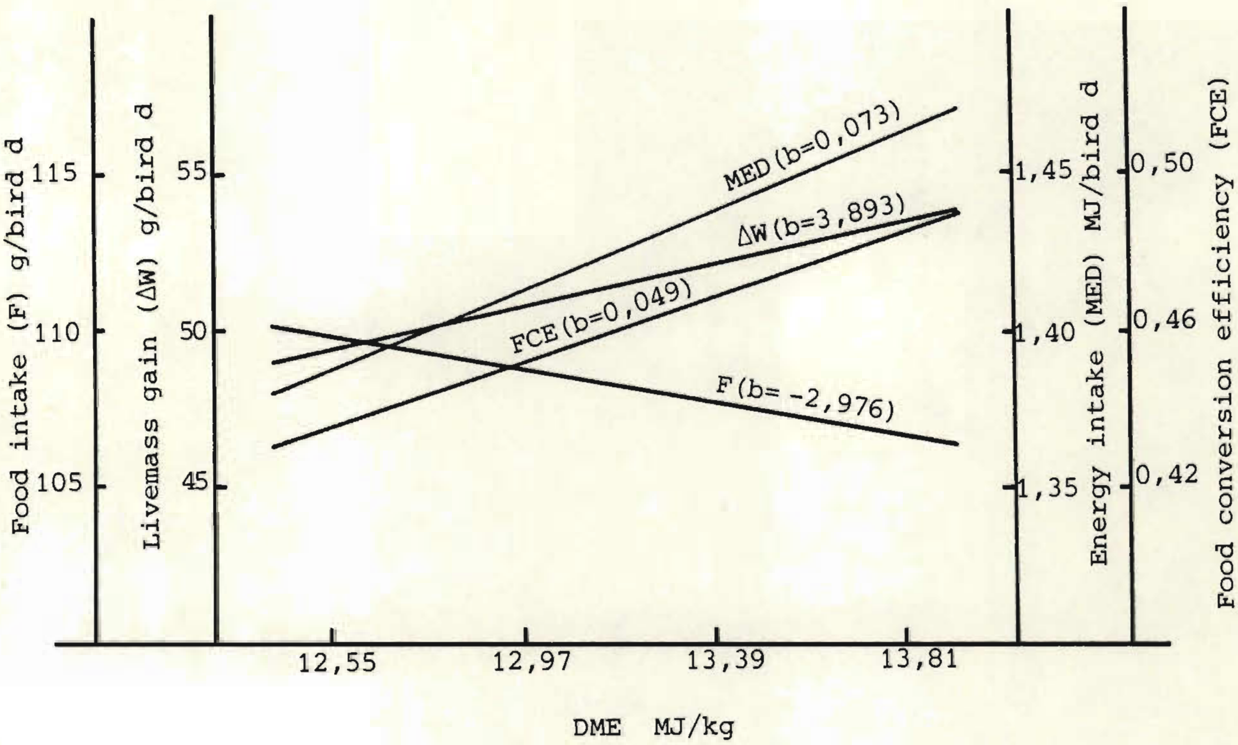

Figure 8.5 Pooled linear regressions of male broiler performance characteristics on dietary metabolisable energy level for the finisher period

Response in carcass composition

Protein and fat content of carcasses, representing the composition of birds on each dietary treatment, were used to calculate the gain in protein and fat of birds during the experimental period. These values are given in Table 8.13. The Reading model was used to describe protein gain in terms of lysine intake, the parameters used in fitting the model being given in Table 8.14 and illustrated in Figure 8.13.

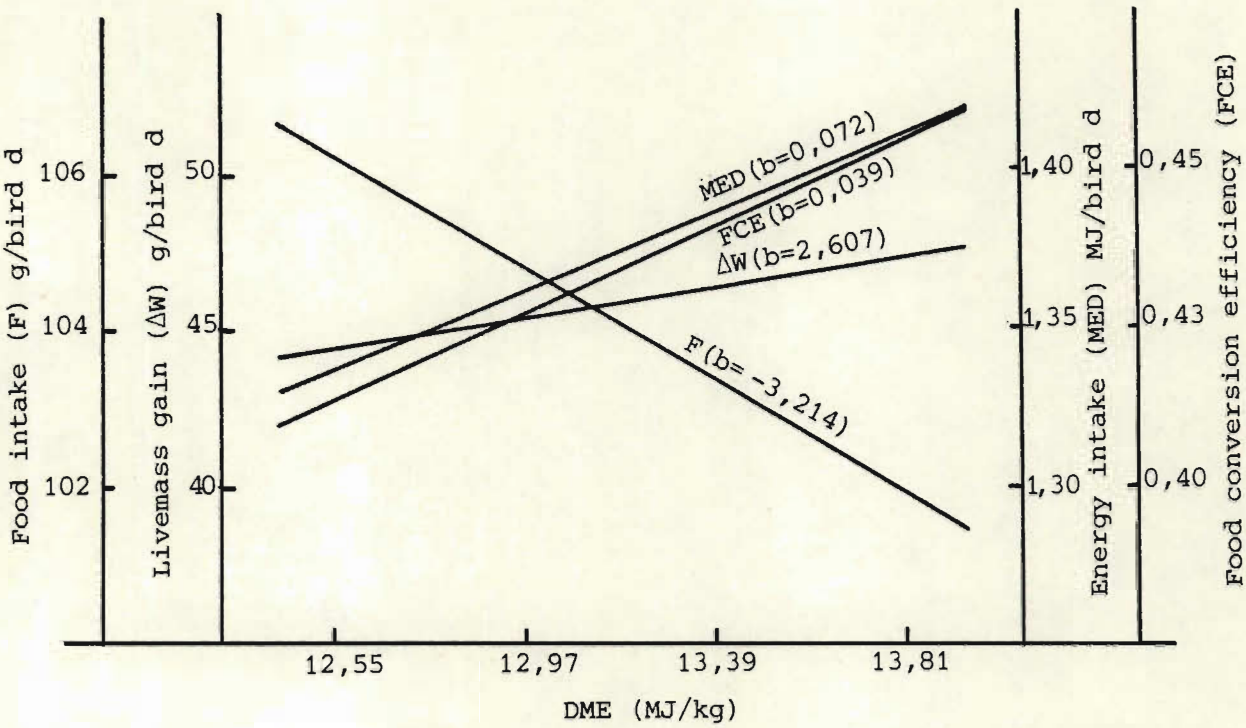

Figure 8.6 Pooled linear regressions of male and female broiler performance characteristics on dietary metabolisable energy level for the finisher period

DISCUSSION

The major objectives of this study were, first to measure the response of male and female chickens to increasing lysine concentrations, using the dilution technique; second to determine the optimum lysine/energy ratio in broiler finisher diets.

Response to dietary lysine concentration

Lysine, which is one of the limiting amino acids in chick diets, had a significant influence on broiler performance as evident from

TABLE 8.11 The regression of mass gain (g/bird d) on lysine/energy ratio (g/kg:MJ/kg) and optimum lysine/energy ratio for male and female chickens from 28 - 45 days of age

DME (MJ/kg)	Constant term	Linear term (b_1)	Quadratic term ($\times 10^{-2}$) (b_2)	SE (b)	Multiple correlation coefficient (R)	Optimum lysine/energy ratio (g/kg:MJ/kg)
Females						
12,55	- 6,72	1,131	-0,661	3,21^{NS}	0,969	0,854
12,97	-13,31	1,307	-0,734	5,53^{NS}	0,944	0,889
13,39	6,15	0,882	-0,525	1,32*	0,990	0,839
13,81	9,18	0,780	-0,447	1,24*	0,991	0,874
Combined	- 1,17	1,025	-0,592	2,61^{NS}	0,976	0,866
Males						
12.55	- 5,75	1,085	-0,524	3,45^{NS}	0,981	1,035
12,97	- 6,39	1,354	-0,825	5,29^{NS}	0,932	0,820
13,39	- 7,21	1,442	-0,863	1,30**	0,996	0,835
13,81	- 8,32	1,464	-0,854	2,47*	0,989	0,857
Combined	- 6,92	1,336	-0,767	1,64*	0,994	0,871
Males and females						
12,55	- 6,37	1,112	-0,597	1,40*	0,996	0,932
12,97	-10,03	1,336	-0,783	5,23^{NS}	0,942	0,852
13,39	- 0,65	1,167	-0,698	1,25*	0,995	0,836
13,81	0,56	1,116	-0,645	1,54*	0,993	0,863
Combined	- 4,12	1,183	-0,681	2,12*	0,988	0,869

^{NS}, * etc see footnote Table 6.4

TABLE 8.12 The regression of food conversion efficiency (ΔW,g/F,g) on lysine/energy ratio (g/kg:MJ/kg) and optimum lysine/energy ratio for male and female chickens from 28 - 45 days of age

DME (MJ/kg)	Constant term	Linear term (b_1)	Quadratic term ($\times 10^{-2}$) (b_2)	SE (b)	Multiple correlation coefficient (R)	Optimum lysine/energy ratio (g/kg:MJ/kg)
Females						
12,55	-5,48	0,011	-0,614	0,02*	0,982	0,865
12,97	-0,16	0,014	-0,809	0,05[NS]	0,958	0,858
13,39	9,35	0,008	-0,431	0,01*	0,994	0,881
13,81	0,10	0,008	-0,428	0,01**	0,997	0,879
Combined	0,01	0,010	-0,571	0,02*	0,986	0,868
Males						
12,55	-0,01	0,009	-0,401	0,03[NS]	0,986	1,078
12,97	-0,04	0,012	-0,731	0,05[NS]	0,924	0,828
13,39	-0,05	0,013	-0,834	0,01*	0,994	0,804
13,81	-0,05	0,013	-0,707	0,02*	0,990	0,891
Combined	-0,04	0,012	-0,668	0,02*	0,993	0,875
Males and females						
12,55	-0,03	0,010	-0,507	0,01**	0,998	0,949
12,97	-0,10	0,013	-0,777	0,05[NS]	0,948	0,842
13,39	0,06	0,009	-0,501	0,02*	0,986	0,874
13,81	0,03	0,010	-0,563	0,01*	0,994	0,887
Combined	-0,01	0,010	-0,587	0,02*	0,989	0,883

[NS], * etc see footnote Table 6.4

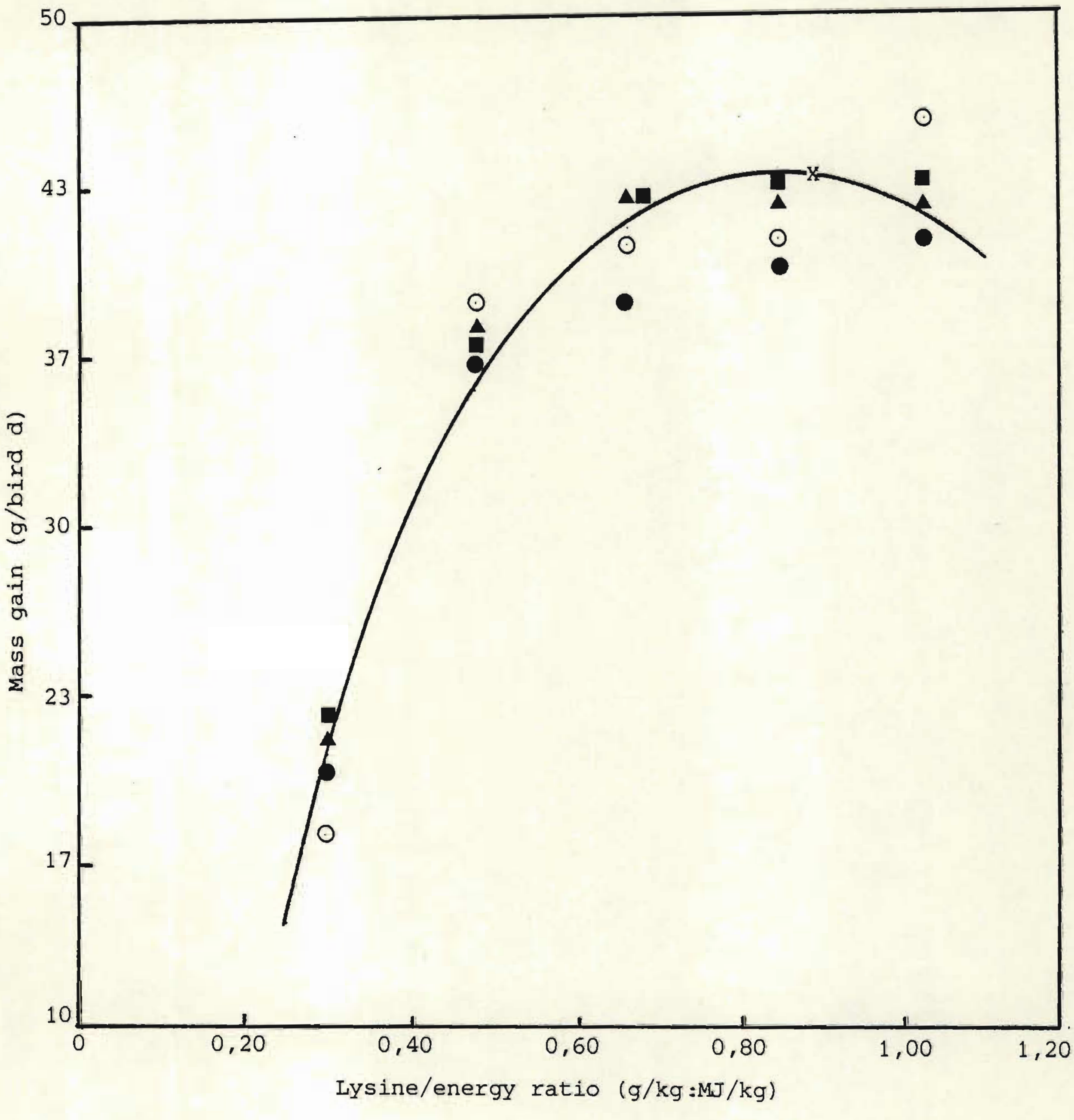

Figure 8.7 Response in mass gain (g/bird d) of female chickens from 28 - 45 days of age to lysine/energy ratio (g/kg:MJ/kg) and estimated optimum lysine/energy ratio (X)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

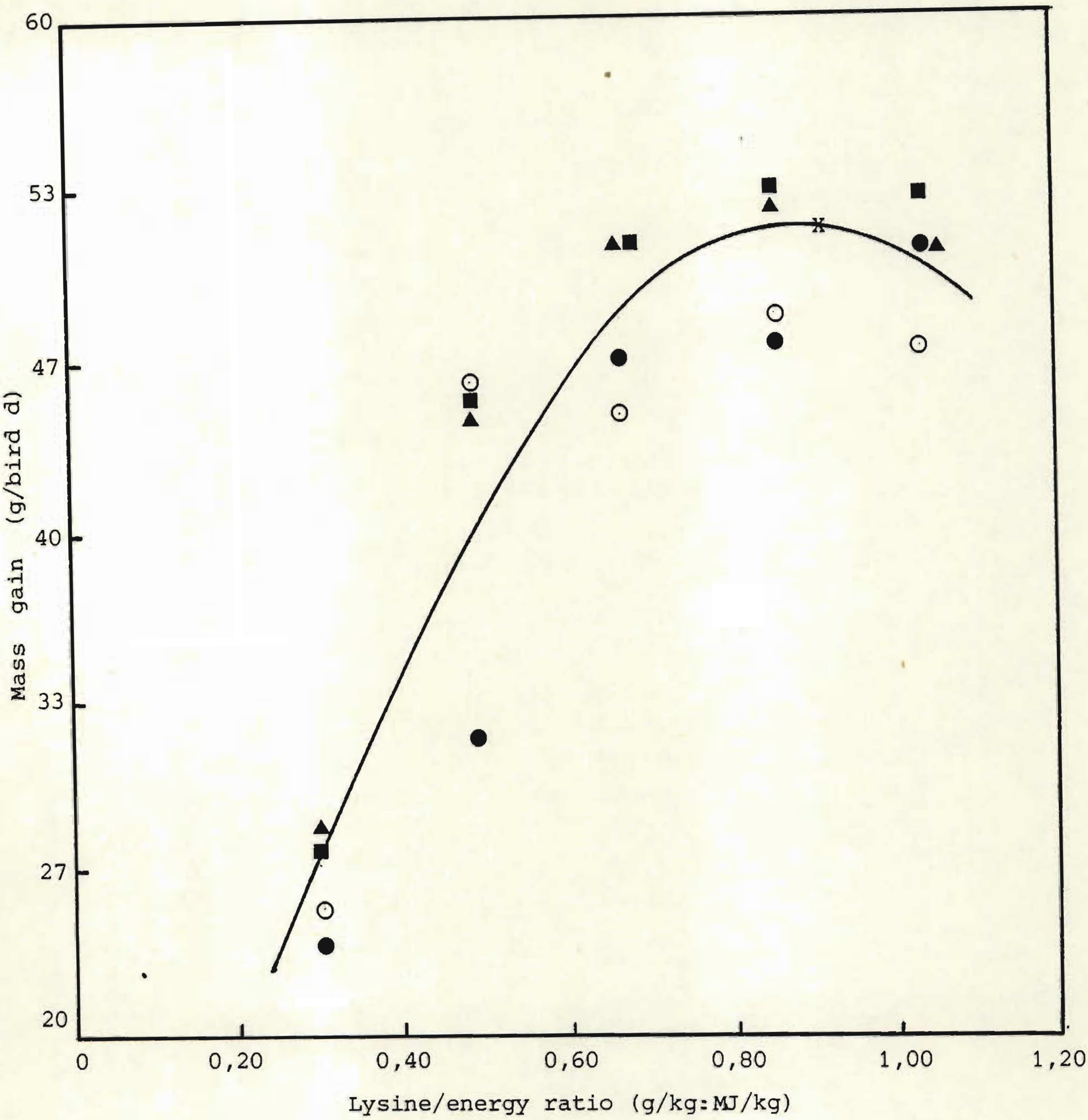

Figure 8.8 Response in mass gain (g/bird d) of male chickens from 28 - 45 days of age to lysine/energy ratio (g/kg:MJ/kg) and estimated optimum lysine/energy ratio (X)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

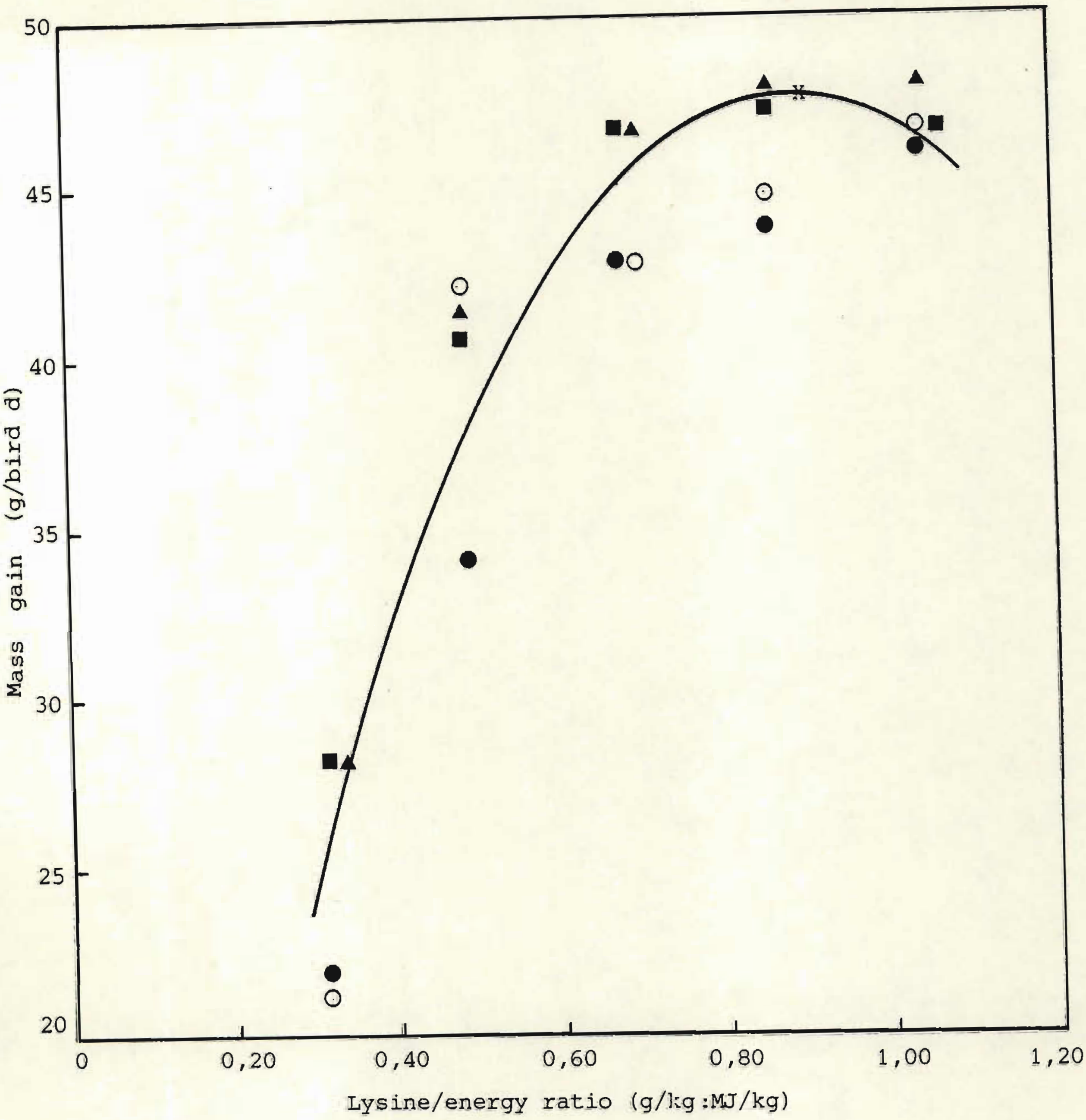

Figure 8.9 Response in mass gain (g/bird d) of male and female chickens from 28 - 45 days of age to lysine/energy ratio (g/kg:MJ/kg) and estimated optimum lysine/energy ratio (X)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

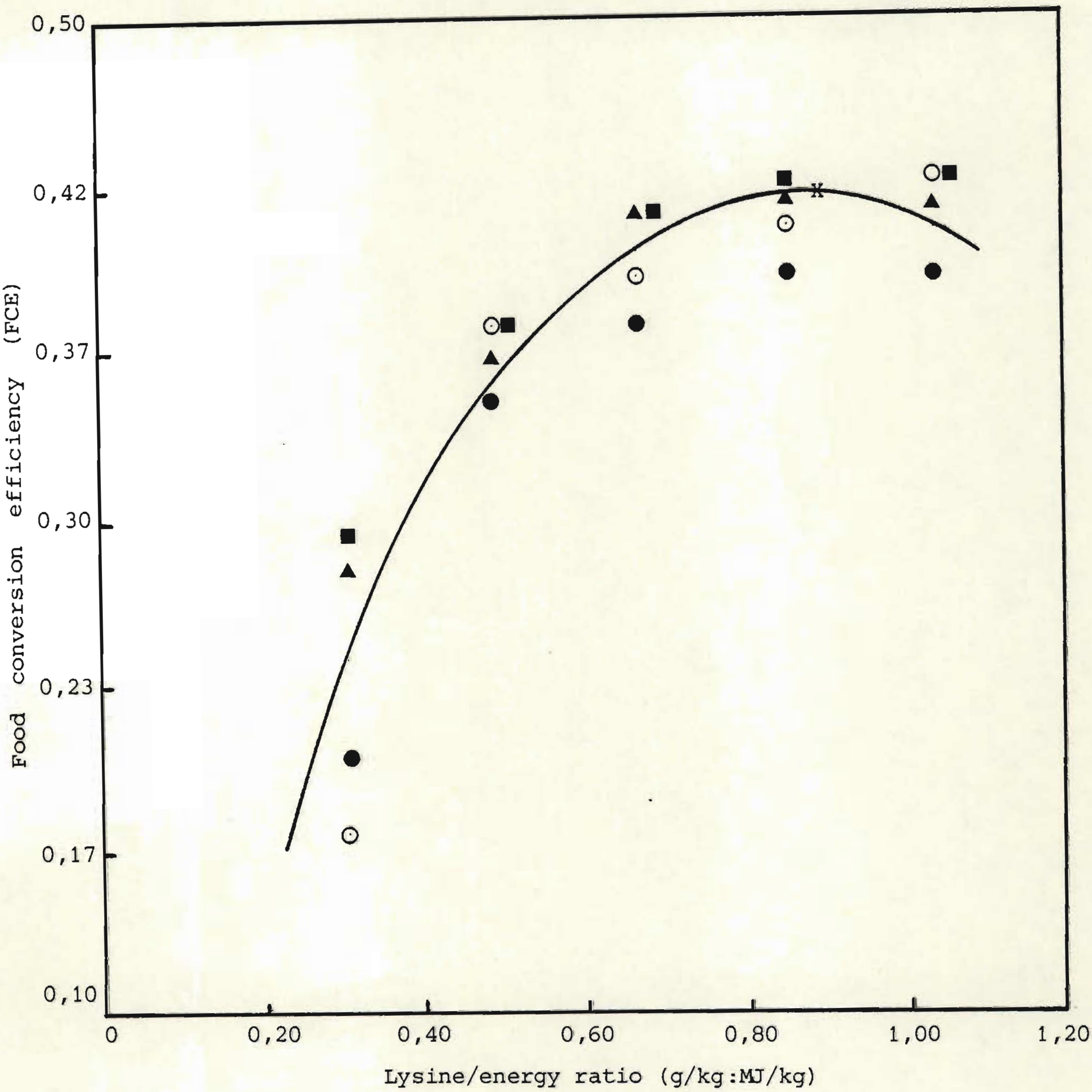

Figure 8.10 Response in food conversion efficiency (ΔW,g/F,g) of female chickens from 28 - 45 days of age to lysine/energy ratio (g/kg:MJ/kg) and estimated optimum lysine/energy ratio (X)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

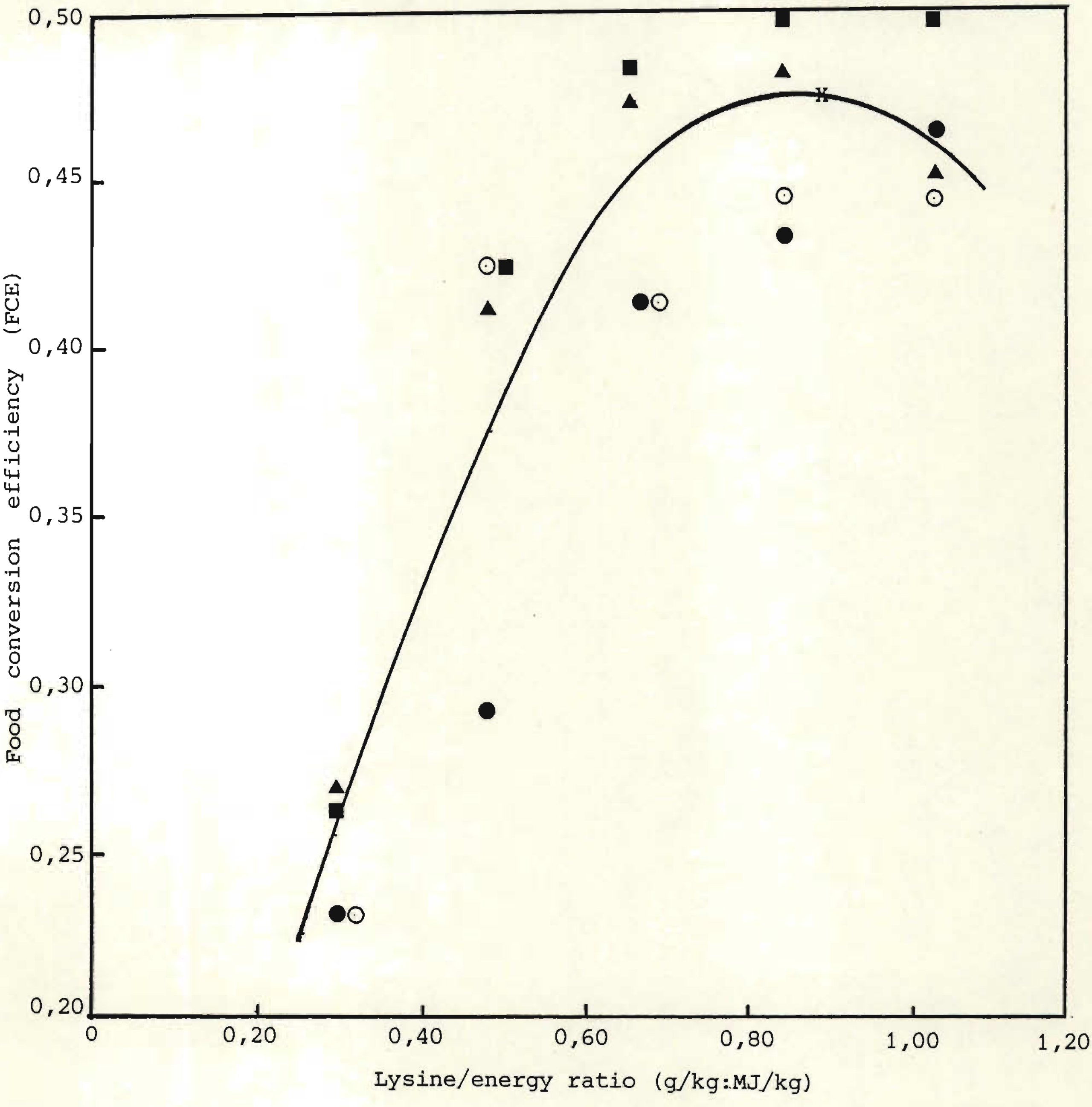

Figure 8.11 Response in food conversion efficiency (ΔW,g/F,g) of male chickens from 28 - 45 days of age to lysine/energy ratio (g/kg:MJ/kg) and estimated optimum lysine/energy ratio (X)

● 12,55 MJ/kg

○ 12,97 MJ/kg

▲ 13,39 MJ/kg

■ 13,81 MJ/kg

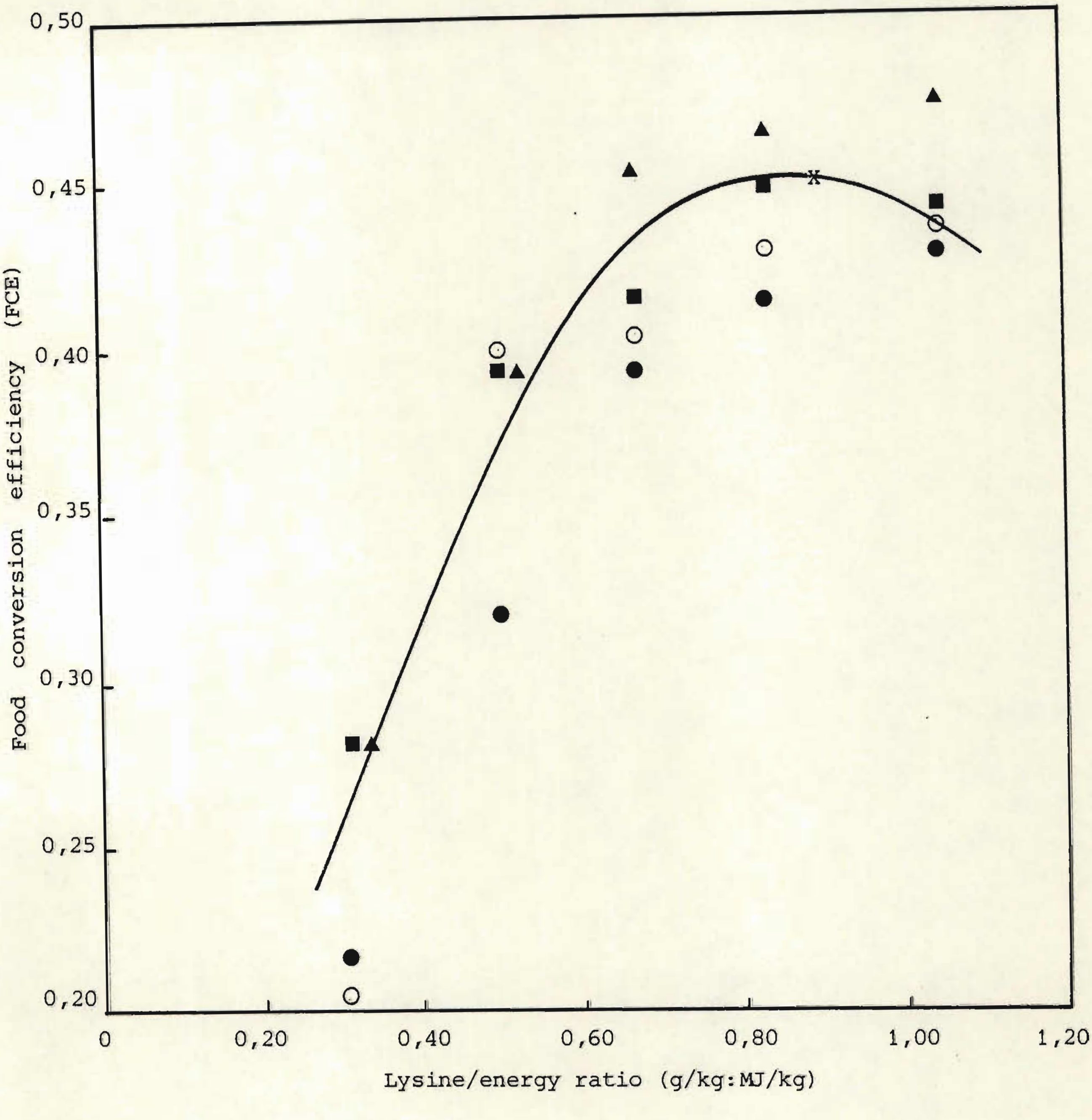

Figure 8.12 Response in food conversion efficiency (ΔW,g/F,g) of male and female chickens from 28 - 45 days of age to lysine/energy ratio (g/kg:MJ/kg) and estimated optimum lysine/energy ratio (X)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

TABLE 8.13 Response in crude protein gain (ΔCP) and carcass fat gain (ΔFT) to dietary lysine and metabolisable energy intakes of male and female chickens from 28 - 45 days of age

DME (MJ/kg)	Protein gain (g/bird d)		Fat gain (g/bird d)	
	Females	Males	Females	Males
12,55				
1	7,35	9,22	7,94	7,92
2	7,42	8,70	7,71	7,10
3	6,05	8,58	7,11	8,03
4	5,86	6,16	8,85	8,68
5	3,40	3,78	5,68	6,25
12,97				
1	7,91	9,31	7,12	7,52
2	7,83	9,34	8,40	8,24
3	6,46	9,16	8,50	9,41
4	6,57	8,31	8,46	8,60
5	4,07	4,63	6,54	6,71
13,39				
1	7,54	7,40	8,31	7,43
2	7,52	8,88	8,28	9,94
3	7,14	9,45	8,31	9,58
4	6,55	8,04	8,38	10,30
5	4,57	4,61	8,13	7,85
13,81				
1	7,86	8,99	8,23	8,59
2	7,27	8,88	7,68	7,42
3	7,75	8,05	8,75	6,72
4	5,85	8,28	8,67	9,60
5	4,15	4,29	5,66	7,06

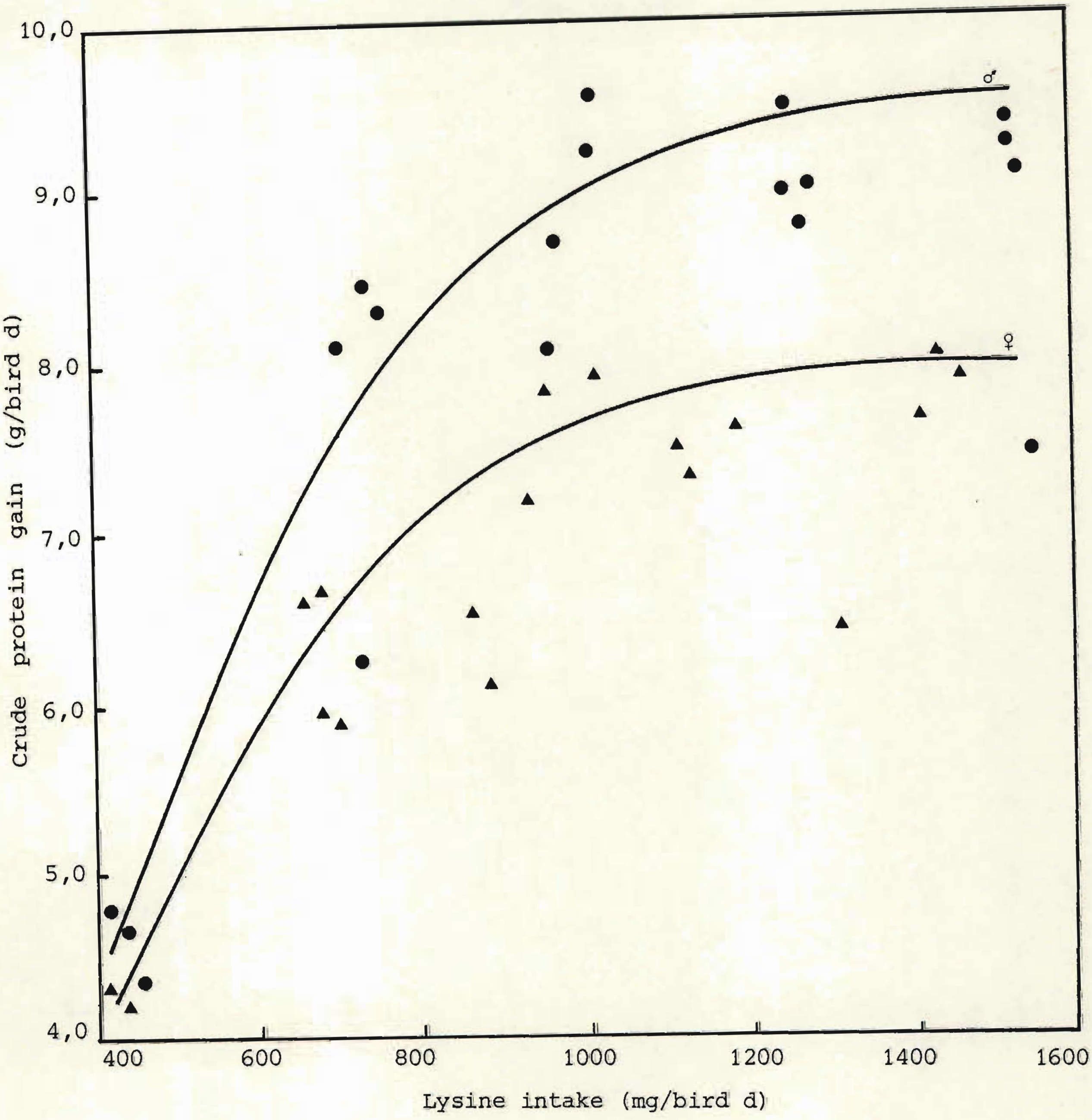

Figure 8.13 Response in crude protein gain (g/bird d) of male (♂,●) and female (♀,▲) chickens from 28 - 45 days of age to lysine intake (mg/bird d) and estimated response using Reading model (Table 8.14)

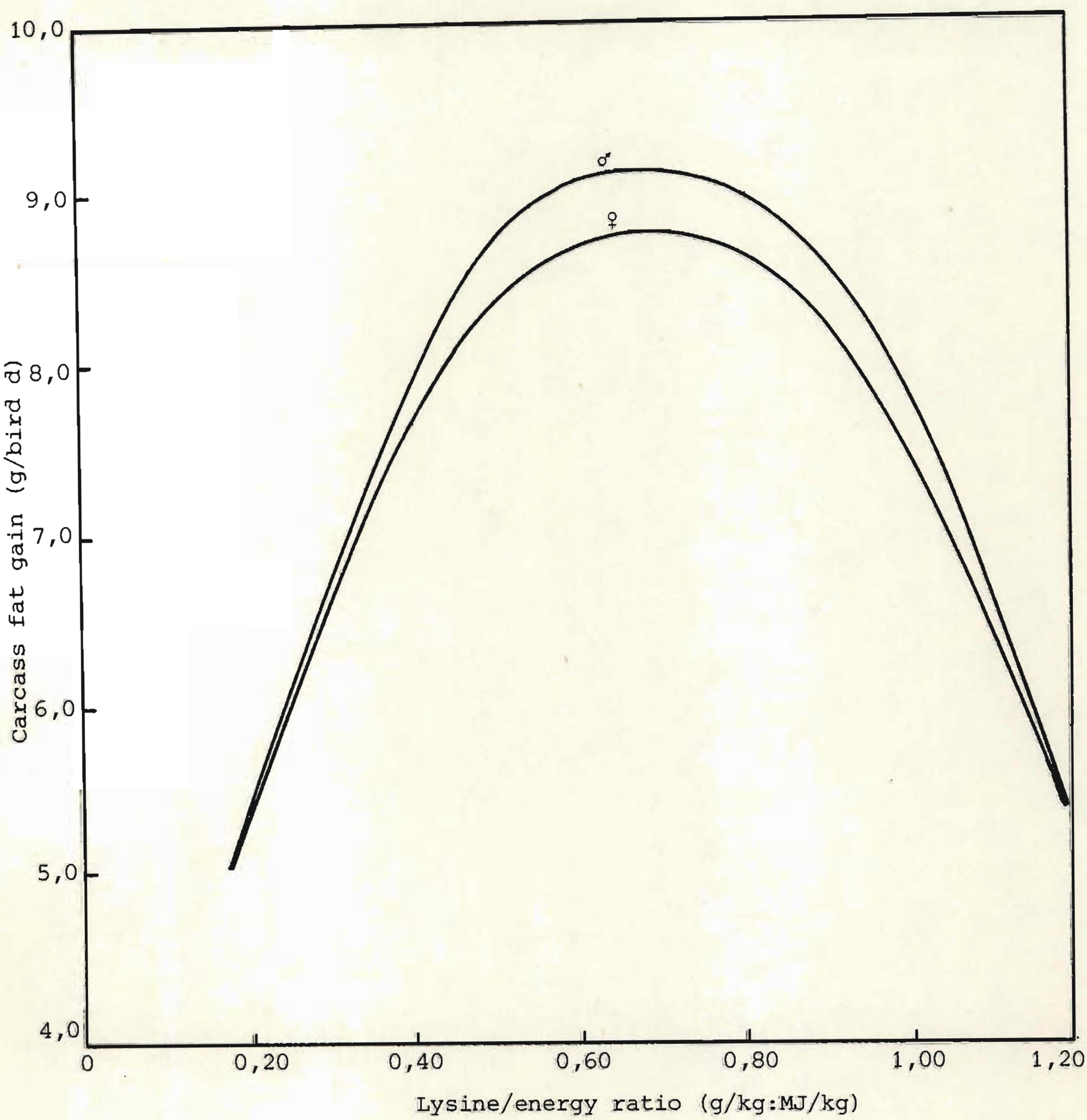

Figure 8.14 Estimated response in carcass fat gain (g/bird d) of male (♂) and female (♀) chickens from 28 - 45 days of age to lysine/energy ratio (g/kg:MJ/kg)

the results of this study. The lysine concentration in the diet governs the biological response, which in turn determines the economic outcome. It is thus essential to have an accurate assessment of the optimum lysine intake throughout the life of the broiler.

Use was made of the Reading model to describe the relationship between mass gain and lysine intake. Equations fitted to the data for each energy concentration and to the combined data over all energy concentrations (Table 8.9) indicate that at marginal costs of 350 c/kg for lysine, and 150 c/kg for broiler mass, the optimum intake of lysine at each energy level for the females would be 802, 1 022, 767, 776; for the males 1 394, 923, 1 001, 1 153; and for the combined sexes 1 171, 1 005, 926 and 1 052 mg/bird d. From the data combining all energy levels (illustrated in Figures 8.1, 8.2 and 8.3) the optimum intakes were calculated to be 914, 1 087 and 909 mg/bird d for females, for males and for combined sexes respectively. The above results will be discussed in greater detail later where data from Chapter 2 will be used together with these results to determine a more universal response curve to lysine in the finisher period.

The tendency for food intake to increase at marginal levels of lysine and to decrease when the deficiency became more severe, was not as clear in the finisher period as it was in the starter period (Chapters 6 and 7). Nevertheless food intake was significantly affected by the lysine content of the diet (Table 8.6).

Response to dietary metabolisable energy concentration

The livemass response to DME in the male broilers (Table 8.4) was significantly greater than the response among the females. There was a downward adjustment of food intake with increasing DME, but the magnitude of this response was not large enough to be significant for either sex. Daily metabolisable energy intake increased with DME in both sexes, the slope being greater among males than among females (0,073 vs 0,054 MJ MED/MJ DME). Fisher and Wilson (1974 a) found the response of MED to DME to be greater among males than among females, their corresponding values being 0,024 and 0,016 MJ MED/MJ DME. This greater increase in MED among males is reflected in a significantly greater response in ΔW for the males (Table 8.4). Because the response to DME differs between the sexes, the separate rearing of broilers should be considered in a broiler enterprise.

Food conversion efficiency of both males and females improved as the DME was increased. Because males adjust intake of DME less efficiently than females and hence show a greater improvement in growth rate as DME is increased, FCE should be expected also to improve more rapidly in males than in females with increasing DME. Responses to DME will be discussed more fully in Chapter 12, when the responses to energy in Chapters 6 to 12 are combined.

Response to lysine/DME ratio

The relationship between the limiting amino acid and the dietary energy level has a highly significant effect on broiler performance, since there is a positive correlation between ΔW and the nutrient density of the diet. If the optimal ratio of lysine to DME is not adhered to, either by increasing or decreasing this ratio, productive efficiency will decline. The optimum ratio between lysine and DME in this experiment was not significantly affected by the DME concentration (Table 8.10), therefore the optimum ratio for the combined data could be used with confidence over the range of DME concentrations used here. This optimum ratio of 0,869 g lysine/MJ compares well with that of 0,84 g lysine/MJ suggested by Thomas *et al.* (1978), but is higher than that of 0,74 g lysine/MJ recommended by Scott *et al.* (1976). The latter figure is based on a finisher period of 4 - 8 weeks requiring less dietary lysine than the finisher period of 3 - 6 weeks favoured by Thomas *et al.* (1978).

TABLE 8.14 Parameters used in determining the relationship between crude protein gain (g/bird d) and lysine intake (mg/bird d) by means of the Reading model using male and female chickens from 28 - 45 days of age

Parameter	Males	Females
$\bar{W}$	115,00	108,00
$\Delta\bar{W}$	9,50	7,90
a	84,85	93,41
b	0,043	0,046
$\sigma\Delta\bar{W}$	2,90	2,60
$\sigma\bar{W}$	6,00	6,00
r	0,60	0,60

Food conversion efficiency was clearly significanly affected by the ratio between lysine and DME (Table 8.12), the optimum ratio for males being 0,868 g lysine/MJ and for females being 0,875 g lysine/MJ. These optimum ratios compare very favourably with the ratios giving maximum growth rate, reiterating the fact that a value of around 0,870 g lysine/MJ would be an ideal ratio between lysine and DME.

Response in crude protein gain and carcass fat gain

In Chapter 7 it was suggested that females apparently exhibit a better net efficiency of lysine utilisation due to the fact that carcass fat contributes more to mass gain in females than in males. In this study, where the two sexes were compared within the same experiment, the net efficiency of lysine utilisation for mass gain was similar in both sexes (a = 13,10 for females and 13,25 for males). However, when protein gain is used as a measure of efficiency of lysine utilisation, male chickens exhibited a far better efficiency than did females (a = 93,41 for females and 84,85 for males). This confirms that males are more efficient than females at converting lysine in carcass protein, but that due to a greater deposition of fat by females the efficiency of lysine utilisation for body mass gain is as good as, or better than for males.

The relationship between carcass fat gain and the lysine/DME ratio was tested by means of a multiple regression analysis. The best fit was achieved at an energy level of 12,97 MJ/kg and these results are presented in Table 8.15 and Figure 8.14. The trend in fat deposition was closely associated with food consumption, which in turn was influenced by the lysine/DME ratio. The food intake increased progressively as the lysine content of the diets decreased until the severe lysine deficiency in the lower dilution series suppressed food consumption. The excess energy ingested was deposited as adipose tissue.

TABLE 8.15 The regression of carcass fat gain (g/bird d) on lysine/DME ratio (g/kg:MJ/kg) for male and female chickens from 28 - 45 days of age

DME (MJ/kg)	Constant term	Linear term (b_1)	Quadratic term (b_2)	SE (b)	Multiple correlation coefficient (R)
Females					
12,97	2,002	19,633	-14,294	3,11*	0,976
Males					
12,97	1,691	21,637	-15,732	4,89*	0,953

* denotes $P < 0,05$

The responses recorded in this study will be collated with the results of Chapters 6 and 7 in a later discussion, where the responses to dietary lysine and energy will be summarised and discussed in relation to their economic importance in broiler feed formulation.

DISCUSSION ON PART TWO

The primary response of broilers to dietary amino acid and metabolisable energy concentrations is seen in food consumption and in productive efficiency rather than in production level. Thus nutrient concentrations in poultry diets should be defined in economic rather than biological terms. The basic requirement for the formulation of practical diets is knowledge, in money terms, of the outputs obtained by feeding diets of different nutrient concentrations. These outputs can then be compared with costs and the nutrient concentration can be selected which optimises returns. This process requires the prior quantification of broiler responses and this study attempts to define the responses of broiler chickens to dietary amino acid and metabolisable energy concentrations from which the optimum intake of these nutrients can be determined for a given set of circumstances.

Use was made of the Reading model to describe the relationship between body mass gain and lysine intake. The results recorded in Chapters 1 and 2 indicated that the model described the data most adequately and confirmed the principle that body mass gain is dependent primarily on the intake of the first-limiting amino acid. A further series of experiments was conducted in order to obtain more data relating intake of lysine to livemass gain in broilers and the results for the starter and finisher periods were presented in Part 2 of this study.

In order to obtain a more general equation relating mass gain to lysine intake, the Reading model was fitted to the combined data from all the lysine experiments. The data used was confined to the first dilution series (initial dilution) described in Chapters 1 and 2 plus the combined data over all energy concentrations described in Chapters 6, 7 and 8. The parameters used in fitting the equation and the resulting coefficients are presented in Fig 8.15.

Optimum daily intakes of lysine, calculated from the Reading model of the combined data in Fig 8.15 (a = 16,529; b = 0,0174), for chickens gaining between 10 and 60 g body mass per day and for marginal costs of broiler mass ranging from 100 c/kg to 200 c/kg and of lysine at 350 c/kg, are shown in Table 8.16. The wide range of optimum intakes of lysine are an indication of the importance of this type of response curve analysis in determining optimal requirements of essential nutrients for broiler chickens.

From the results of the lysine trials in which dietary lysine and energy concentrations were tested, the optimum lysine/energy ratio for maximum growth rate appears to be 1,102 g lysine/MJ for the starter period and 0,869 g lysine/MJ for the finisher period, for maximum FCE this ratio is 1,227 g lysine/MJ and 0,871 g lysine/MJ for the starter and finisher periods respectively.

An interesting aspect of the lysine experiment was the effect of the lysine concentration in the diets on feathering and cannibalism in the experimental birds. In the first and second dilution series, which had adequate levels of dietary lysine, the number of feathers appearing on the litter appeared to be normal. Feather eating was evident among birds fed the third dilution series and increased progressively as the lysine deficiency became greater in the remaining dilution series. In the fifth dilution series, which had the lowest lysine concentration, the incidence of feather pulling, eating and cannibalism was very high, resulting in many poorly feathered and bare-backed birds in this treatment. These observations concur with the results of Thomas *et al.* (1976) who studied the relationship between the adequacy of a broiler diet and the number of feathers appearing on the litter. Thus feather eating suggests a lysine deficiency and the degree of feather consumption is indicative of the severity of the lysine deficiency.

TABLE 8.16 Calculated lysine requirements as affected by body mass gain ($\Delta\bar{W}$) and the ratio between cost of lysine and value of broiler mass (k)

$\bar{W}$ (g/bird d)	Lysine requirements[1] (mg/bird d) k[2] 0,0035	0,0028	0,0023	0,0020	0,0018
10	247	252	257	260	263
15	369	376	383	388	393
20	490	501	510	516	522
25	612	625	636	644	652
30	739	755	768	777	587
35	860	879	894	905	917
40	982	1 003	1 021	1 033	1 046
45	1 104	1 128	1 147	1 161	1 176
50	1 231	1 258	1 279	1 294	1 311
55	1 352	1 382	1 406	1 422	1 440
60	1 474	1 506	1 532	1 551	1 570

The following values were assumed in all cases:

[1] a = 16,529; b = 0,0174; $r\Delta\bar{W}.\bar{W}$ = 0,6;

$\bar{W}$(for $\Delta\bar{W}$ from 10-20 g) = 250 g;

$\bar{W}$(for $\Delta\bar{W}$ from 25-40 g) = 500 g;

$\bar{W}$(for $\Delta\bar{W}$ from 45-60 g) = 800 g;

$\sigma\Delta\bar{W}$ = 0,3 $\Delta\bar{W}$; $\sigma\bar{W}$ = 0,12 $\bar{W}$

[2] The five values of k corresponding to broiler mass values of 100, 125, 150, 175 and 200 c/kg and a lysine cost of 350 c/kg

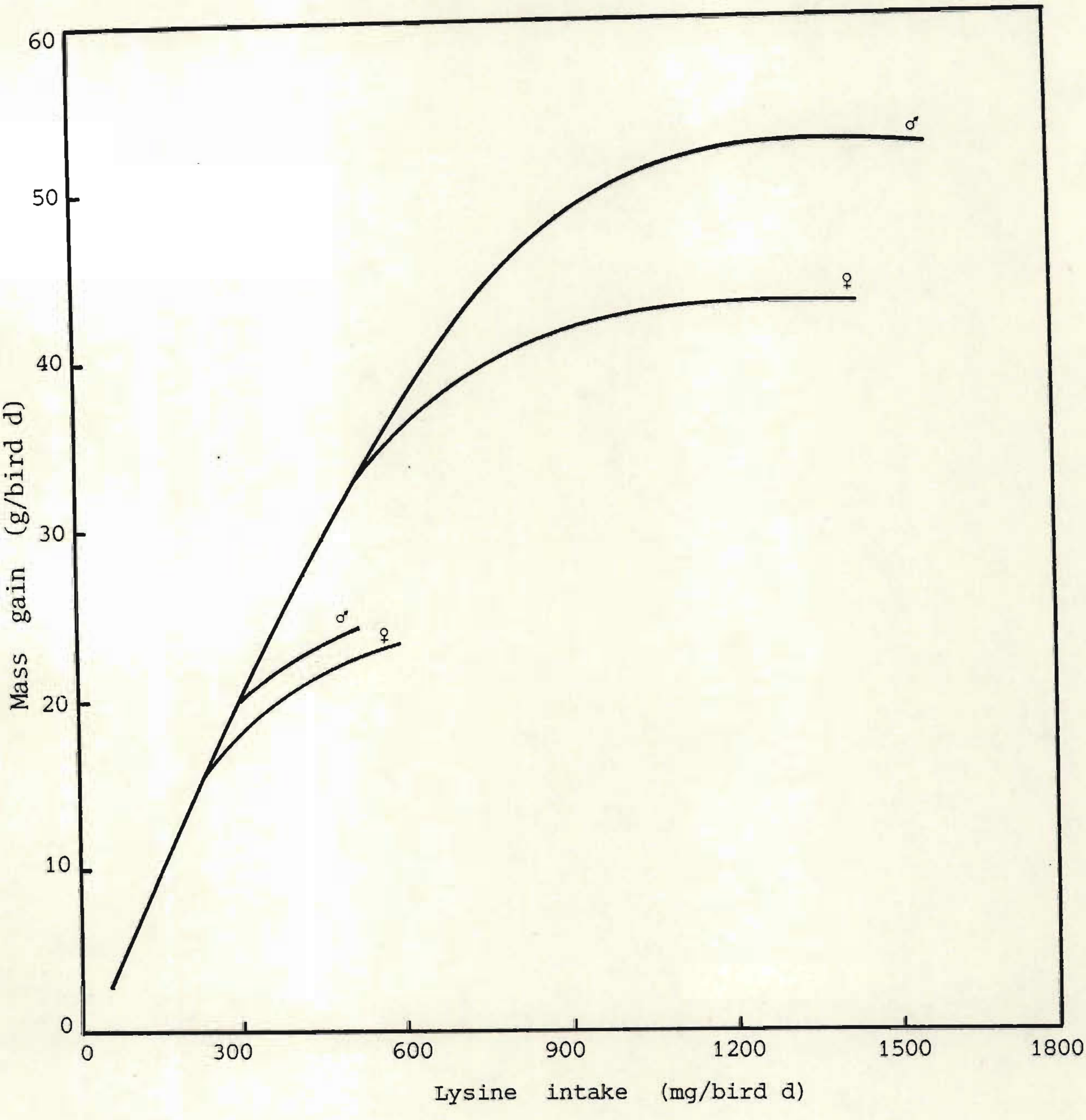

Figure 8.15 The relationship between mass gain and lysine intake using data from experiments reported in Chapters 1, 2, 6, 7 and 8. The fitted curves illustrate treatments that resulted in growth rates in the starter and finisher trials by male and female chickens respectively and are based on the following parameters: a = 16,529; b = 0,017; $\Delta\bar{W}$(♂F) 52 g/bird d; $\Delta\bar{W}$(♀F) 42 g/bird d; $\Delta\bar{W}$(♂S) 25 g/bird d; $\bar{W}$(♀S) 22 g/bird d; $\bar{W}$(starter) 200 g; $\bar{W}$(finisher) 800 g; r = 0,6; $\sigma\Delta\bar{W} = 0,3\ \Delta\bar{W}$; $\sigma\bar{W} = 1,12\ \bar{W}$

S U M M A R Y   O N   P A R T   T W O

Quantitative estimates of the responses of broiler chickens to dietary lysine and energy concentrations were made using the dilution technique. Responses in livemass gain (ΔW), food intake (F), metabolisable energy intake (MED), food conversion efficiency (FCE), crude protein gain (ΔCP) and carcass fat gain (ΔFT) were calculated. The Reading model was fitted to the data relating mass gain and crude protein gain to lysine intake to determine the optimum daily lysine intake .

Linear regressions of ΔW, F, MED and FCE on lysine/DME ratio were calculated on the data recorded for the first and second dilution series. The optimum lysine/DME ratios for the variates ΔW and FCE were determined.

From further analyses of suitable estimates of response relationships between dietary lysine and energy levels and ΔW, F and FCE were defined for use in the practical formulation of broiler diets for marginal costs of dietary lysine and broiler mass.

P A R T   T H R E E

RESPONSE TO DIETARY METHIONINE AND METABOLISABLE ENERGY CONCENTRATIONS

CHAPTER 9

RESPONSE OF MALE BROILERS TO DIETARY METHIONINE AND METABOLISABLE ENERGY CONCENTRATIONS DURING THE PERIOD 11 TO 21 DAYS OF AGE

INTRODUCTION

The commercial production of synthetic methionine has overcome the need to include excessive amounts of protein into diets in an attempt to overcome the deficiency of methionine in maize-soyabean diets. Such inclusion of the limiting amino acid improves the balance between the essential amino acids and results in a diet that is more efficient in promoting growth in broilers than a diet containing excessive concentrations of some amino acids and deficiencies of others.

In spite of the fact that synthetic methionine is readily obtainable, it is nevertheless necessary to define the response of broiler chickens to dietary methionine intake in order to determine the optimum level of supplementation of a diet based on maize and soyabean. Indeed, such a response curve is invaluable irrespective of the ingredients available to the feed formulator, as such a curve allows for the determination of the optimum methionine intake under changing economic conditions.

The purpose of the present study was to measure the response of male broiler chickens to dietary methionine and metabolisable energy concentrations during the starter period, using the dilution technique, whereafter the optimum local requirements of methionine and energy in broiler starter diets were determined.

MATERIALS AND METHODS

Ross male broiler chickens were used in the experiment. They were reared to 11 days of age in wire-floored brooders on a commercial starter diet. Management of the chickens to 11 days of age, and the handling of chickens in order to obtain equal body mass groups per replication, have been outlined previously (Chapter 1). The same procedure was used in this experiment.

In choosing the range of methionine contents of the experimental diets it was intended that as wide a range as was practically feasible would be used in order that a full response curve could be plotted. The required range of methionine contents was obtained by formulating a summit diet calculated to contain 1,22 times the requirement, and a dilution diet calculated to contain 0,36 times the requirement for methionine, the intermediate methionine contents being obtained by blending the basal diets in appropriate proportions as shown in Table 9.3. The net result of the dilution technique was the formation of five experimental diets with methionine contents of 1,22; 1,01; 0,79; 0,58 and 0,36 times the requirements recommended by Thomas *et al.* (1978).

In order to determine the effect of DME on broiler performance four energy levels were included in the design. Summit and dilution diets were formulated at 12,13; 12,55; 12,97 and 13,39 MJ/kg and blended proportionally to form the 20 experimental diets presented in Table 9.3. The ratio between methionine and metabolisable energy was kept constant in the summit and dilution diets, which were formulated with similar net energy contents for reasons previously discussed.

The composition of the summit and dilution diets is shown in Table 9.1. Specific protein contents were not used in formulation but the minimum contents of all essential amino acids except methionine were set at 1,75 times for the summits and 0,70 times

TABLE 9.1 Composition (g/kg) of the summit and dilution diets

	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet
Diet code	1	5	6	10	11	15	16	20
DME (MJ/kg)	12,13		12,55		12,97		13,39	
NE (MJ/kg)	8,50	8,50	8,75	8,75	9,27	9,27	9,33	9,33
Ingredients								
Yellow maize meal	410,5	165,0	333,5	197,0	289,4	246,0	253,0	273,5
Maize gluten meal (60%)							21,0	
Wheat bran		405,2		347,0		235,0		179,5
Lucerne meal		7,0		22,0		49,0		62,0
Groundnut meal	51,0	44,0		42,0	135,0	71,0		90,0
Sunflower meal	213,0		196,0				70,0	
Soyabean unextracted	123,0		272,0		360,0		439,0	
Fish meal	104,0		104,0		126,0		128,0	
Blood meal	25,0	20,0	25,0	25,0	25,0	25,0	25,0	25,0
Bone meal		8,0		30,0		37,0		40,0
Monocalcium phosphate		8,0					10,0	
Limestone flour	12,0	21,0	13,0	12,0	10,0	7,0		5,0
Salt	1,0	4,5	1,0	4,5	0,5	4,5	0,5	4,5
Starch		255,0		255,0		257,0		255,0
Feather meal	53,0	6,0	50,0	9,0	50,0	12,0	50,0	14,0
Sunflower oil		50,0		50,0		50,0		45,0
Lysine HCl	4,0	2,8	2,0	3,0	0,6	3,0		3,0
Vitamins and trace minerals *	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0
Anti-coccidial	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5

TABLE 9.1 continued

	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet
Diet code	1	5	6	10	11	15	16	20
DME (MJ/kg)	12,13		12,55		12,97		13,39	
NE (MJ/kg)	8,50	8,50	8,75	8,75	9,27	9,27	9,33	9,33
Calculated analysis								
Arginine	21,5	8,1	22,4	8,3	24,6	8,9	23,7	9,5
Histidine	7,3	2,8	7,8	3,0	8,2	3,1	8,6	3,2
Isoleucine	11,4	3,2	12,8	3,2	13,8	3,5	14,8	3,7
Leucine	26,5	9,3	27,8	9,9	29,6	10,7	31,7	11,3
Lysine	17,9	7,2	18,7	7,8	20,0	8,0	20,5	8,2
Methionine	5,9	1,7	6,1	1,8	6,3	1,9	6,5	1,9
Cystine	5,9	2,7	6,2	2,6	6,0	2,6	6,3	2,6
Phenylalanine	14,2	5,3	15,2	5,5	16,3	6,0	17,0	6,3
Tyrosine	7,9	3,4	8,5	3,4	10,5	3,7	10,3	4,0
Threonine	11,6	3,9	12,7	4,2	13,3	4,5	14,4	4,7
Tryptophan	3,6	1,4	4,0	1,4	4,3	1,5	4,5	1,5
Valine	16,4	6,1	17,4	6,3	18,3	6,6	19,3	6,8
Calcium	10,0	12,0	10,0	12,0	10,0	12,0	10,0	12,0
Phosphorus	7,0	7,0	7,0	7,0	7,0	7,0	7,0	7,0
Crude protein (gN x 6,25kg)	320,0	120,0	336,0	127,0	355,0	136,0	371,0	143,0

Supplies per kg of diet: Vit A 7 027 IU, Vit D_3 2 000 IU, Vit E 20 IU, Hetrazine 3 ppm, Thiamine hydrochloride 0,985 ppm, Riboflavin 3 ppm, Calcium pantothenate 7,846 ppm, Niacin 29,823 ppm, Folic acid 0,95 ppm, Biotin 0,08 ppm, Choline chloride 300 ppm

Copper 125 ppm, Iron 40 ppm, Zinc 28 ppm, Iodine 2 ppm, Manganese 78 ppm, Selenium 0,1 ppm

TABLE 9.2 Calculated amino acid contents of the summit and dilution diets relative to the requirements of broilers during the starter period

	Requirements according to Thomas *et al.* (1978) (g/kg)	Amino acid contents expressed as multiples of requirement	
		Summit diet	Dilution diet
Diet code		1	5
Arginine	11,59	1,86	0,70
Histidine	4,21	1,73	0,67
Isoleucine	7,91	1,44	0,40
Leucine	14,76	1,80	0,43
Lysine	11,86	1,51	0,61
Methionine	4,83	1,22	0,36
Cystine	3,56	1,66	0,76
Phenylalanine	7,38	1,92	0,72
Tyrosine	6,33	1,25	0,54
Threonine	6,99	1,66	0,56
Tryptophan	2,11	1,71	0,66
Valine	8,96	1,83	0,68
Diet code		6	10
Arginine	12,00	1,87	0,69
Histidine	4,36	1,79	0,69
Isoleucine	8,18	1,56	0,40
Leucine	15,28	1,82	0,65
Lysine	12,28	1,52	0,64
Methionine	5,00	1,22	0,36
Cystine	3,68	1,68	0,71
Phenylalanine	7,64	1,99	0,72
Tyrosine	6,55	1,30	0,52
Threonine	7,23	1,76	0,58
Tryptophan	2,18	1,83	0,64
Valine	9,28	1,88	0,68

TABLE 9.2 continued

	Requirements according to Thomas *et al.* (1978) (g/kg)	Amino acid contents expressed as multiples of requirement	
		Summit diet	Dilution diet
Diet code		11	15
Arginine	12,40	1,98	0,72
Histidine	4,51	1,82	0,69
Isoleucine	8,45	1,63	0,41
Leucine	15,78	1,87	0,68
Lysine	12,68	1,58	0,63
Methionine	5,17	1,22	0,36
Cystine	3,80	1,58	0,68
Phenylalanine	7,89	2,07	0,76
Tyrosine	6,76	1,55	0,55
Threonine	7,47	1,78	0,60
Tryptophan	2,25	1,91	0,67
Valine	9,58	1,84	0,69
Diet code		16	20
Arginine	12,80	1,85	0,74
Histidine	4,66	1,85	0,69
Isoleucine	8,73	1,70	0,42
Leucine	16,30	1,94	0,69
Lysine	13,10	1,56	0,63
Methionine	5,34	1,22	0,36
Cystine	3,93	1,60	0,66
Phenylalanine	8,15	2,09	0,77
Tyrosine	6,98	1,48	0,57
Threonine	7,71	1,87	0,61
Tryptophan	2,33	1,93	0,64
Valine	9,89	1,95	0,69

TABLE 9.3 Summary of dilution technique and calculated analysis of the experimental diets

Diet code	Blending ratio			Calculated dietary methionine (g/kg)	Calculated dietary protein (g/kg)
	Summit diet		Dilution diet		
12,13 MJ/kg					
1	100	+	0	5,90	320,0
2	75	+	25	4,85	269,0
3	50	+	50	3,80	219,0
4	25	+	75	2,75	168,0
5	0	+	100	1,70	120,0
12,55 MJ/kg					
6	100	+	0	6,10	336,0
7	75	+	25	5,03	284,0
8	50	+	50	3,95	231,0
9	25	+	75	2,88	179,0
10	0	+	100	1,80	127,0
12,97 MJ/kg					
11	100	+	0	6,30	355,0
12	75	+	25	5,20	300,0
13	50	+	50	4,10	245,0
14	25	+	75	3,00	190,0
15	0	+	100	1,90	136,0
13,39 MJ/kg					
16	100	+	0	6,50	371,0
17	75	+	25	5,35	314,0
18	50	+	50	4,20	257,0
19	25	+	75	3,05	200,0
20	0	+	100	1,90	143,0

for the dilutions of the requirements proposed by Thomas *et al.* (1978). In a number of instances it was impossible to formulate to the minimum amino acid specifications outlined above with the raw ingredients available, so it was necessary to reduce the levels of the limiting amino acids in order to obtain feasible diets.

The experimental diets were fed from 11 to 21 days of age, and the criteria studied were growth rate and food intake during the ten day experimental period. The data on each variate were subjected to statistical analysis, using the method of Rayner (1967). Means, standard errors of the means (SEM) and least significant differences (LSD) at $P < 0,05$ and $P < 0,01$ were calculated.

RESULTS

Means of the twenty treatments for the variates mass gain, lysine intake, food intake, dietary metabolisable energy intake and food conversion efficiency are given in Tables 9.4 to 9.8 respectively. The main effects of DME and the methionine/DME ratio are also shown, together with standard errors of the means; LSD's; and an analysis of variance table for each variate.

Response to dietary methionine concentration

The Reading model, described in Chapter 1, was used to fit an equation to the data relating body mass gain to methionine intake. The parameters used in fitting the model were estimated as described earlier (Chapter 6), use being made of the iterative procedure for determining values of a and b (Curnow, 1973). Equations were fitted to the data from each energy level, as well as to the combined data. The parameters used in fitting these equations, and the resulting coefficients, are given in Table 9.9 The

TABLE 9.4 Mass gain (g/bird d) for male broilers from 11 - 21 days of age

Mass Gain

	DME MJ/kg	Methionine/DME Ratio 0,49	0,40	0,31	0,23	0,14	Mean
Males	12,13	23,7	23,7	21,7	17,8	9,9	19,4
	12,55	22,4	23,6	21,7	17,2	10,0	19,0
	12,97	23,4	23,3	22,1	20,1	10,9	20,0
	13,39	22,0	23,3	20,9	17,2	11,3	18,9
Mean		22,9	23,5	21,6	18,1	10,5	19,3

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,364	1,04	1,40
Mean of 5 entries	0,407	1,17	1,56
Mean of 20 entries	0,813	2,33	3,12
	CV	7,30%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	2	7,30	3,65	1,84 NS
Methionine	4	1369,63	342,41	172,93 **
Energy	3	10,19	3,40	1,72 NS
Methionine x energy	12	20,18	1,68	0,85 NS
Error	38	75,36	1,98	
Total	59	1482,66		

SEM, CV etc see footnote Table 6.4

TABLE 9.5 Methionine intake (mg/bird d) for male broilers from 11 - 21 days of age

Methionine Intake

	ME MJ/kg	Methionine/DME Ratio 0,49	0,40	0,31	0,23	0,14	Mean
Males	12,13	278	228	188	134	76	181
	12,55	288	236	192	137	79	186
	12,97	289	230	190	142	85	187
	13,39	286	233	191	137	77	185
Mean		285	232	190	138	79	185

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	1,864	5,34	7,15
Mean of 5 entries	2,083	5,97	7,99
Mean of 20 entries	4,167	11,93	15,99
	CV	3,90%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	2	305,76	152,88	2,93 NS
Methionine	4	308666,08	77166,52	1481,41 **
Energy	3	337,08	112,36	2,16 NS
Methionine x energy	12	250,03	20,84	0,40 NS
Error	38	1979,54	52,09	
Total	59	311538,48		

SEM, CV etc see footnote Table 6.4

TABLE 9.6 Food intake (g/bird d) for male broilers from 11 - 21 days of age

Food Intake

	DME MJ/kg	Methionine/DME Ratio 0,49	0,40	0,31	0,23	0,14	Mean
Males	12,13	47,1	47,6	49,6	48,0	44,7	47,4
	12,55	47,2	46,3	47,9	47,4	43,6	46,5
	12,97	45,9	44,2	46,4	47,3	44,5	45,7
	13,39	44,0	43,2	45,6	45,6	40,7	43,8
Mean		46,0	45,3	47,4	47,1	43,4	45,8

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,505	1,45	1,94
Mean of 5 entries	0,565	1,62	2,17
Mean of 20 entries	1,129	3,23	4,33
	CV	4,27%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	2	29,07	14,53	3,80 *
Methionine	4	120,97	30,24	7,92 *
Energy	3	104,01	34,67	9,08 *
Methionine x energy	12	19,06	1,59	$0,42^{NS}$
Error	38	145,34	3,82	
Total	59	418,44		

SEM, CV etc see footnote Table 6.4

TABLE 9.7 Dietary metabolisable energy (MJ/bird d) intake for male broilers from 11 - 21 days of age

MED Intake

	DME MJ/kg	Methionine/DME Ratio 0,49	0,40	0,31	0,23	0,14	Mean
Males	12,13	0,57	0,58	0,60	0,58	0,54	0,57
	12,55	0,59	0,58	0,60	0,59	0,55	0,58
	12,97	0,59	0,57	0,60	0,61	0,57	0,59
	13,39	0,59	0,58	0,61	0,61	0,54	0,58
Mean		0,58	0,57	0,60	0,60	0,55	0,58

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,006	0,02	0,02
Mean of 5 entries	0,007	0,02	0,03
Mean of 20 entries	0,014	0,04	0,05
	CV	4,25%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	2	0,0046	0,0023	3,83 *
Methionine	4	0,0197	0,0049	8,17 **
Energy	3	0,0019	0,0006	1,00 NS
Methionine x energy	12	0,0031	0,0003	0,50 NS
Error	38	0,0232	0,0006	
Total	59	0,0525		

SEM, CV etc see footnote Table 6.4

TABLE 9.8 Food conversion efficiency of male broilers from 11 - 21 days of age

F C E

	DME MJ/kg	Methionine/DME Ratio 0,49	0,40	0,31	0,23	0,14	Mean
Males	12,13	0,50	0,50	0,44	0,37	0,22	0,41
	12,55	0,47	0,51	0,45	0,37	0,23	0,41
	12,97	0,51	0,53	0,47	0,43	0,25	0,44
	13,39	0,50	0,54	0,46	0,38	0,28	0,43
Mean		0,50	0,52	0,46	0,38	0,24	0,42

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,008	0,02	0,03
Mean of 5 entries	0,009	0,03	0,03
Mean of 20 entries	0,018	0,05	0,07
	CV	7,49%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	2	0,0086	0,0043	0,43 NS
Methionine	4	0,5946	0,1486	15,01 **
Energy	3	0,0108	0,0036	0,36 NS
Methionine x energy	12	0,0084	0,0007	0,07 NS
Error	38	0,0376	0,0099	
Total	59	0,6599		

SEM, CV etc see footnote Table 6.4

combined equation, together with the means for each energy level, are illustrated in Figure 9.1.

TABLE 9.9 Parameters used in determining the relationship between mass gain (g/bird d) and methionine intake (mg/bird d) by means of the Reading model using male chickens from 11 - 21 days of age

Parameter	Energy concentration (MJ/kg)				
	12,13	12,55	12,97	13,39	Combined
$\bar{W}$	250,00	250,00	250,00	250,00	250,00
$\Delta\bar{W}$	23,70	23,16	23,70	22,55	23,17
a	6,72	6,88	5,18	6,95	6,58
b	0,03	0,04	0,08	0,02	0,04
$\sigma\Delta\bar{W}$	5,95	5,93	8,90	5,88	5,91
$\sigma\bar{W}$	40,00	40,00	40,00	40,00	40,00
r	0,60	0,60	0,60	0,60	0,60

Response to dietary energy concentration

Responses to DME were tested by means of a linear regression analysis and the data used in the analysis consisted of the results of the first and second dilution series only. There were thus 24 values that were pooled for each of the variates ΔW, F, MED and FCE. The equations are presented in Table 9.10 and are illustrated in Figure 9.2.

Response ot methionine/DME ratio

Curvilinear regressions of mass gain and FCE on methionine/DME ratio were calculated using a multiple regression analysis. The coefficients at each energy level and for the combined data over all energy concentrations are given in Tables 9.11 and 9.12 and illustrated in Figures 9.3 and 9.4 respectively.

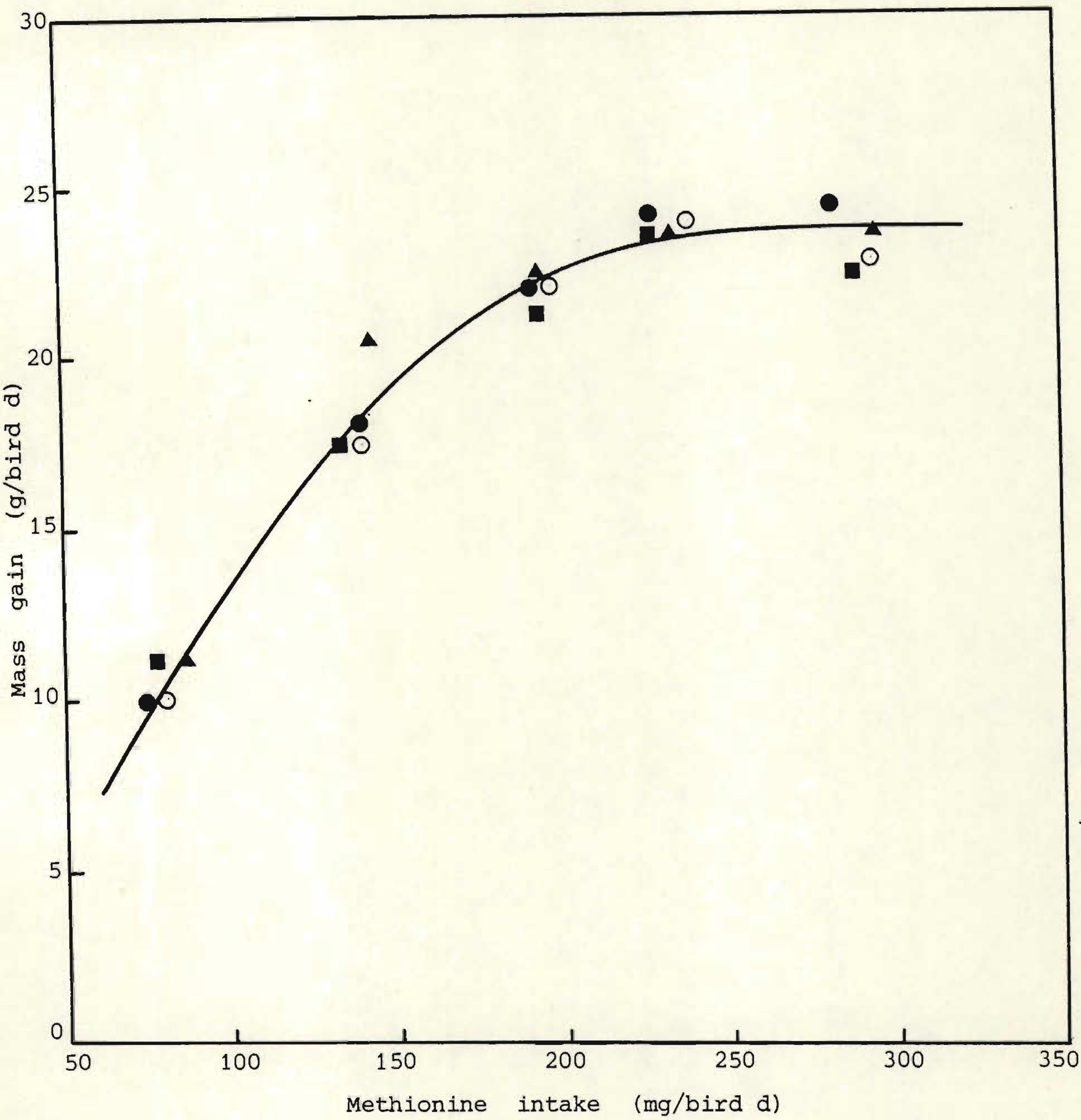

Figure 9.1 Response in mass gain (g/bird d) of male broilers from 11 - 21 days of age to methionine intake (mg/bird d) and estimated response using the Reading model (Table 9.9)

● 12,13 MJ/kg ▲ 12,97 MJ/kg

○ 12,55 MJ/kg ■ 13,39 MJ/kg

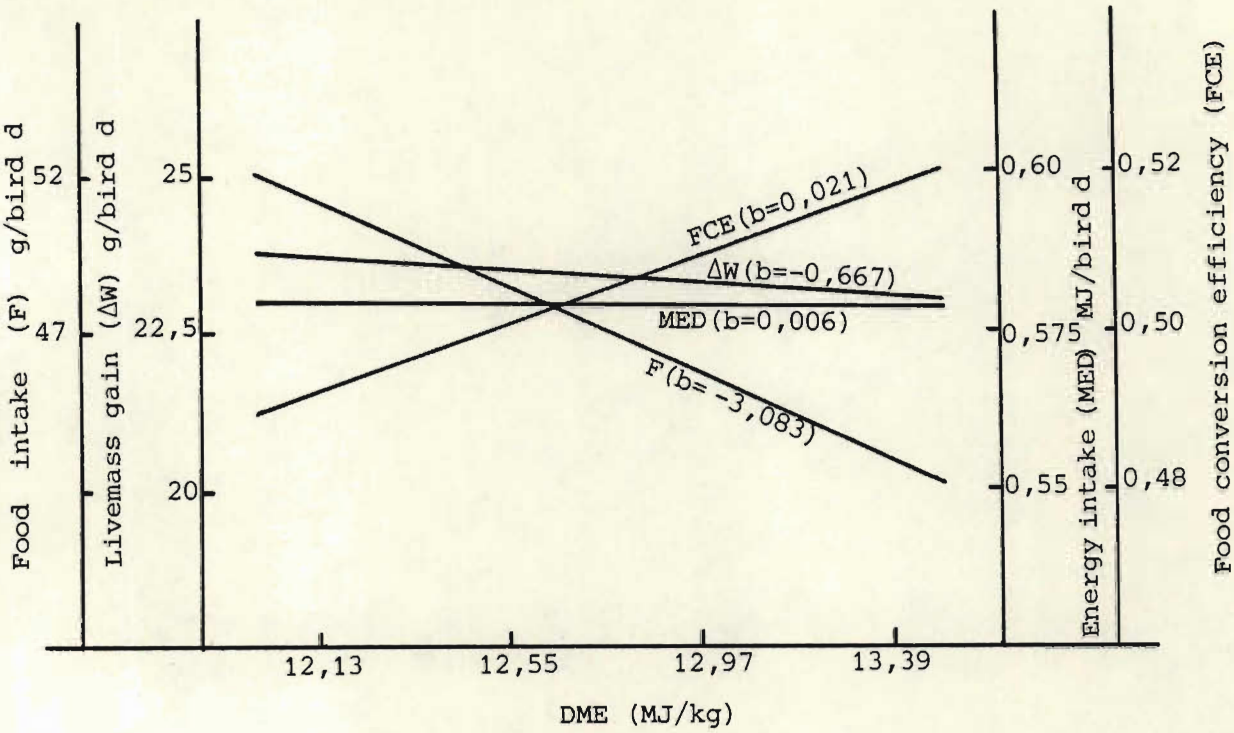

Figure 9.2 Pooled linear regressions of male broiler performance characteristics on dietary metabolisable energy level for the starter period

TABLE 9.10 Pooled linear regression coefficients of male broiler performance characteristics on dietary metabolisable energy level for the period 11 - 21 days of age (units are per MJ/kg)

Character regressed on DME	Constant term	Regression coefficient (b)	SE (b)
Livemass gain (ΔW)	31,682	-0,667	1,837[NS]
Food intake (F)	85,031	-3,083	1,041**
Energy intake (MED)	0,505	0,006	0,013[NS]
Food conversion efficiency (FCE)	0,234	0,021	0,017[NS]

[NS], **, see footnote Table 6.4

TABLE 9.11 The regression of mass gain (g/bird d) on methionine/energy ratio, and optimum methionine/energy ratio for male chickens from 11 - 21 days of age

DME (MJ/kg)	Constant term	Linear term (b_1)	Quadratic term ($\times 10^{-2}$) (b_2)	SE (b)	Multiple correlation coefficient (R)	Optimum methionine/energy ratio (g/kg:MJ/kg)
12,13	-6,78	1,435	-1,666	0,70**	0,999	0,431
12,55	-7,62	1,506	-1,824	0,53**	0,999	0,413
12,97	-5,35	1,454	-1,795	2,96*	0,984	0,405
13,39	-3,41	1,246	-1,478	1,13**	0,997	0,422
Combined	-5,79	1,410	-1,691	0,62**	0,999	0,417

*, **, see footnote Table 6.4

TABLE 9.12 The regression of food conversion efficiency on methionine/energy ratio and optimum methionine/energy ratio for male broilers from 11 - 21 days of age

DME (MJ/kg)	Constant term	Linear term (b_1)	Quadratic term ($\times 10^{-3}$) (b_2)	SE (b)	Multiple correlation coefficient (R)	Optimum methionine/energy ratio (g/kg:MJ/kg)
12,13	-7,96	0,026	-0,279	0,02**	0,998	0,457
12,55	-0,12	0,030	-0,358	0,03*	0,996	0,416
12,97	-0,10	0,031	-0,373	0,04*	0,993	0,411
13,39	-0,01	0,024	-0,261	0,06^{NS}	0,983	0,448
Combined	-0,08	0,027	-0,318	0,03**	0,997	0,431

^{NS}, * etc see footnote Table 6.4

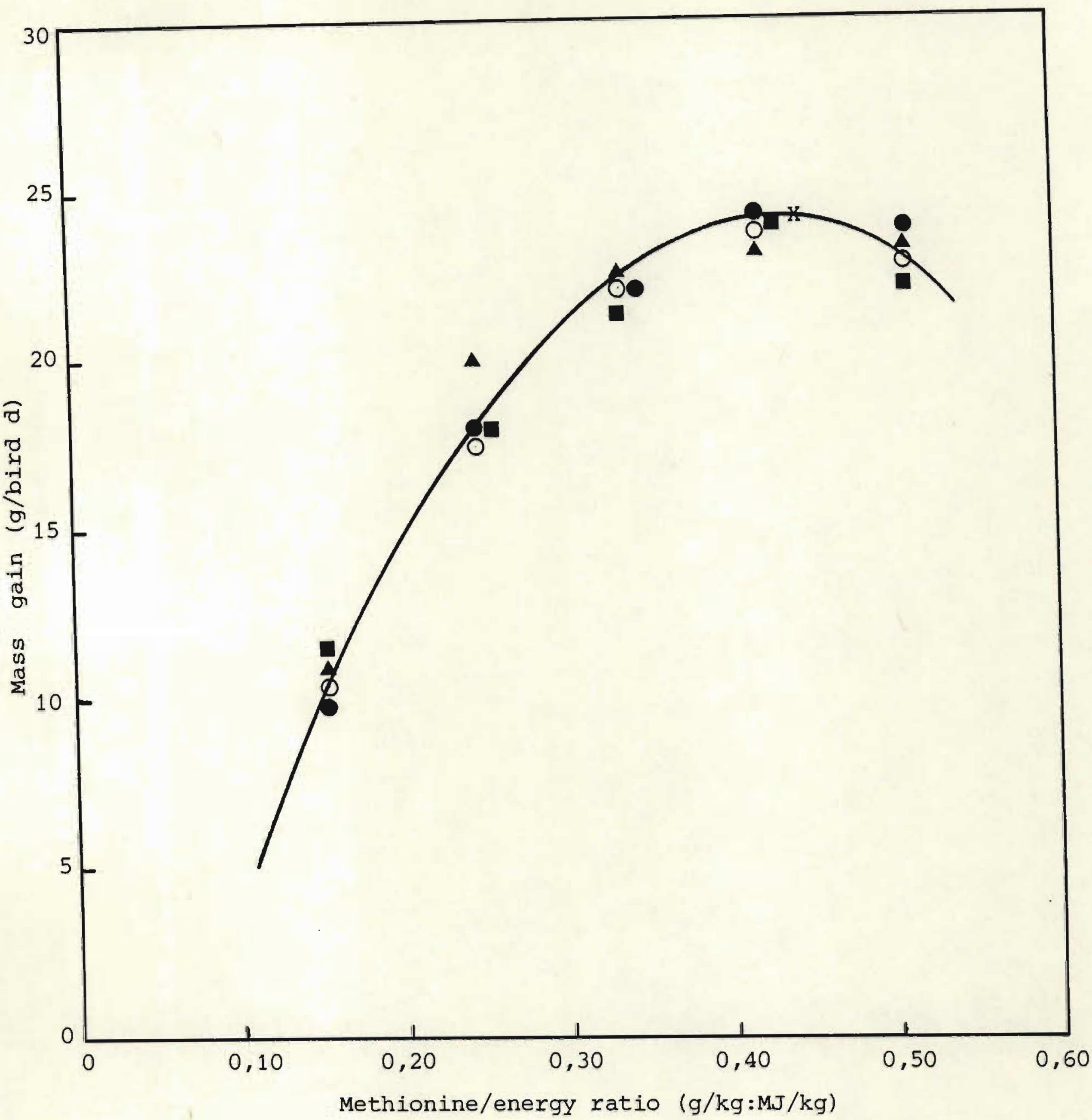

Figure 9.3 Response in mass gain (g/bird d) of male broilers from 11 - 21 days of age to methionine/energy ratio and estimated optimum methionine/energy ratio (X)

● 12,13 MJ/kg ▲ 12,97 MJ/kg

○ 12,55 MJ/kg ■ 13,39 MJ/kg

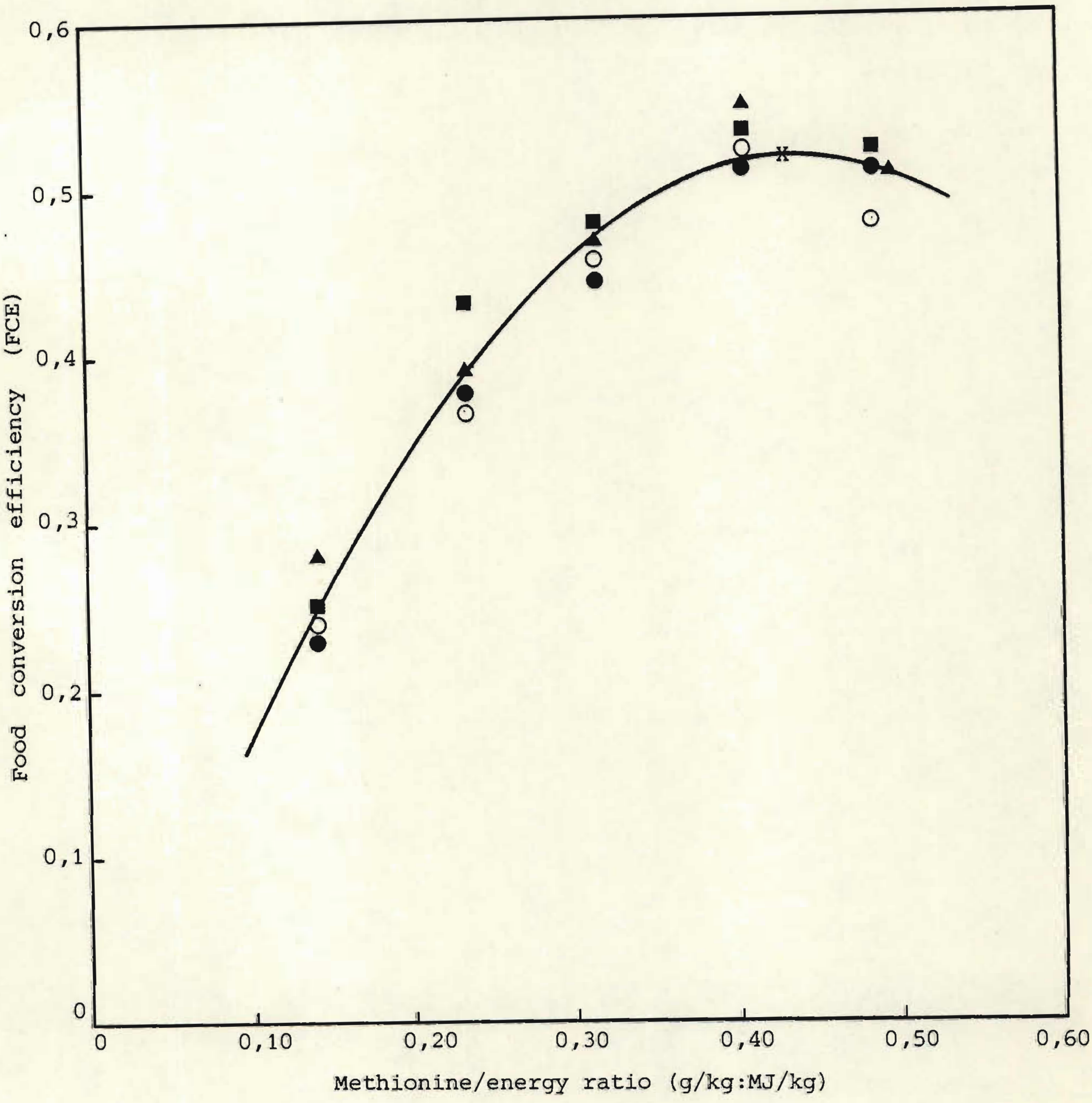

Figure 9.4 Response in food conversion efficiency of male broilers from 11 - 21 days of age to methionine/energy ratio and estimated optimum methionine/energy ratio (X)

● 12,13 MJ/kg ▲ 12,97 MJ/kg

○ 12,55 MJ/kg ■ 13,39 MJ/kg

DISCUSSION

The major objective of this experiment was to measure the response of broiler chickens to methionine intake using the dilution technique, and to determine the optimum methionine intake in practical diets for male chickens during the starter period.

Response to dietary methionine concentration

In practical broiler diets based on maize, soyabeans and fishmeal methionine is one of the limiting amino acids, thus it is essential, from an economic standpoint to have an accurate assessment of the response of broilers to methionine intake.

The reading model was used to describe the relationship between body mass gain and methionine intake. Equations fitted to the data for each energy concentration and to the combined data over all energy concentrations (Table 9.9) indicate that at marginal cost of 280 c/kg for methionine and 150 c/kg for broiler mass, the optimum intake of methionine at each energy level would be 274, 276, 229 and 269 mg/bird d. On the combined data which is shown in Figure 9.1 the optimum intake was 266 mg/bird d. The optimum intakes at each energy level are very similar to one another, with no obvious linear trend in any direction, indicating that growth rate is in this case dependent on the methionine intake of the chickens irrespective of the energy concentration of the diet. Further discussion on the subject of optimum intakes of methionine will be discussed in a later chapter (Chapter 11) where the Reading model is used to describe the combined response of broiler chickens of different ages and sexes to dietary methionine concentrations.

The concentration of the first-limiting amino acid in a diet has a significant effect on food intake. It has been observed that chickens respond to a moderate deficiency of essential amino acids by increasing their food intake, however, when the deficiency becomes severe then food intake is suppressed. The above observations are shown in Table 9.6 where the effect of methionine on food intake is recorded. The highest intake was recorded on diets in the third dilution series, with intakes declining at an increasing rate as the methionine concentration was diluted further.

The effect of the methionine content of the diets on FCE is presented in Table 9.8. The high concentration of methionine in the first dilution series had a detrimental effect on performance since the highest FCE was recorded in the second dilution series. The response in FCE then declined progressively as the methioinine level decreased in the remaining dilution series diets.

Response to dietary energy concentration

The effect of DME on ΔW, F, MED and FCE was examined by means of linear regression analyses and the pooled results are presented in Table 9.10 and Figure 9.2 respectively. The data used in the analyses were restricted to the results recorded for diets of the first and second dilution series for reasons previously discussed.

DME had a highly significant effect on food consumption, intake decreasing progressively as the energy content of the diets increased. This phenomenon was responsible for the non significant effect of DME on MED and mass gain, since the birds did not consume excess energy at the higher energy levels. In this experiment male broilers regulated their energy intake during the starter period which thus accounts for the non significant effect of DME on MED and mass gain. Notwithstanding the fact that DME had a significant effect on food intake, the energy intake was not constant on all energy concentrations which was therefore responsible for the significant effect of MED on FCE. FCE improved as the energy content of the diets increased.

Response to methionine/energy ratio

Chickens tend to regulate their daily food intake so as to maintain an appropriate level of energy intake, consequently the mass of food consumed generally decreases when balanced diets of increasingly higher energy concentrations are offered. For this reason it is important that an appropriate balance should be maintained between energy content and the levels of other essential nutrients in the diet. Any deviation from this perfect balance is accompanied by a concomitant deterioration in broiler performance. The results presented in Table 9.4 show that the maximum response in ΔW to methionine/DME ratio was recorded in the diets of the second dilution series. The response in ΔW decreased progressively as the methionine/DME ratio narrowed in the lower dilutions. The high methionine concentrations in the first dilution series had an adverse effect on mass gain.

The effect of varying the methionine/energy ratio on mass gain and FCE was examined by means of a multiple regression analysis, the results of which are presented in Tables 9.11 and 9.12 and Figures 9.3 and 9.4 respectively. For the combined data illustrated in Figure 9.4 the optimum ratio for FCE was 0,43 g methionine/MJ. These ratios compare favourably with the optimum methionine/energy ratio of 0,40 g methionine/MJ recommended by Thomas *et al.* (1978) and 0,42 g methionine/MJ recommended by AEC (1978).

The responses measured in this study will be collated with the results of proceeding methionine and energy experiments in Chapter 11, where the responses to dietary methionine and energy will be discussed.

CHAPTER 10

RESPONSE OF FEMALE BROILERS TO DIETARY METHIONINE AND METABOLISABLE ENERGY CONCENTRATIONS DURING THE PERIOD 10 TO 21 DAYS OF AGE

INTRODUCTION

The experiment described in this chapter is similar to the study conducted in the previous chapter, the difference being that females were used in place of the male broiler chickens used previously.

The objective of the study was to determine the response of female broilers to dietary methionine and metabolisable energy from 10 to 21 days of age using the dilution technique. This data would then be used to determine the optimum intake of these nutrients for broiler females during the starter period.

MATERIALS AND METHODS

The Ross female broiler chickens were subjected to the same housing, management and experimental procedures as those described in Chapter 9. The experimental diets were the same for both trials, but were mixed separately for each trial. The composition of the experimental diets was shown in Tables 9.1, 9.2 and 9.3 respectively.

The experimental diets were fed from 10 to 21 days of age, and the parameters measured were growth rate and food intake during the 11 day experimental period. The data on each variate were

TABLE 10.1 Mass gain (g/bird d) for female broilers from 10 - 21 days of age

Mass Gain

	DME MJ/kg	Methionine/DME Ratio 0,49	0,40	0,31	0,23	0,14	Mean
Females	12,13	26,3	23,3	21,7	18,5	9,2	19,8
	12,55	24,4	24,2	22,5	17,8	9,8	19,7
	12,97	25,7	22,0	23,7	17,9	11,9	20,2
	13,39	22,2	25,0	22,3	18,8	10,5	19,8
Mean		24,7	23,6	22,5	18,3	10,4	19,9

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,412	1,18	1,58
Mean of 5 entries	0,461	1,32	1,77
Mean of 20 entries	0,921	2,64	3,53
	CV	8,83%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	2	2,40	1,20	0,47 NS
Methionine	4	1361,28	340,32	133,46 **
Energy	3	2,00	0,67	0,26 NS
Methionine x energy	12	52,47	4,37	1,71 NS
Error	38	96,78	2,55	
Total	59	1514,93		

SEM, CV etc see footnote Table 6.4

TABLE 10.2 Methionine intake (mg/bird d) for female broilers from 10 - 21 days of age

Methionine Intake

	DME MJ/kg	Methionine/DME Ratio 0,49	0,40	0,31	0,23	0,14	Mean
Females	12,13	221	191	154	120	65	150
	12,55	244	205	159	112	69	158
	12,97	240	193	163	117	74	157
	13,39	237	213	167	121	70	161
Mean		235	201	161	117	70	157

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	1,933	5,54	7,42
Mean of 5 entries	2,161	6,19	8,29
Mean of 20 entries	4,322	12,38	16,58
	CV	4,78%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	2	42,33	21,16	0,38 NS
Methionine	4	207395,16	51848,79	925,05 **
Energy	3	1040,18	346,73	6,19 **
Methionine x energy	12	1459,22	121,61	2,17 *
Error	38	2129,85	56,05	
Total	59	212066,73		

SEM, CV etc see footnote Table 6.4

TABLE E 10.3 Food intake (g/bird d) for female broilers from 10 - 21 days of age

Food Intake

	DME MJ/kg	Methionine/DME Ratio 0,59	0,40	0,31	0,23	0,14	Mean
Females	12,13	34,0	36,1	36,8	38,9	34,8	36,1
	12,55	36,4	36,6	36,0	35,0	35,1	35,8
	12,97	34,7	33,8	36,0	35,3	35,5	35,1
	13,39	33,1	35,8	36,2	36,5	33,3	35,0
Mean		34,5	35,6	36,3	36,4	34,6	35,5

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,398	1,14	1,53
Mean of 5 entries	0,445	1,27	1,71
Mean of 20 entries	0,891	2,55	3,42
	CV	4,35%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	2	3,17	1,58	0,66 NS
Methionine	4	37,63	9,41	3,95 **
Energy	3	13,80	4,60	1,93 NS
Methionine x energy	12	53,72	4,48	1,88 NS
Error	38	90,45	2,38	
Total	59	198,77		

SEM, CV etc see footnote Table 6.4

TABLE 10.4 Dietary metabolisable energy intake (MJ/bird d) for female broilers from 10 - 21 days of age

MED Intake

	DME MJ/kg	Methionine/DME Ratio 0,49	0,40	0,31	0,23	0,14	Mean
Females	12,13	0,45	0,48	0,49	0,52	0,46	0,48
	12,55	0,50	0,50	0,50	0,48	0,48	0,49
	12,97	0,49	0,48	0,51	0,50	0,50	0,50
	13,39	0,48	0,52	0,53	0,53	0,49	0,51
Mean		0,48	0,50	0,51	0,51	0,48	0,50

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,005	0,01	0,02
Mean of 5 entries	0,006	0,02	0,02
Mean of 20 entries	0,012	0,03	0,05
	CV	4,30%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	2	0,0006	0,0003	0,60 NS
Methionine	4	0,0073	0,0018	3,60 *
Energy	3	0,0076	0,0025	5,00 **
Methionine x energy	12	0,0104	0,0009	1,80 NS
Error	38	0,0172	0,0005	
Total	59	0,0431		

SEM, CV etc see footnote Table 6.4

TABLE 10.5 Food conversion efficiency for female broilers from 10 - 21 days of age

F C E

	DME MJ/kg	Methionine/DME Ratio 0,49	0,40	0,31	0,23	0,14	Mean
Females	12,13	0,70	0,59	0,54	0,43	0,24	0,50
	12,55	0,61	0,60	0,57	0,46	0,25	0,50
	12,97	0,67	0,59	0,60	0,46	0,30	0,53
	13,39	0,61	0,64	0,56	0,47	0,29	0,51
Mean		0,65	0,60	0,57	0,46	0,27	0,51

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 4 entries	0,011	0,03	0,04
Mean of 5 entries	0,013	0,04	0,05
Mean of 20 entries	0,025	0,07	0,10
	CV	8,57%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	2	0,0007	0,0004	0,21 NS
Methionine	4	1,0987	0,2747	144,58 **
Energy	3	0,0066	0,0022	1,16 NS
Methionine x energy	12	0,0338	0,0028	1,47 NS
Error	38	0,0723	0,0019	
Total	59	1,2120		

SEM, CV etc see footnote Table 6.4

subjected to statistical analysis, using the method of Rayner (1967). Means, standard errors of means (SEM) and least significant differences (LSD) at P < 0,05 and P < 0,01 were calculated.

RESULTS

Means of the twenty treatments for the variates mass gain, lysine intake, food intake, dietary metabolisable energy intake and food conversion efficiency are given in Tables 10.1 to 10.5 respectively. The main effects of DME and the methionine/DME ratio are also shown, together with standard errors of the means; LSD's and an analysis of variance table for each variate.

Response to dietary methionine concentration

The Reading model was used to describe the relationship between body mass gain and methionine intake. Equations were fitted to the data from each energy level, as well as to the combined data. The parameters used in fitting these equations and the resulting coefficients, are given in Table 10.6. The combined equation, together with the means for each energy level, are illustrated in Figure 10.1.

TABLE 10.6 Parameters used in determining the relationship between mass gain (g/bird d) and methionine intake (mg/bird d) by means of the Reading model using male chickens from 10 - 21 days of age

Parameter	Energy concentration (MJ/kg)				
	12,13	12,55	12,97	13,39	Combined
$\bar{W}$	250,00	250,00	250,00	250,00	250,00
$\Delta\bar{W}$	25,02	24,42	24,37	23,57	24,19
a	6,49	5,81	5,86	5,78	5,75
b	0,02	0,03	0,02	0,03	0,04
$\sigma\Delta\bar{W}$	3,00	5,07	5,28	5,17	5,15
$\sigma\bar{W}$	40,00	40,00	40,00	40,00	40,00
r	0,60	0,60	0,60	0,60	0,60

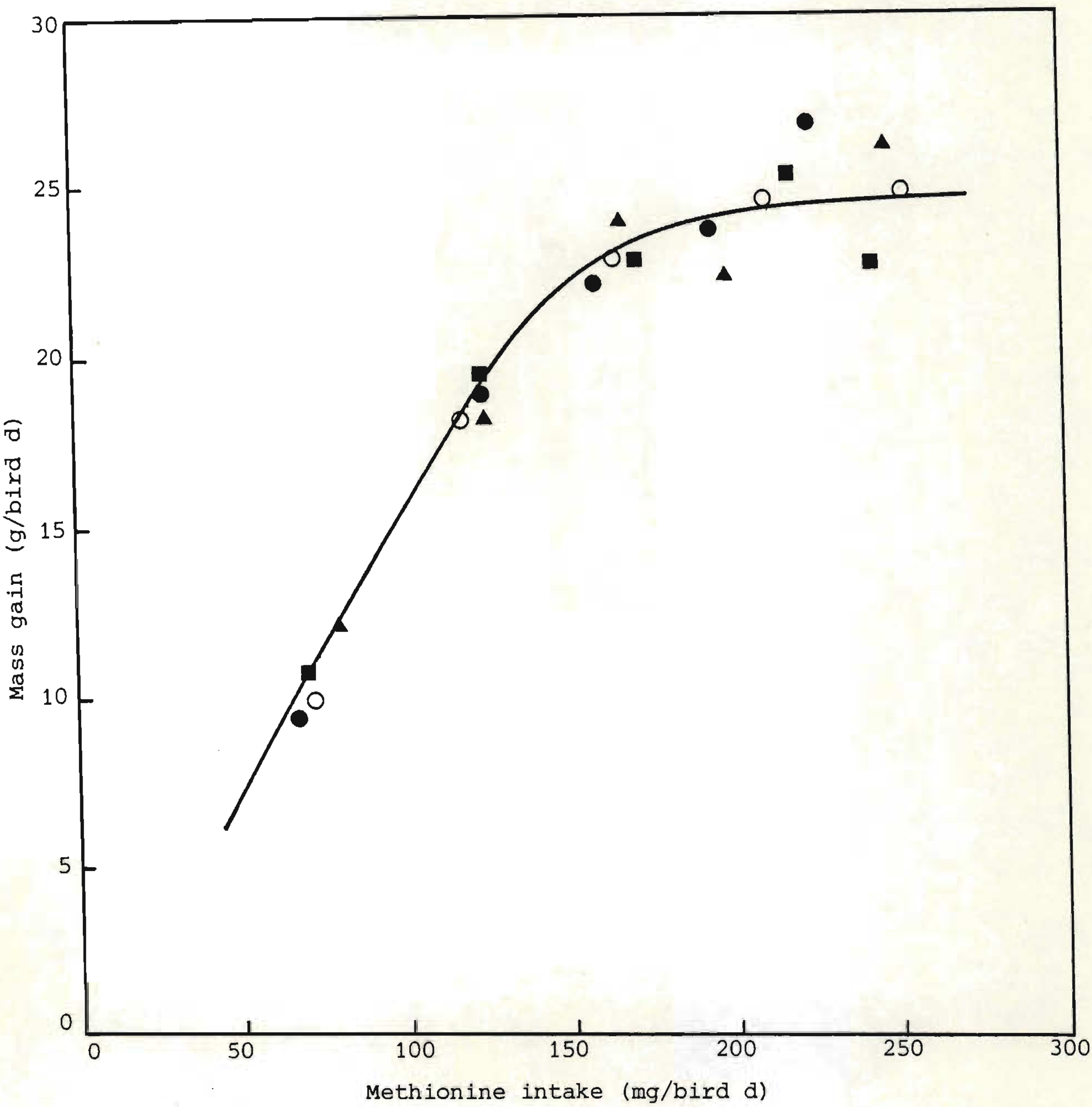

Figure 10.1 Response in mass gain (g/bird d) of female broilers from 10 - 21 days of age to methionine intake (mg/bird d) and estimated response using the Reading model (Table 10.6)

● 12,13 MJ/kg ▲ 12,97 MJ/kg

○ 12,55 MJ/kg ■ 13,39 MJ/kg

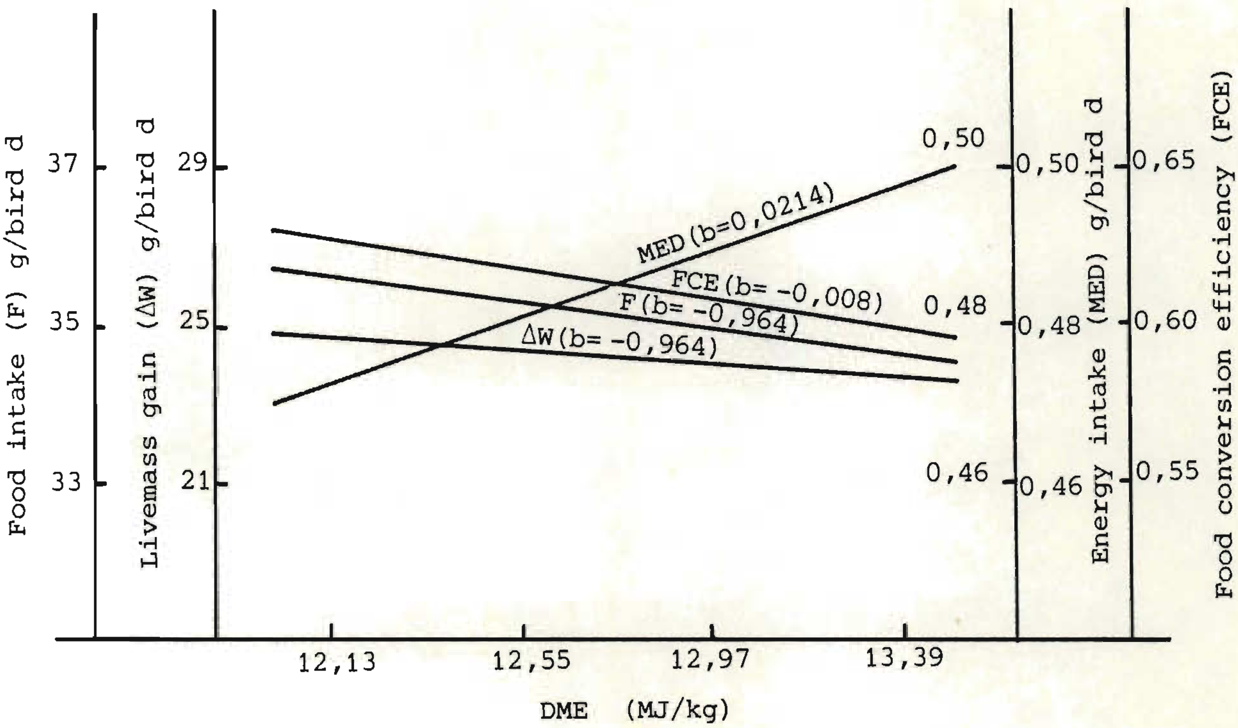

Figure 10.2 Pooled linear regressions of female broiler performance characteristics on dietary metabolisable energy level for the starter period

TABLE 9.10 Pooled linear regression coefficients of female broiler performance characteristics on dietary metabolisable energy level for the starter period (units are per MJ/kg)

Character regressed on DME	Constant term	Regression coefficient (b)	SE (b)
Livemass gain (ΔW)	36,442	-0,964	0,849[NS]
Food intake (F)	47,367	-0,964	0,821[NS]
Energy intake (MED)	0,214	0,021	0,011[NS]
Food conversion efficiency (FCE)	0,733	-0,008	0,023[NS]

[NS] denotes non significant

TABLE 10.8 The regression of mass gain on methionine/energy ratio and optimum methionine/energy ratio for female chickens from 10 - 21 days of age

DME (MJ/kg)	Constant term	Linear term (b_1)	Quadratic term ($\times 10^{-2}$) (b_2)	SE (b)	Multiple correlation coefficient (R)	Optimum Methionine/energy ratio (g/kg:MJ/kg)
12,13	-5,64	1,294	-1,337	3,37^{NS}	0,987	0,484
12,55	-7,84	1,510	-1,747	0,69**	0,999	0,432
12,97	-0,70	1,071	-1,119	4,68^{NS}	0,963	0,479
13,39	-9,14	1,705	-2,171	1,55**	0,996	0,393
Combined	-5,83	1,395	-1,594	1,60*	0,996	0,438

^{NS}, * etc see footnote Table 6.4

TABLE 10.9 The regression of food conversion efficiency on methionine/energy ratio and optimum methionine/energy ratio for female chickens from 10 - 21 days of age

DME (MJ/kg)	Constant term	Linear term (b_1)	Quadratic term ($\times 10^{-3}$) (b_2)	SE (b)	Multiple correlation coefficient (R)	Optimum Methionine/energy ratio (g/kg:MJ/kg)
12,13	-5,430	0,025	-0,190	0,07*	0,992	0,646
12,55	-0,186	0,038	-0,444	0,04**	0,997	0,426
12,97	-1,463	0,027	-0,266	0,08^{NS}	0,983	0,504
13,39	-0,124	0,035	-0,407	0,03**	0,998	0,429
Combined	-0,095	0,031	-0,327	0,04**	0,997	0,475

^{NS}, * etc see footnote Table 6.4

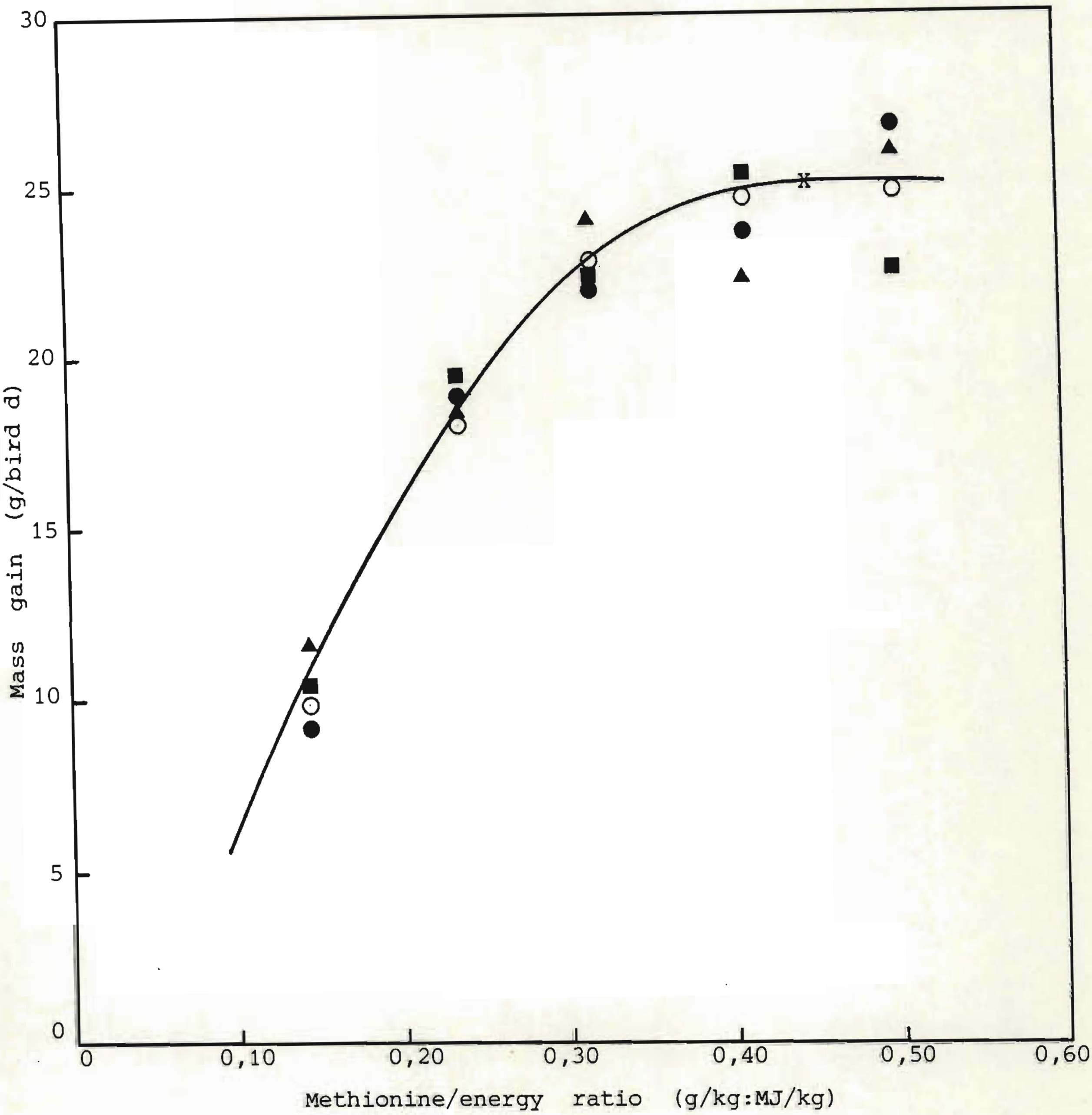

Figure 10.3 Response in mass gain (g/bird d) of female broilers from 11 - 21 days of age to methionine/energy ratio and estimated optimum methionine/energy ratio (X)

● 12,13 MJ/kg ▲ 12,97 MJ/kg

○ 12,55 MJ/kg ■ 13,39 MJ/kg

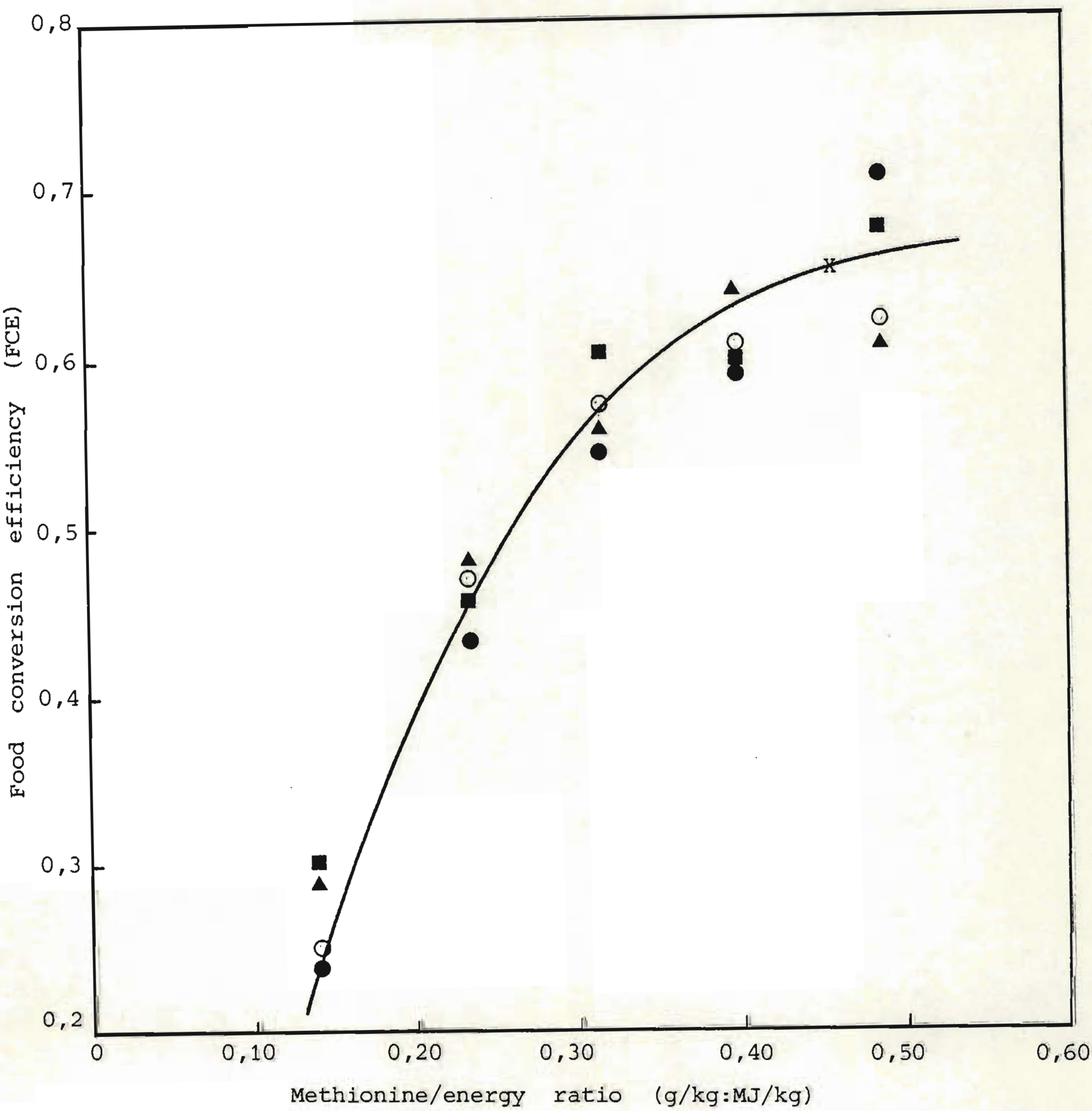

Figure 10.4 Response in food conversion efficiency of female broilers from 10 - 21 days of age to methionine/energy ratio and estimated optimum methionine/energy ratio (X)

● 12,13 MJ/kg ▲ 12,97 MJ/kg

○ 12,55 MJ/kg ■ 13,39 MJ/kg

Response to dietary energy concentration

Data used to measure the response to DME consisted of the results of the first and second dilution series only. There were thus 24 values that were pooled for each of the variates ΔW, F, MED and FCE. Linear responses were calculated and the equations are presented in Table 10.7 and illustrated in Figure 10.2.

Response to methionine/DME ratio

Curvilinear regressions of mass gain and FCE on methionine/DME ratio were calculated using multiple regression analysis. The coefficients at each energy level and for the combined data over all energy concentrations are given in Tables 10.8 and 10.9 and illustrated in Figures 10.3 and 10.4 respectively.

DISCUSSION

The major objective of this experiment was to measure the response of growing chickens to methionine intake using the dilution technique and to determine the optimum methionine intake in practical diets for female broilers during the starter period.

Response to dietary methionine concentration

Equations relating mass gain to methionine intake, fitted to the data for each energy level and to the combined data over all energy concentrations (Table 10.6) indicate that at marginal costs of 280 c/kg for methionine, and 150 c/kg for broiler mass, the optimum intake of methionine at each energy level for the females would be 293, 247, 247 and 238 mg/bird d. As in the case of the male chickens fed these diets the optimum intakes at each energy level show no trend nor do they vary much one from the other, indicating that methionine intake was of importance in determining growth rate irrespective of the energy concentration of the diet. From the combined data (illustrated in Figure 10.1) the optimum intake was calculated to be 245 mg/bird d. This intake is lower than that of 266 mg/bird d determined for males (Chapter 9) in spite of the fact that the females grew more rapidly than did the males (19.9 vs 19.3 g/bird d).

A comparison between the responses obtained with male and female broilers in this and the previous experiment should be viewed with caution. Because the trials were not conducted simultaneously, nor were the diets for the two trials made at the same time, variations in ingredient composition and chicken quality could account for the differences that were evident between the sexes.

The combined *a* coefficient, indicating the efficiency with which chickens utilise methionine for growth, was higher for males (6,58 mg/g body mass) than for females (5,75 mg/g body mass). This indicates that females were more efficient than males in utilising the dietary methionine specified in Chapters 9 and 10. The maintenance requirement was similar for both sexes (b = 0,04 mg/g body mass).

The effect of the methionine concentration in the diet on food intake was almost identical for both sexes. In the females the food consumption increased progressively in relation to the induced methionine deficiency, until maximum intake was reached in the fourth dilution series. The intake then decreased in the fifth dilution series which had the lowest methionine content.

The effect of the methionine content of the diets on FCE is presented in Table 10.5. The highest FCE was recorded in the first dilution series, the response in FCE then declined progressively as the methionine level decreased in the remaining dilution series. The females responded to the high methionine concentration in the first dilution series diets whereas the males recorded their peak FCE in the second dilution series.

Response to dietary energy concentration

The effect of DME on ΔW, F, MED and FCE was examined by means of linear regression analysis, and the pooled results for the first and second dilution series are presented in Table 10.7 and in Figure 10.2.

The livemass response to DME in the female broiler was non significant, a similar response being recorded for males. Unlike the males, the females did not regulate their energy intake during the starter period (Table 10.4) this accounting for the increase in MED intake as the energy content of the diets was raised.

Food conversion efficiency of both females and males improved as the DME was increased. However, the magnitude of the response was not large enough to be significant. Because females adjusted intake of DME less efficiently than did males and hence showed a greater improvement in growth rate as DME was increased, FCE was expected also to improve more rapidly in females than in males with increasing DME.

Response to methionine/DME ratio

The results presented in Table 10.1 show that the maximum response in ΔW to methionine/DME ratio was recorded in the diets of the first dilution series. The response in ΔW decreased progressively as the methionine/DME ratio narrowed in the lower dilutions. For the combined data illustrated in Figure 10.3 the optimum ratio was 0,438 g methionine/MJ. This figure is slightly higher than the optimum ratio determined for males (0,417 g methionine/MJ). These optimum ratios are slightly higher than the optimum methionine/energy ratio of 0,40 g methionine/MJ recommended by Thomas *et al.* (1978) for broilers during the starter period.

The effect of varying the methionine/energy ratio on food conversion efficiency was examined by means of a multiple regression analysis, the results of which are presented in Table 10.9 and illustrated in Figure 10.4. The optimum methionine/DME ratio for the combined data was 0,475 g methionine/MJ which is higher than the optimum of 0,43 g methionine/MJ determined for males. The optimum ratios determined for both males and females confirm the opinion that higher dietary concentrations of methionine are required for optimum FCE than for optimum body mass gain. The results of this study indicate that a methionine/DME ratio of 0,427 g methionine/MJ would be ideal for practical diets during the starter period. A wider methionine/energy ratio would elicit greater responses in FCE, but it is doubtful whether these higher ratios would be the most profitable.

The results of this trial will be used in a later chapter (Chapter 12) where the experimental results relating dietary methionine and DME to broiler performance will be summarised and discussed.

CHAPTER 11

RESPONSE OF MALE AND FEMALE BROILERS TO DIETARY METHIONINE AND METABOLISABLE ENERGY CONCENTRATIONS DURING THE PERIOD 21 TO 45 DAYS OF AGE

INTRODUCTION

Methionine is one of the limiting essential amino acids in chicken nutrition, especially in broiler diets based on soyabean and maize. Thus numerous studies have been conducted, using the classical method to determine the optimum methionine intake of broilers during all stages of their production cycle. The diet dilution technique of Fisher and Morris (1970) has been applied by Freeman (1979) to determine the available tryptophan requirement of broilers, and by Gous (1980) to measure the response to lysine intake. The aim of this experiment was, therefore, to increase the data pertaining to the response of broilers to methionine using the dilution technique and to compare the results obtained with other reports where the classical technique was used. In this experiment the response to dietary methionine and energy of male and female broilers was tested, in order to determine the optimum intake of these two nutrients during the finisher period.

MATERIALS AND METHODS

Day-old chickens of the Ross broiler strain were allocated at random to 80 pens, such that 40 pens each contained 180 male chicks, and 40 pens contained 180 female chicks. The stocking density was 19,4 birds/m^2. The pens were in an environmentally controlled broiler house of a commercial design and management procedures that conformed as closely as possible with commercial practice were adopted. The chickens were reared on a commercial broiler starter diet to 21 days of age, at which time the numbers in each pen were equalised, and the initial mass of the birds in each pen was determined.

A summit and a dilution diet based on the principles applied by Pilbrow and Morris (1974) were formulated at a DME level of 12,55 MJ/kg with amino acid levels based on the recommendations of Thomas *et al.* (1978) for the finisher period. The available lysine and methionine figures quoted were converted to total requirements based on an availability of 90 percent. The dilution diet was formulated at the same net energy level as the summit diet for reasons previously discussed (Chapter 6). The wide range of methionine contents required in the experimental diets was obtained by formulating the summit diet calculated to contain 1,20 times and the dilution diet 0,36 times the recommended level, and the intermediate methionine contents were obtained by blending these diets in appropriate proportions as shown in Table 11.3. The above formulation procedure was repeated at DME levels of 12,97; 13,39 and 13,81 MJ/kg as shown in Table 11.2. The composition and analysis of the summit and dilution diets is presented in Table 11.1 and their blended intermediate diets are shown in Table 11.3.

Specific protein contents were not used in formulation but the minimum contents of all essential amino acids except methionine were set at 1,75 times for the summit and 0,7 times for the dilution, of the requirements proposed by Thomas *et al.* (1978). In some instances it was not possible to achieve the minimum levels of some of the amino acids, in which case the most feasible levels were then accepted.

The 20 experimental diets were mixed according to the procedure

TABLE 11.1 Composition (g/kg) of the summit and dilution diets

	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet
Diet code	1	5	6	10	11	15	16	20
DME (MJ/kg)	12,55		12,97		13,39		13,81	
DNE (MJ/kg)	8,77	8,77	9,15	9,15	9,41	9,41	9,77	9,77
Ingredients								
Yellow maize meal	549,7	139,7	596,5	191,7	520,5	204,0	459,3	247,0
Wheat bran		560,0		497,0		430,0		355,0
Soyabean unextracted	41,0				164,0		250,0	
Sunflower meal	172,0		5,0					
Groundnut meal	6,0	16,0	92,0	26,0	36,0	79,0	8,0	114,0
Fish meal	133,0		205,0		206,0		198,0	
Blood meal	25,0		25,0	5,0				
Feather meal	52,0		64,0		62,0		68,0	
Bone meal	7,0	31,0	2,0	32,0		28,0	13,0	18,0
Monocalcium phosphate						3,0		7,0
Limestone flour	8,0	6,0	7,0	5,0	8,0	7,0		10,0
Salt		4,0		4,0		4,0		4,0
Sunflower oil		100,0		100,0		100,0		100,0
Starch		137,0		133,0		138,0		138,0
Lysine HCl	2,8	2,8		2,8		3,5		3,5
DL - Methionine	0,03						0,22	
Vitamins and trace minerals *	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0
Anti-coccidial	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5

TABLE 11.1 continued

	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet	Summit diet	Dilution diet
Diet code	1	5	6	10	11	15	16	20
DME (MJ/kg)	12,55		12,97		13,39		3,81	
DNE (MJ/kg)	8,77	8,77	9,15	9,15	9,41	9,41	9,77	9,77
Calculated analysis								
Arginine	17,5	7,9	17,4	8,0	18,1	9,5	19,2	10,2
Histidine	6,5	2,1	6,4	2,3	6,1	2,5	6,4	2,6
Isoleucine	9,9	3,1	10,4	3,1	12,0	3,6	12,8	3,8
Leucine	24,9	7,1	26,9	7,9	26,4	8,3	27,5	9,0
Lysine	15,5	6,3	14,9	6,6	16,0	7,2	17,3	7,3
Methionine	5,9	1,7	6,1	1,8	6,3	1,9	6,5	1,9
Cystine	5,3	2,7	5,0	2,7	5,2	2,7	5,5	2,8
Phenylalanine	12,3	4,1	12,6	4,4	12,5	4,9	13,2	5,3
Tyrosine	7,4	3,1	8,4	3,3	8,8	3,8	9,0	4,2
Threonine	10,5	3,5	10,8	3,7	11,4	3,8	12,3	3,9
Tryptophan	3,1	1,4	3,1	1,4	3,4	1,5	3,7	1,5
Valine	14,9	5,0	15,8	5,3	15,6	5,4	16,4	5,7
Calcium	10,0	10,0	10,0	10,0	10,0	10,0	10,0	10,0
Phosphorus	7,0	8,0	7,0	8,0	7,0	8,0	8,0	8,0
Crude protein (gN×6,25/kg)	287,0	114,0	300,0	116,0	317,0	124,0	328,0	128,0

Supplies per kg of diet: Vit A 7 025 IU, Vit D_3 2 000 IU, Vit E 8,5 IU, Hetrazine 3 ppm, Thiamine hydrochloride 0,969 ppm, Riboflavin 8 ppm, Calcium pantothenate 7,837 ppm, Niacin 24, 42 ppm, Folic acid 0,95 ppm

Copper 125 ppm, Iron 40 ppm, Zinc 28 ppm, Iodine 2 ppm, Manganese 78 ppm, Selenium 0,1 ppm

TABLE 11.2 Calculated amino acid contents of the summit and dilution diets relative to the requirements of broilers during the finisher period

	Requirements according to Thomas *et al.* (1978) (g/kg)	Amino acid contents expressed as multiples of requirement	
		Summit diet	Dilution diet
Diet code		1	5
Arginine	11,32	1,55	0,70
Histidine	4,09	1,59	0,51
Isoleucine	7,77	1,27	0,40
Leucine	14,32	1,74	0,50
Lysine	10,91	1,42	0,58
Methionine	5,00	1,18	0,34
Cystine	3,41	1,55	0,79
Phenylalanine	7,23	1,70	0,57
Tyrosine	6,14	1,20	0,50
Threonine	7,23	1,45	0,48
Tryptophan	1,64	1,89	0,85
Valine	8,73	1,71	0,57
Diet code		6	10
Arginine	11,69	1,49	0,68
Histidine	4,23	1,51	0,54
Isoleucine	8,03	1,30	0,39
Leucine	14,79	1,82	0,53
Lysine	11,27	1,32	0,59
Methionine	5,17	1,18	0,35
Cystine	3,52	1,42	0,77
Phenylalanine	7,47	1,69	0,59
Tyrosine	6,34	1,32	0,52
Threonine	7,47	1,45	0,50
Tryptophan	1,69	1,83	0,83
Valine	9,02	1,75	0,59

TABLE 11.2 continued

	Requirements according to Thomas *et al.* (1978) (g/kg)	Amino acid contents expressed as multiples of requirement	
		Summit diet	Dilution diet
Diet code		11	15
Arginine	12,08	1,50	0,79
Histidine	4,37	1,40	0,57
Isoleucine	8,29	1,45	0,43
Leucine	15,28	1,73	0,54
Lysine	11,64	1,37	0,62
Methionine	5,34	1,18	0,36
Cystine	3,64	1,43	0,74
Phenylalanine	7,71	1,62	0,64
Tyrosine	6,55	1,34	0,58
Threonine	7,71	1,48	0,49
Tryptophan	1,75	1,94	0,86
Valine	9,31	1,68	0,58
Diet code		16	20
Arginine	12,45	1,54	0,82
Histidine	4,50	1,42	0,58
Isoleucine	8,55	1,50	0,44
Leucine	15,75	1,75	0,57
Lysine	12,00	1,44	0,61
Methionine	5,50	1,18	0,35
Cystine	3,75	1,47	0,75
Phenylalanine	7,95	1,66	0,67
Tyrosine	6,75	1,33	0,62
Threonine	7,95	1,55	0,49
Tryptophan	1,80	2,06	0,83
Valine	9,60	1,71	0,59

TABLE 11.3 Summary of dilution technique and calculated analysis of the experimental diets

Diet code	Blending ratio			Calculated dietary methionine (g/kg)	Calculated dietary protein (g/kg)
	Summit diet		Dilution diet		
12,55 MJ/kg					
1	100	+	0	5,90	287,0
2	75	+	25	4,85	243,0
3	50	+	50	3,80	200,0
4	25	+	75	2,75	157,0
5	0	+	100	1,70	114,0
12,97 MJ/kg					
6	100	+	0	6,10	300,0
7	75	+	25	5,03	254,0
8	50	+	50	3,95	208,0
9	25	+	75	2,88	162,0
10	0	+	100	1,80	116,0
13,39 MJ/kg					
11	100	+	0	6,30	317,0
12	75	+	25	5,20	269,0
13	50	+	50	4,10	220,0
14	25	+	75	3,00	174,0
15	0	+	100	1,90	124,0
13,81 MJ/kg					
16	100	+	0	6,50	328,0
17	75	+	25	5,35	278,0
18	50	+	50	4,20	228,0
19	25	+	75	3,05	178,0
20	0	+	100	1,90	128,0

described in Chapter 8 to minimise any raw ingredient variation between diets. The treatments were allocated such that there were two pens of each sex fed each diet. Pelleted food in tube feeders and water were provided *ad lib.*. Body mass of the birds was measured at 21 and at 45 days of age and food consumption was recorded during this period.

The criteria studied were growth rate and food intake during the finisher period. The data on each variate were subjected to statistical analysis using the method of Rayner (1967). Means, standard errors of the means (SEM) and least significant differences (LSD) at $P < 0,05$ and $P < 0,01$ were calculated.

RESULTS

Means of the twenty treatments for the variates mass gain, methionine intake, food intake, dietary metabolisable energy intake and food conversion efficiency for the females, males and combined sexes are given in Tables 11.4 to 11.8 respectively. The main effects of DME and the methionine/DME ratio are also shown, together with standard errors of the means; LSD's and an analysis of variance table for each variate.

Response to dietary methionine concentration

The Reading model described in Chapter 1 was used to fit an equation to the data relating body mass gain to methionine intake. Equations for the females, males and combined sexes were fitted to data from each energy level, as well as to the combined data. The parameters used in fitting these equations, and the resulting coefficients, estimated and calculated as described in Chapter 6, are given in Table 11.9. The combined equations, together with the means for each energy level are illustrated in Figures 11.1, 11,2 and 11.3 respectively.

TABLE 11.4 Mass gain (g/bird d) for male and female broilers from 21 - 45 days of age

Mass Gain

	ME	Methionine/DME Ratio					Mean
	MJ/kg	0,47	0,39	0,30	0,22	0,14	
Females	12,55	41,7	41,9	38,5	36,0	25,0	36,6
	12,97	41,5	42,2	40,5	38,0	28,3	38,1
	13,39	41,3	42,3	39,9	35,8	27,3	37,3
	13,81	42,6	42,9	40,1	36,6	25,6	37,6
Mean		41,8	42,3	39,7	36,6	26,6	37,4
Males	12,55	47,9	47,6	46,1	41,8	25,7	41,8
	12,97	47,7	47,9	46,6	42,2	31,9	43,2
	13,39	43,6	47,4	44,1	41,2	31,8	41,6
	13,81	47,8	48,6	46,6	40,7	30,1	42,7
Mean		46,7	47,8	45,8	41,5	29,9	42,4
Mean of Females and Males Combined	12,55	44,8	44,8	42,3	38,9	25,4	39,2
	12,97	44,6	45,0	43,5	40,1	30,1	40,7
	13,39	42,5	44,9	42,0	38,5	29,5	39,5
	13,81	45,1	45,7	43,3	38,6	27,9	40,2
Mean		44,3	45,1	42,8	39,0	28,2	39,8

TABLE 11.4 continued

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 2 entries	0,192	0,55	0,74
Mean of 4 entries	0,271	0,78	1,04
Mean of 5 entries	0,303	0,87	1,16
Mean of 20 entries	0,606	1,73	2,33
	CV	3,04%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	1	5,32	5,32	3,62 NS
Methionine	4	3067,98	767,00	521,77 **
Energy	3	25,84	8,61	5,86 **
Sex	1	488,25	488,25	332,14 **
Methionine x energy	12	61,38	5,12	3,48 **
Methionine x sex	4	17,10	4,28	2,91 *
Energy x sex	3	2,78	0,93	0,63 NS
Methionine x energy x sex	12	25,38	2,11	1,44 NS
Error	39	57,28	1,47	
Total	79	3751,31		

SEM, CV etc see footnote Table 6.4

TABLE 11.5 Methionine intake (mg/bird d) for male and female broilers from 21 - 45 days of age

Methionine Intake

	ME	Methionine/DME Ratio					Mean
	MJ/kg	0,47	0,39	0,30	0,22	0,14	
Females	12,55	532	442	337	254	157	344
	12,97	538	447	342	267	176	354
	13,39	540	436	364	270	167	355
	13,81	535	442	359	277	165	355
Mean		536	442	351	267	166	352
Males	12,55	594	486	391	288	169	386
	12,97	608	474	393	287	188	390
	13,39	625	499	406	301	187	404
	13,81	619	504	406	313	183	405
Mean		611	491	399	297	182	396
Mean of Females and Males Combined	12,55	563	464	364	271	163	365
	12,97	573	461	368	277	182	372
	13,39	583	468	385	286	177	380
	13,81	577	473	382	295	174	380
Mean		574	466	375	282	174	374

TABLE 11.5 continued

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 2 entries	1,456	4,17	5,58
Mean of 4 entries	2,059	5,89	7,89
Mean of 5 entries	2,302	6,59	8,82
Mean of 20 entries	4,604	13,17	17,64
	CV	2,46%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	1	549,21	549,21	6,48*
Methionine	4	1551138,62	387784,66	4574,01**
Energy	3	3105,41	1035,14	$2,21^{NS}$
Sex	1	38145,00	38145,00	449,93**
Methionine × energy	12	1478,78	123,23	8,45**
Methionine × sex	4	8079,76	2019,94	23,83**
Energy × sex	3	615,41	205,14	$2,42^{NS}$
Methionine × energy × sex	12	951,59	79,30	$0,94^{NS}$
Error	39	3306,56	84,78	
Total	79	1607370,30		

SEM, CV etc see footnote Table 6.4

TABLE 11.6 Food intake (g/bird d) for male and female broilers from 21 - 45 days of age

Food Intake

	ME MJ/kg	Methionine/DME Ratio 0,47	0,39	0,30	0,22	0,14	Mean
Females	12,55	90,2	90,3	88,8	90,6	92,3	90,4
	12,97	88,1	89,4	87,8	92,1	97,8	91,1
	13,39	85,8	83,9	88,7	93,2	87,9	87,9
	13,81	82,3	83,4	85,5	89,4	86,6	85,4
Mean		86,6	86,8	87,7	91,3	91,2	88,7
Males	12,55	100,7	99,2	102,9	102,9	99,2	101,0
	12,97	99,6	94,8	100,9	98,9	104,4	99,7
	13,39	99,2	96,0	99,0	103,9	98,4	99,3
	13,81	95,2	95,1	96,6	101,1	96,1	96,9
Mean		98,7	96,3	99,8	101,7	99,5	99,2
Mean of Females and Males Combined	12,55	95,4	94,7	95,8	96,8	95,8	95,7
	12,97	93,9	92,1	94,4	95,5	101,1	95,4
	13,39	92,5	90,0	93,9	98,5	93,2	93,6
	13,81	88,8	89,3	91,0	95,3	91,4	91,1
Mean		92,6	91,5	93,8	96,5	95,4	94,0

TABLE 11.6 continued

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 2 entries	0,370	1,06	1,42
Mean of 4 entries	0,523	1,50	2,00
Mean of 5 entries	0,585	1,67	2,24
Mean of 20 entries	1,169	3,34	4,48
	CV	2,50%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	1	59,33	59,33	10,85 **
Methionine	4	259,30	64,82	11,85 **
Energy	3	262,72	87,57	16,01 **
Sex	1	2208,57	2208,57	403,76 **
Methionine x energy	12	197,72	16,48	3,01 **
Methionine x sex	4	43,50	10,87	1,99 NS
Energy x sex	3	25,53	8,51	1,56 NS
Methionine x energy x sex	12	48,16	4,01	0,73 NS
Error	39	213,33	5,47	
Total	79	3318,15		

SEM, CV etc see footnote Table 6.4

TABLE 11.7 Dietary metabolisable energy intake (MJ/bird d) for male and female broilers from 21 - 45 days of age

MED Intake

	ME MJ/kg	Methionine/DME Ratio 0,47	0,39	0,30	0,22	0,14	Mean
Females	12,55	1,13	1,13	1,11	1,13	1,15	1,13
	12,97	1,14	1,15	1,13	1,19	1,26	1,17
	13,39	1,14	1,12	1,18	1,24	1,17	1,17
	13,81	1,14	1,15	1,18	1,23	1,20	1,18
Mean		1,14	1,14	1,15	1,20	1,20	1,16
Males	12,55	1,26	1,24	1,29	1,29	1,24	1,26
	12,97	1,29	1,22	1,30	1,28	1,35	1,29
	13,39	1,32	1,28	1,32	1,38	1,31	1,32
	13,81	1,31	1,31	1,33	1,40	1,33	1,34
Mean		1,29	1,26	1,31	1,34	1,31	1,30
Mean of Females and Males Combined	12,55	1,19	1,18	1,20	1,21	1,20	1,20
	12,97	1,21	1,19	1,22	1,23	1,30	1,23
	13,39	1,23	1,20	1,25	1,31	1,24	1,24
	13,81	1,23	1,23	1,26	1,31	1,26	1,26
Mean		1,21	1,20	1,23	1,27	1,25	1,23

TABLE 11.7 continued

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 2 entries	0,005	0,01	0,02
Mean of 4 entries	0,007	0,02	0,03
Mean of 5 entries	0,008	0,02	0,03
Mean of 20 entries	0,015	0,05	0,06
	CV	2,48%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	1	0,010	0,010	10,00 **
Methionine	4	0,046	0,011	11,00 **
Energy	3	0,042	0,014	14,00 **
Sex	1	0,382	0;382	382,00 **
Methionine x energy	12	0,035	0,003	3,00 **
Methionine x sex	4	0,007	0,002	2,00 NS
Energy x sex	3	0,007	0,002	2,00 NS
Methionine x energy x sex	12	0,008	0,007	7,00 **
Error	39	0,036	0,001	
Total	79	0,573		

SEM, CV etc see footnote Table 6.4

TABLE 11.8 Food conversion efficiency for male and female broilers from 21 - 45 days of age

F C E

	ME MJ/kg	Methionine/DME Ratio 0,47	0,39	0,30	0,22	0,14	Mean
Females	12,55	0,46	0,46	0,43	0,40	0,27	0,41
	12,97	0,47	0,47	0,46	0,41	0,29	0,42
	13,39	0,48	0,50	0,45	0,38	0,31	0,43
	13,81	0,52	0,52	0,47	0,41	0,30	0,44
Mean		0,48	0,49	0,45	0,40	0,29	0,42
Males	12,55	0,48	0,48	0,45	0,41	0,26	0,41
	12,97	0,48	0,51	0,46	0,43	0,31	0,44
	13,39	0,44	0,49	0,45	0,40	0,32	0,42
	13,81	0,50	0,51	0,48	0,40	0,31	0,44
Mean		0,47	0,50	0,46	0,41	0,30	0,43
Mean of Females and Males Combined	12,55	0,47	0,47	0,44	0,40	0,27	0,41
	12,97	0,48	0,49	0,46	0,42	0,30	0,43
	13,39	0,46	0,50	0,45	0,39	0,32	0,42
	13,81	0,51	0,51	0,48	0,41	0,30	0,44
Mean		0,48	0,49	0,46	0,40	0,30	0,43

TABLE 11.8 continued

SEM's and LSD's

	SEM	LSD (0,05)	LSD (0,01)
Mean of 2 entries	0,002	0,01	0,01
Mean of 4 entries	0,003	0,01	0,01
Mean of 5 entries	0,003	0,01	0,01
Mean of 20 entries	0,006	0,02	0,02
	CV	2,91%	

Analysis of variance

Source	DF	SS	MS	F
Replicates	1	0,0033	0,0033	21,71 **
Methionine	4	0,4077	0,1019	664,83 **
Energy	3	0,0104	0,0035	22,64 **
Sex	1	0,0003	0,0003	1,93 NS
Methionine x energy	12	0,0093	0,0008	5,03 **
Methionine x sex	4	0,0009	0,0002	1,51 NS
Energy x sex	3	0,0012	0,0004	2,62 NS
Methionine x energy x sex	12	0,0030	0,0002	1,61 NS
Error	39	0,0060	0,0002	
Total	79	0,4421		

SEM, CV etc see footnote Table 6.4

TABLE 11.9 Parameters used in determining the relationship between mass gain (g/bird d) and methionine intake (mg/bird d) by means of the Reading model using male and female chickens from 21 - 45 days of age

Parameter	Energy concentration (MJ/kg)				
	12,55	12,97	13,39	13,81	Combined
Females					
$\bar{W}$	800,00	800,00	800,00	800,00	800,00
$\Delta\bar{W}$	41,90	42,20	41,27	43,33	41,87
a	5,13	4,58	4,12	5,42	4,73
b	0,01	0,03	0,03	0,01	0,03
$\sigma\Delta\bar{W}$	20,95	21,10	26,15	21,45	21,15
$\sigma\bar{W}$	80,00	80,00	80,00	80,00	80,00
r	0,60	0,60	0,60	0,60	0,60
Males					
$\bar{W}$	800,00	800,00	800,00	800,00	800,00
$\Delta\bar{W}$	48,38	47,90	43,74	47,75	47,31
a	4,75	4,00	3,35	4,74	4,43
b	0,03	0,03	0,04	0,03	0,02
$\sigma\Delta\bar{W}$	23,95	30,95	35,70	24,30	27,90
$\sigma\bar{W}$	80,00	80,00	80,00	80,00	80,00
r	0,60	0,60	0,60	0,60	0,60
Males and females					
$\bar{W}$	800,00	800,00	800,00	800,00	800,00
$\Delta\bar{W}$	44,80	45,00	43,00	45,25	44,66
a	4,67	4,27	3,90	5,04	4,71
b	0,03	0,02	0,03	0,02	0,03
$\alpha\Delta\bar{W}$	22,40	27,50	30,45	22,85	22,55
$\sigma\bar{W}$	80,00	80,00	80,00	80,00	80,00
r	0,60	0,60	0,60	0,60	0,60

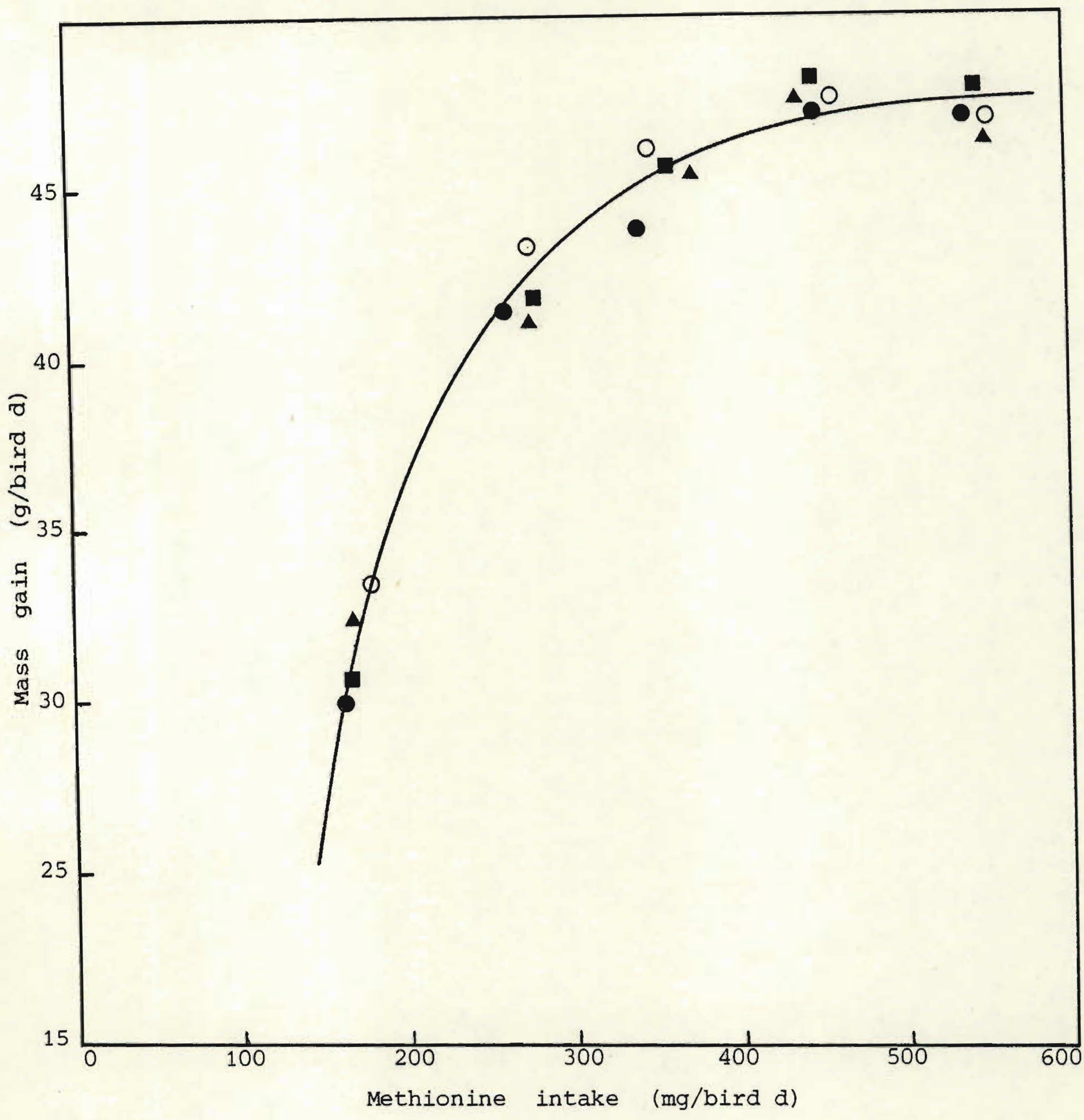

Figure 11.1 Response in mass gain (g/bird d) of female chickens from 21 - 45 days of age to methionine intake (mg/bird d) and estimated response using Reading model (Table 11.9)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

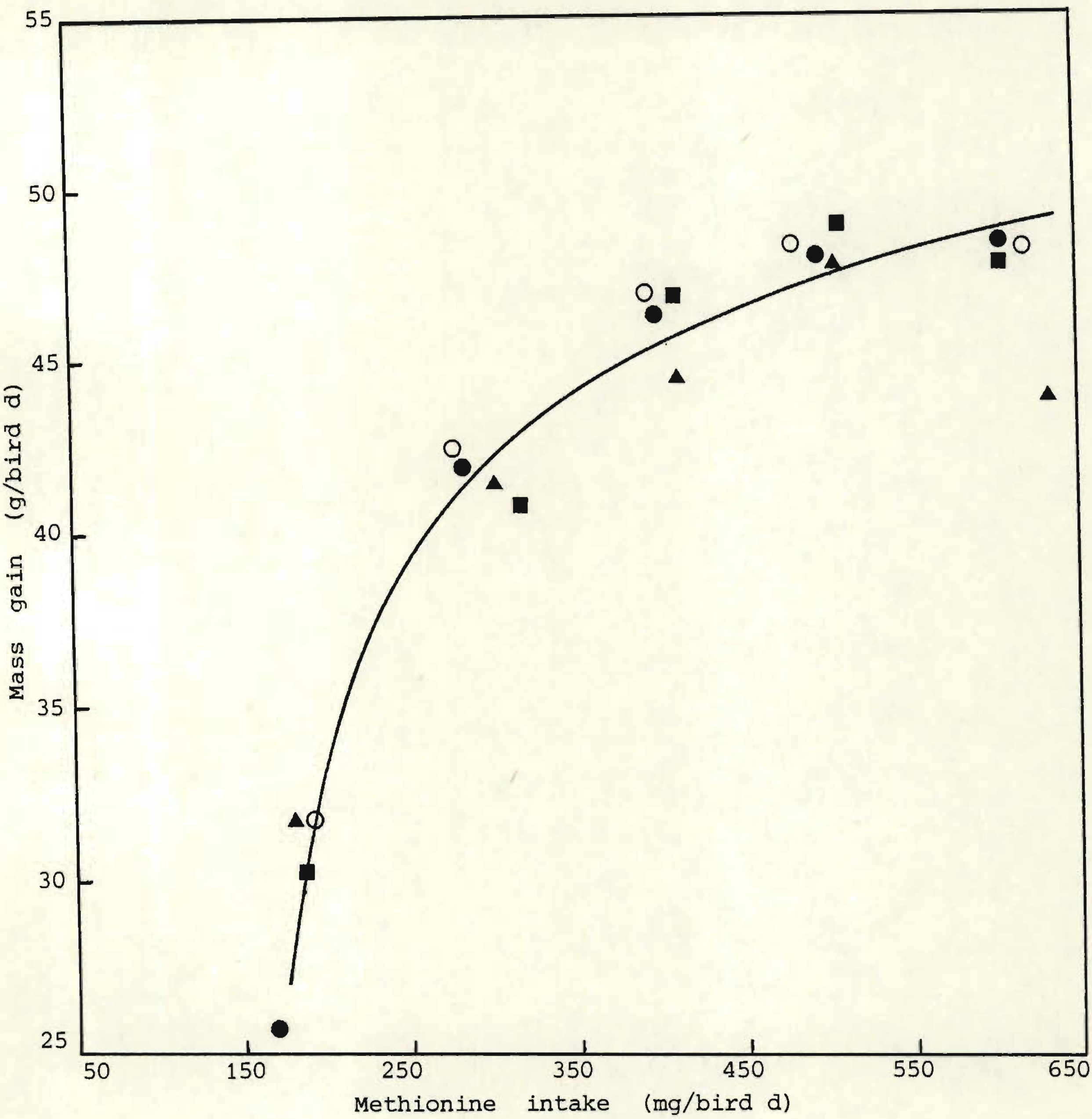

Figure 11.2 Response in mass gain (g/bird d) of male chickens from 21 - 45 days of age to methionine intake (mg/bird d) and estimated response using Reading model (Table 11.9)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

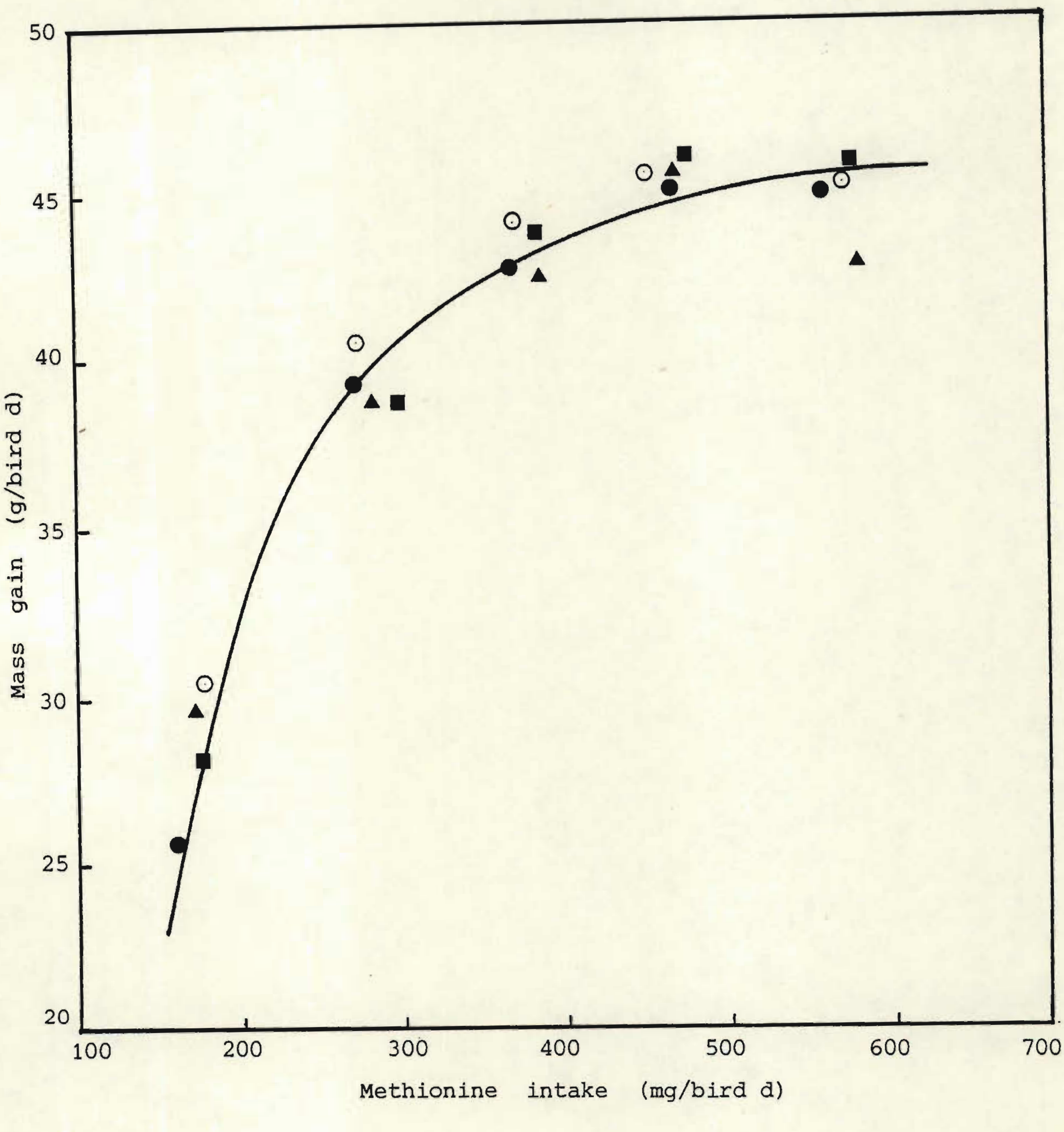

Figure 11.3 Response in mass gain (g/bird d) of male and female chickens from 21 - 45 days of age to methionine intake (mg/bird d) and estimated response using Reading model (Table 11.9)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,31 MJ/kg

TABLE 11.10 Pooled responses of performance characteristics on dietary metabolisable energy concentration for males, females and for the sexes combined in the finisher period (units are per MJ/kg)

Character regressed on DME	Constant term	Regression coefficient (b)	SE (b)
Livemass gain (ΔW, g/bird d)			
males	50,294	-0,226	0,645 NS
females	33,263	0,667	0,646 NS
combined	42,478	0,167	0,646 NS
Food intake (F, g/bird d)			
males	141,410	-3,333	1,245*
females	168,580	-6,214	1,245**
combined	154,065	-4,702	1,245**
Energy intake (MED, MJ/bird d)			
males	0,573	0,054	0,016**
females	1,043	0,007	0,016 NS
combined	0,737	0,036	0,016*
Food conversion efficiency (FCE, ΔW, g/F,g)			
males	0,345	0,011	0,040 NS
females	-0,143	0,048	0,040 NS
combined	0,125	0,027	0,040 NS

NS, * etc see footnote Table 6.4

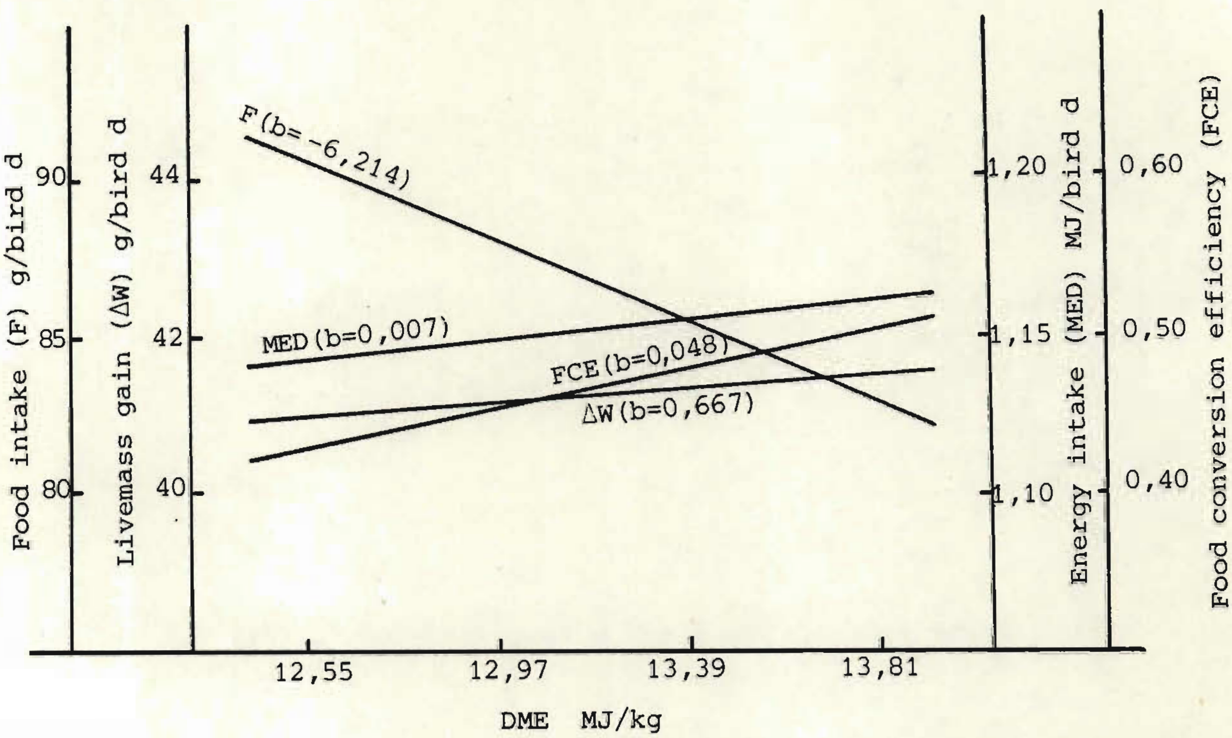

Figure 11.4 Pooler linear regressions of female broiler performance characteristics on dietary metabolisable energy level for the finisher period

Response to dietary energy concentration

Data used to measure the response to DME consisted of the results of the first and second dilution series only. There were thus 24 values that were pooled for each of the variates ΔW, F, MED and FCE. Linear responses to DME were calculated separately for each sex and for both sexes combined, the equations being presented in Table 11.10 and illustrated in Figures 11.4, 11.5 and 11.6 respectively.

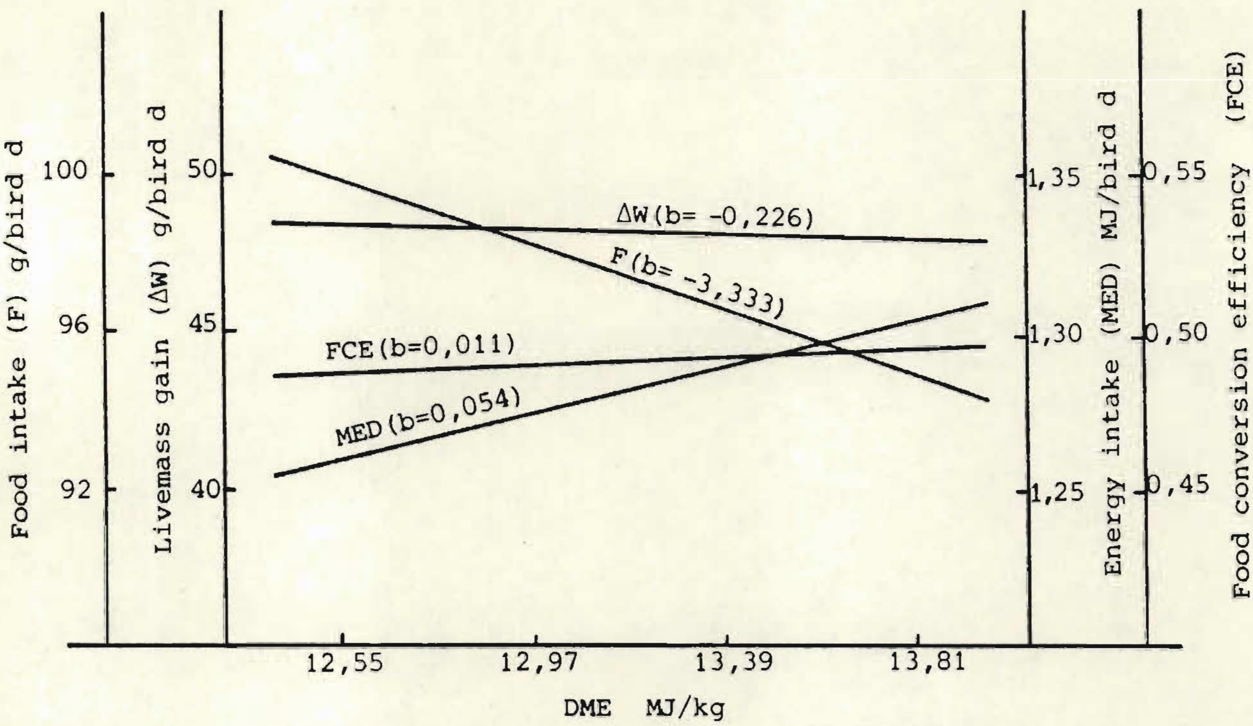

Figure 11.5 Pooled linear regressions of male broiler performance characteristics on dietary metabolisable energy level for the finisher period

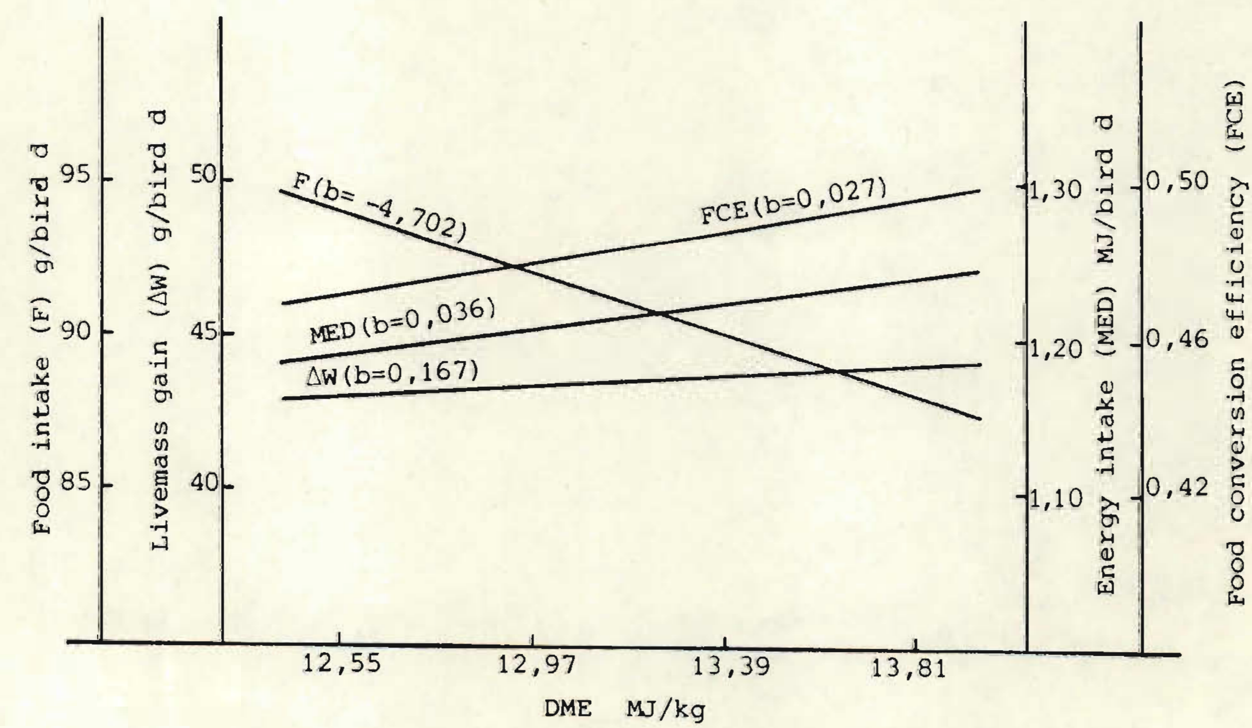

Figure 11.6 Pooled linear regressions of male and female broiler performance characteristics on dietary metabolisable energy level for the finisher period

TABLE 11.11 The regression of mass gain (g/bird d) on methionine/energy ratio, and optimum methionine/energy ratio for male and female chickens from 21 - 45 days of age

DME (MJ/kg)	Constant term	Linear coefficient (b_1)	Quadratic coefficient ($\times 10^{-2}$) (b_2)	SE (b)	Multiple correlation (R)	Optimum methionine/energy ratio (g/kg:MJ/kg)
Females						
12,55	-3,60	2,713	-3,483	5,14*	0,986	0,389
12,97	10,78	1,917	-2,426	1,94*	0,996	0,395
13,39	10,42	1,939	-2,609	2,72*	0,990	0,372
13,81	6,17	2,139	-2,674	0,70**	0,999	0,400
Combined	7,17	1,761	-2,189	2,22*	0,995	0,402
Males						
12,55	5,45	1,782	-2,162	3,33*	0,989	0,412
12,97	9,96	1,689	-2,181	2,57*	0,991	0,387
13,39	8,63	1,668	-2,071	0,66**	0,999	0,403
13,81	4,64	1,903	-2,342	2,48*	0,994	0,406
Combined	5,95	2,177	-2,798	1,90**	0,997	0,389
Males and females						
12,55	10,09	2,244	-2,817	3,99*	0,989	0,398
12,97	10,48	1,794	-2,289	2,22*	0,994	0,392
13,39	9,39	1,810	-2,345	1,68**	0,996	0,386
13,81	5,56	2,011	-2,498	1,32**	0,999	0,403
Combined	6,61	1,965	-2,487	2,03*	0,996	0,395

*, **, see footnote Table 6.4

TABLE 11.12 The regression of food conversion efficiency on methionine/energy ratio and optimum methionine/energy ratio for male and female chickens from 21 - 45 days of age

DME (MJ/kg)	Constant term	Linear term (b_1)	Quadratic term ($\times 10^{-3}$) (b_2)	SE (b)	Multiple correlation coefficient (R)	Optimum methionine/energy ratio (g/kg:MJ/kg)
Females						
12,55	-0,01	0,025	0,309	0,04*	0,992	0,401
12,97	0,06	0,022	0,287	0,03*	0,993	0,392
13,39	0,10	0,019	0,253	0,05^{NS}	0,972	0,375
13,81	0,07	0,021	0,246	0,02*	0,996	0,421
Combined	0,06	0,020	0,246	0,01**	0,999	0,418
Males						
12,55	0,04	0,021	0,264	0,03*	0,992	0,450
12,97	0,04	0,023	0,290	0,03*	0,994	0,390
13,39	0,11	0,017	0,195	0,04*	0,988	0,444
13,31	0,06	0,021	0,234	0,01**	0,999	0,443
Combined	0,06	0,022	0,274	0,02**	0,998	0,398
Males and females						
12,55	0,01	0,023	0,286	0,04*	0,992	0,403
12,97	0,05	0,023	0,289	0,02**	0,996	0,391
13,39	0,01	0,018	0,223	0,05^{NS}	0,981	0,409
13,81	0,07	0,021	0,241	0,01**	0,999	0,431
Combined	0,06	0,021	0,260	0,01**	0,999	0,408

*, **, ^{NS}, see footnote Table 6.4

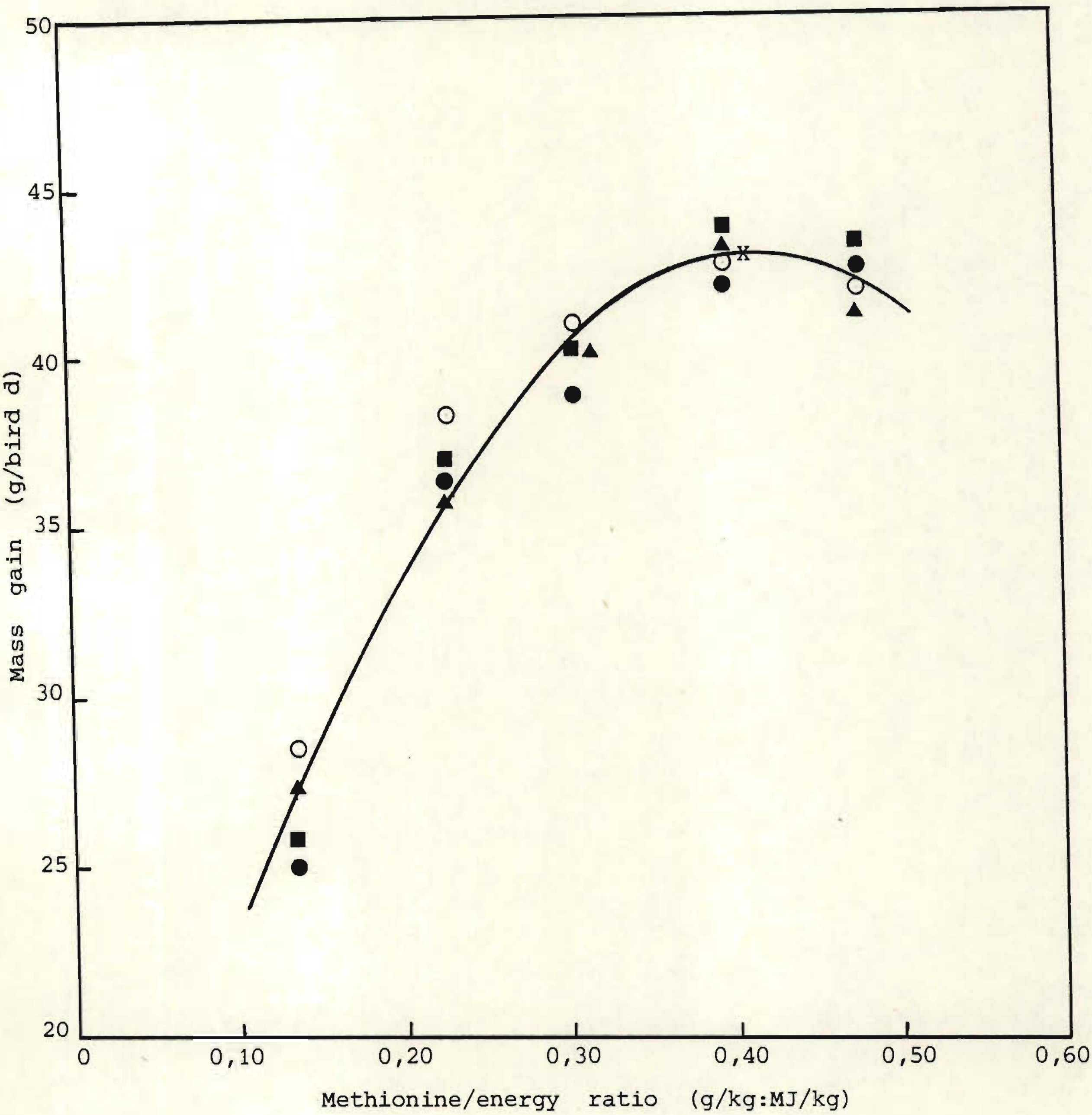

Figure 11.7 Response in mass gain (g/bird d) of female chickens from 21 - 45 days of age to methionine/energy ratio and estimated optimum methionine/energy ratio (X)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

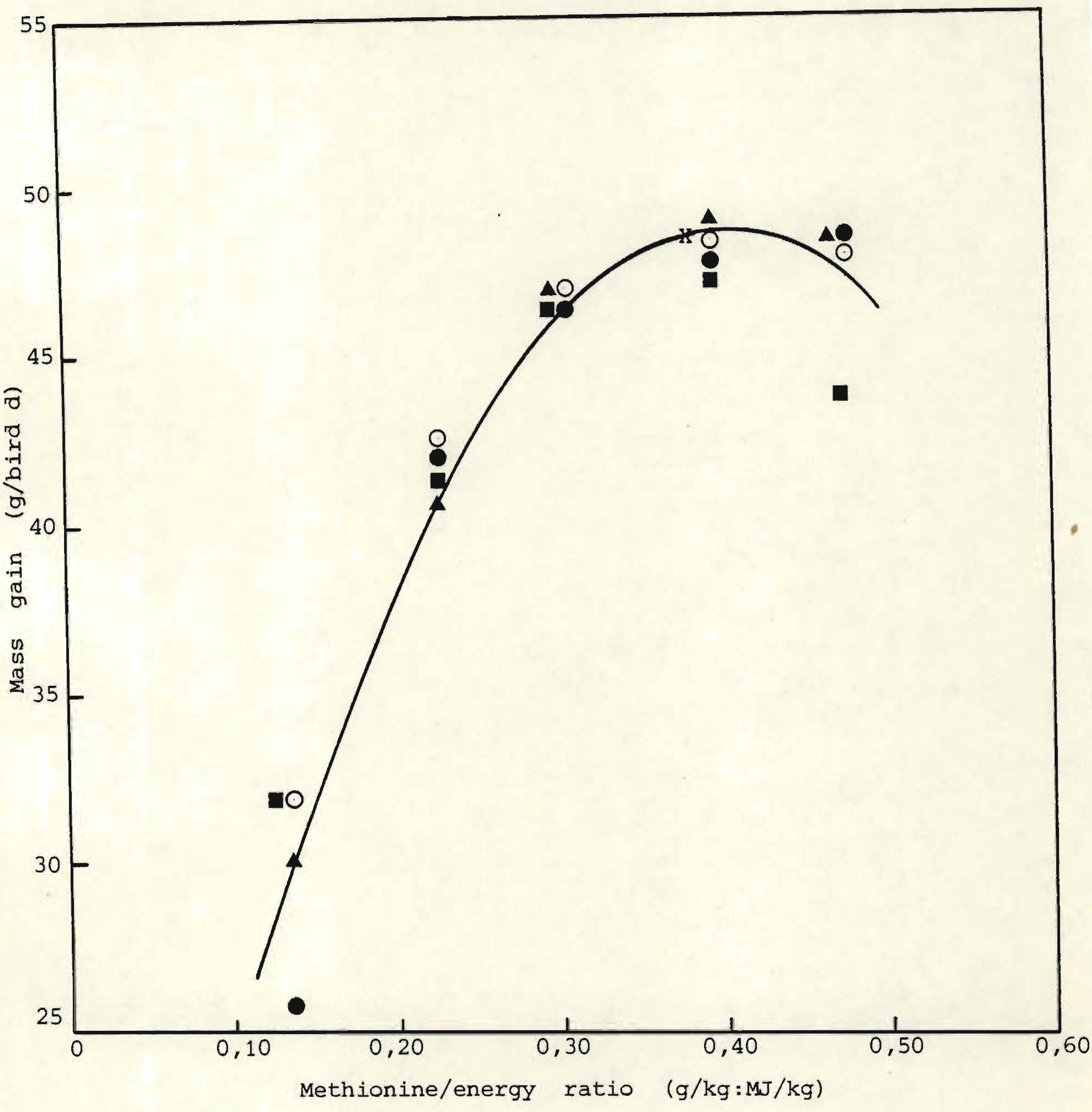

Figure 11.8 Response in mass gain (g/bird d) of male chickens from 21 - 45 days of age to methionine/energy ratio and estimated optimum methionine/energy ratio (X)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

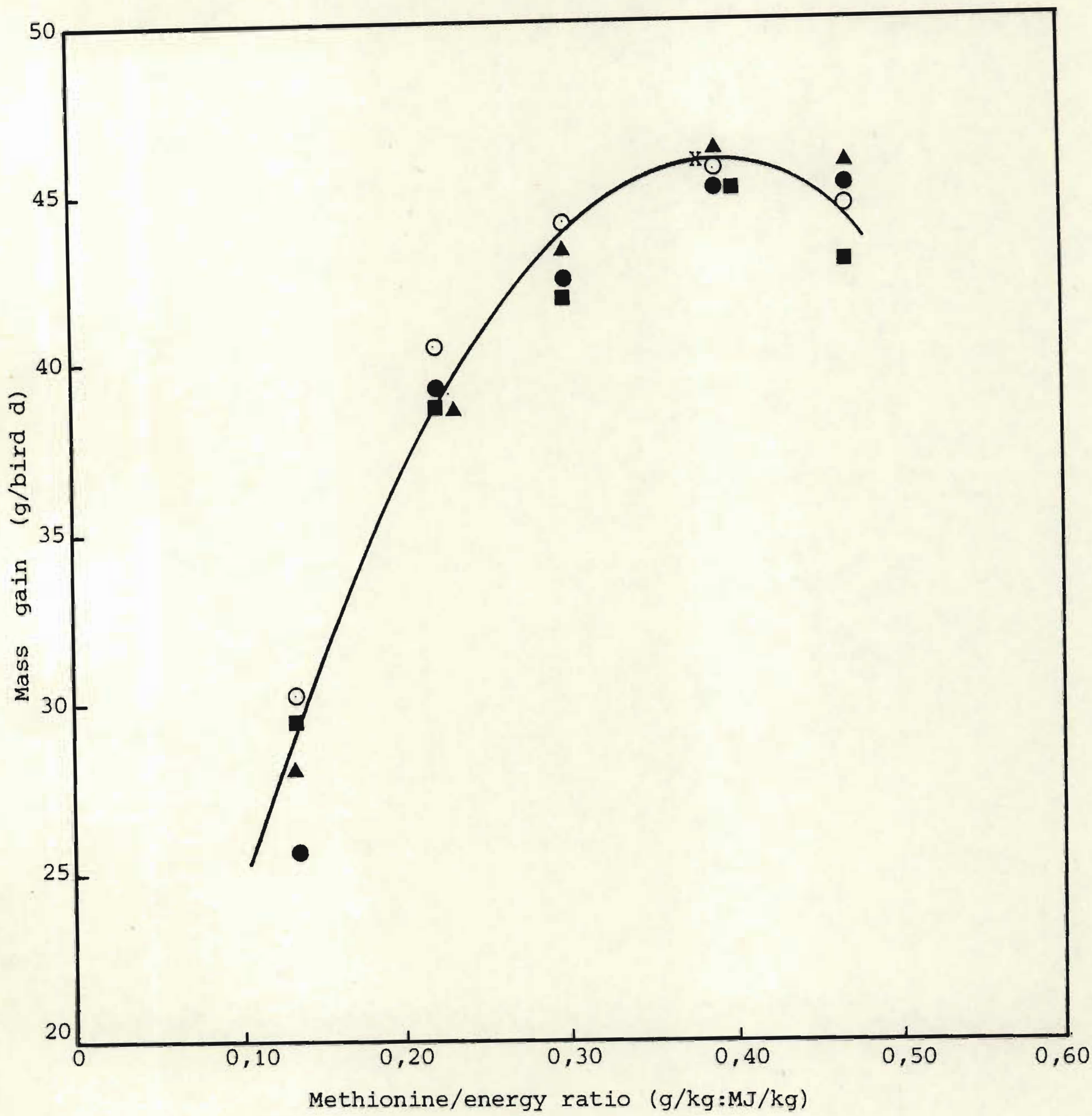

Figure 11.9 Response in mass gain (g/bird d) of male and female chickens from 21 - 45 days of age to methionine/energy ratio and estimated optimum methionine/energy ratio (X)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

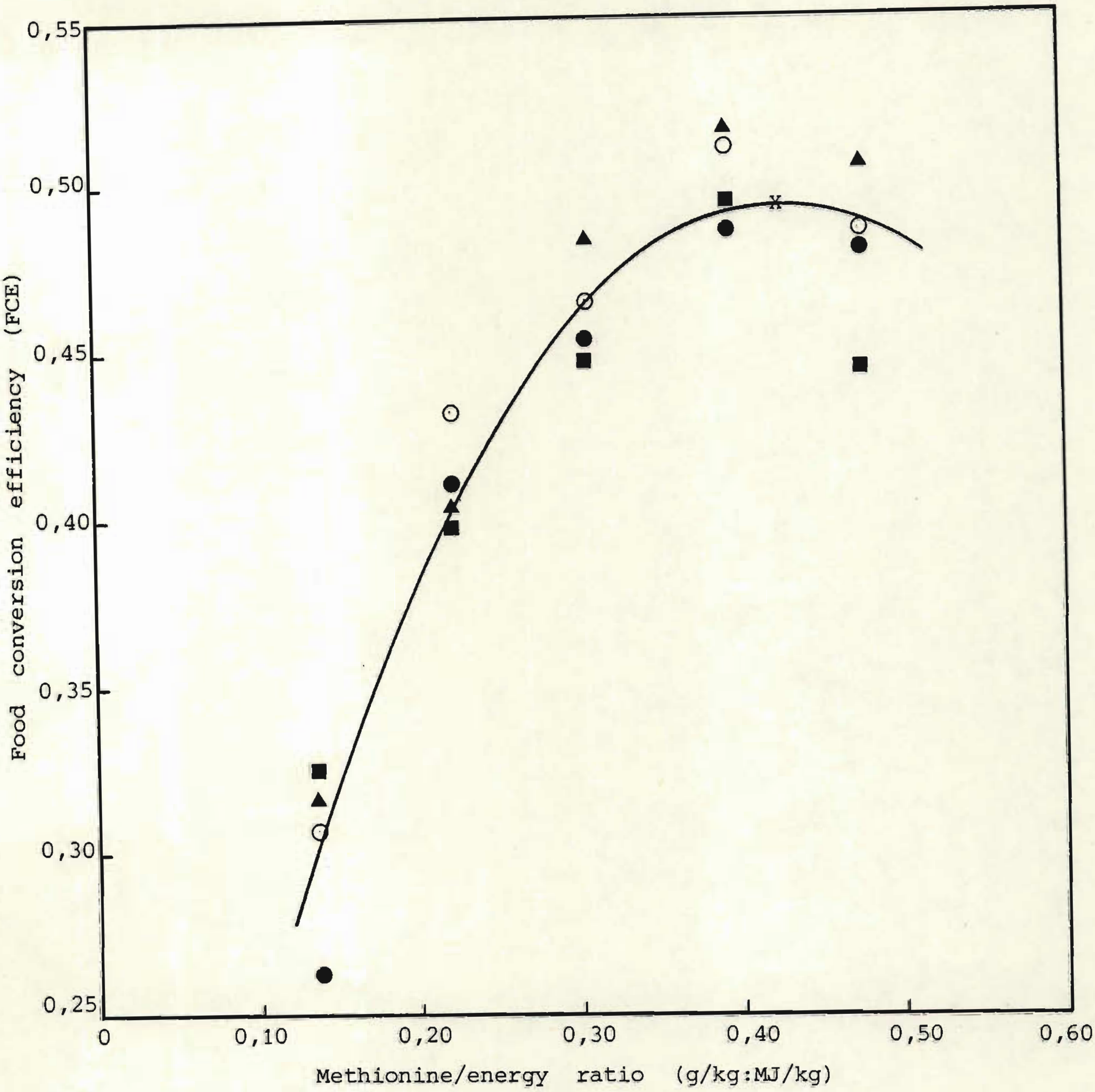

Figure 11.10 Response in food conversion efficiency of female chickens from 21 - 45 days of age to methionine/energy ratio and estimated optimum methionine/energy ratio (X)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

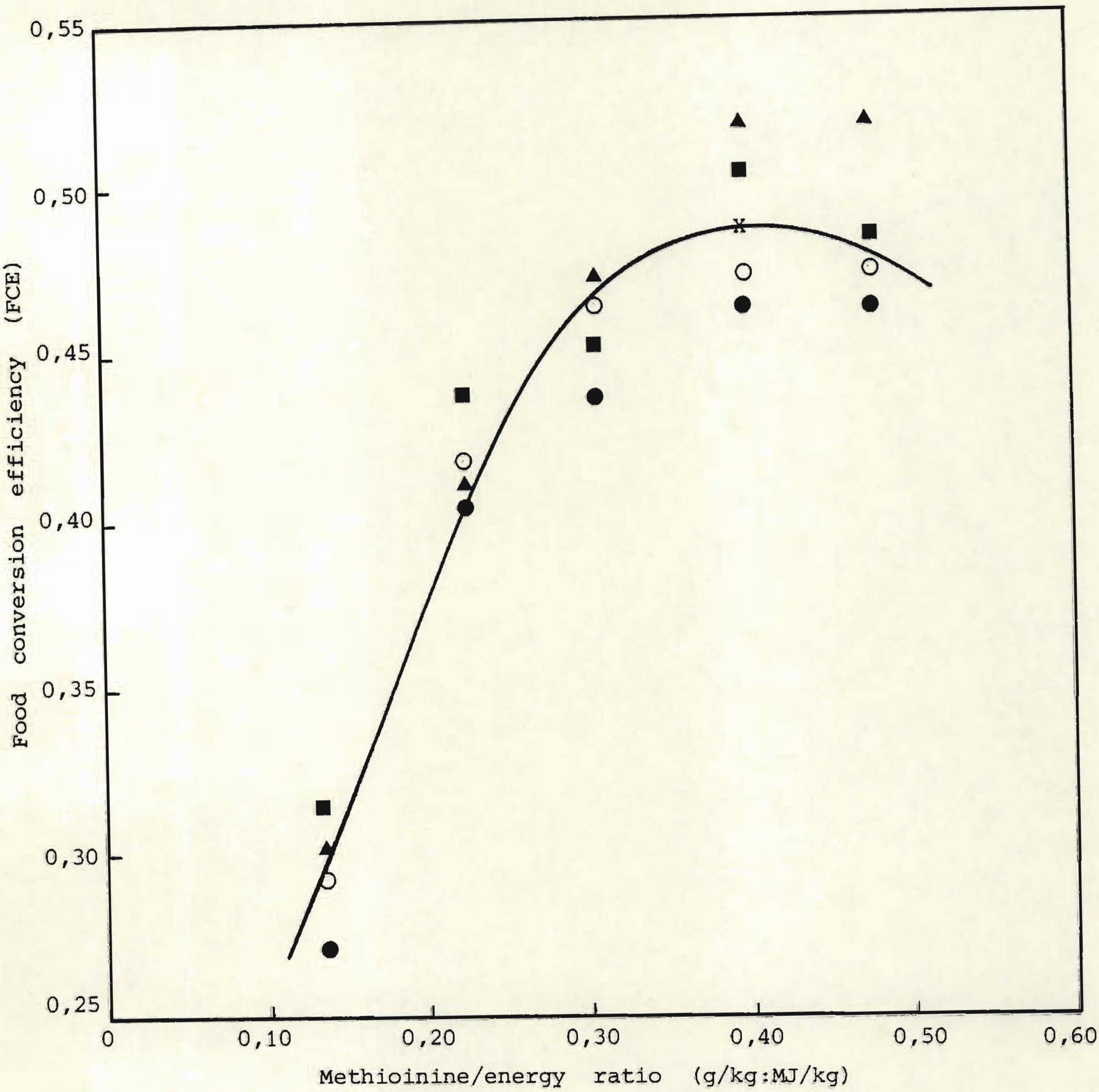

Figure 11.11 Response in food conversion efficiency of male chickens from 21 - 45 days of age to methionine/energy ratio and estimated optimum methionine/energy ratio (X)

● 12,55 MJ/kg ▲ 13,39 MJ/kg

○ 12,97 MJ/kg ■ 13,81 MJ/kg

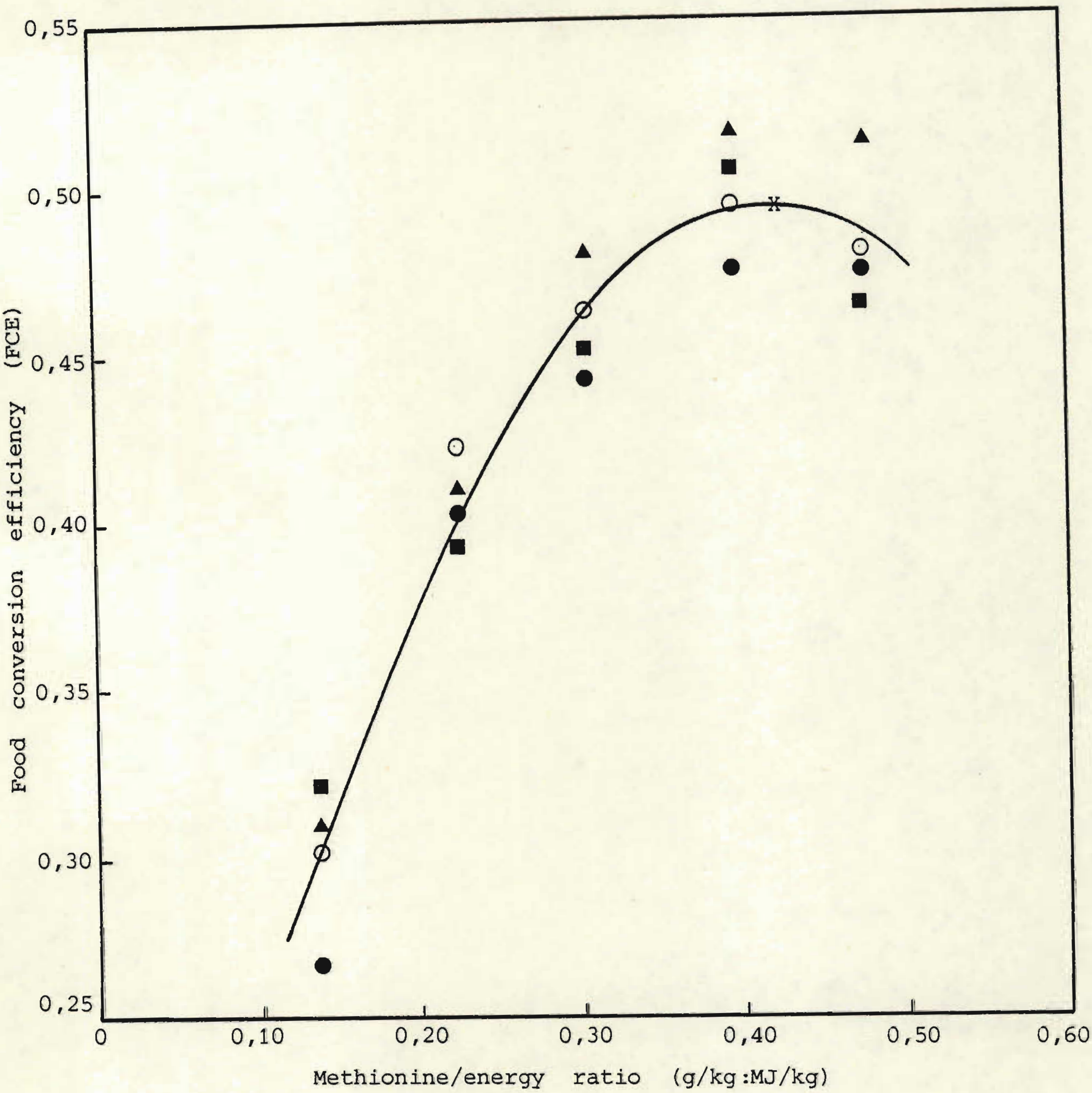

Figure 11.12 Response in food conversion efficiency of male and female chickens from 21 - 45 days of age to methionine/energy ratio and estimated optimum methionine/energy ratio (X)

● 12,55 MJ/kg
○ 12,97 MJ/kg
▲ 13,39 MJ/kg
■ 13,81 MJ/kg

Response to methionine/DME ratio

Curvilinear regressions of mass gain and FCE on methionine/DME ratio were calculated using a multiple regression analysis. Male and female data were analysed separately and combined, the results of these analyses being given in Tables 11.11 and 11.12 and illustrated in Figures 11.7 to 11.12.

DISCUSSION

The major objectives of this study were, first to measure the response of male and female chickens to increasing methionine concentrations, using the dilution technique; second to determine the optimum methionine intake for broilers in the finisher period; and third to calculate the optimum methionine/energy ratio in broiler finisher diets.

Response to dietary methionine concentration

Equations fitted to the data for each energy concentration and to the combined data over all energy concentrations (Table 11.9) indicate that at marginal costs of 280 c/kg for methionine, and 150 c/kg for broiler mass, the optimum intake of methionine at each energy level for the females would be 381, 339, 301, 401; for the males 401, 340, 266, 395 and for the combined sexes 364, 337, 299 and 395 mg/bird d. From the combined data (illustrated in Figures 11.1, 11.2 and 11.3) The optimum intakes were calculated to be 347, 366 and 368 mg/bird d for females, males and combined sexes respectively. The above results will be discussed in greater detail later where data from Chapter 4 will be used together with the present data to determine a more universal response curve to methionine in the finisher period.

The amino acid content of a chicken diet has a significant effect on food intake, as shown in the results presented in Table 11.6. During the finisher period, as in the starter period (Chapter 9 and 10), there was a clear tendency for food intake to increase progressively as the induced methionine deficiency increased in the dilution series.

Response to dietary metabolisable energy content

The livemass response to DME in the male broiler (Table 11.4) was significantly greater than the response among the females. The consistent downward adjustment of food intake with increasing DME resulted in a highly significant effect of DME on F for both sexes. Daily metabolisable energy intake increased with DME in both sexes, the slope being greater among males than among females (0,054 vs 0,007 MJ/bird d:MJ/kg)

The greater increase in MED is reflected in a significantly greater response in ΔW for the males. Because the response to DME differs between the sexes, the separate rearing of broilers should receive practical consideration.

The increase in MED, albeit significant for both sexes, was not large enough to have a significant effect on ΔW or FCE, both of which are influenced by the dietary energy consumed. FCE of both males and females improved significantly as the DME was increased. Responses to DME will be discussed more fully later, (Chapter 12) when the responses to DME and methionine intake covering the preceding experiments will be combined.

Response to methionine/DME ratio

The ratio between the limiting amino acid and the dietary energy concentration has a major influence on livemass gain and food conversion efficiency. If the optimal methionine/energy ratio is not adhered to, either by increasing or decreasing this ratio broiler performance will decline. The optimum combined ratios between methionine and DME in this experiment for the females, males and combined sexes were 0,402; 0,389 and 0,395 g methionine/MJ respectively. The optimum ratio of 0,395 g methionine/MJ compares favourably with that of 0,39 g methionine/MJ recommended by Thomas *et al.* (1978) for the finisher period. A ratio of 0,395 g methionine/MJ is higher than many of the recommendations quoted in the literature, but cognisance must be taken of the age group for which the particular recommendation is made, since younger birds require wider amino acid/DME ratios than older birds.

Food conversion efficiency was clearly significantly affected by the ratio between methionine and DME (Table 11.8), the optimum ratio for males being 0,398 g methionine/MJ and for females being 0,418 g methionine/MJ. These optimum ratios compare very favourably with the ratios giving maximum growth rate, reiterating the fact that a value of around 0,40 g methionine/MJ would be an ideal ratio between methionine and DME.

The responses recorded in this study will be collated with the results of the preceding methionine and DME studies in a later discussion where the responses to dietary methionine and energy will be summarised and discussed in relation to their economic importance in broiler performance and feed formulation.

D I S C U S S I O N   O N   P A R T   T H R E E

The basic requirement for the fomulation of practical broiler diets is knowledge, in economic terms, of the outputs obtained by feeding diets of different nutrient concentrations. These outputs can then be compared with costs and the nutrient concentration can be selected which optimises returns.

Use was made of the Reading model to describe the relationship between body mass gain and methionine intake. The results recorded in Chapters 3 and 4 indicated that the model described the data most adequately and confirmed the principle that body mass gain is dependent primarily on the intake of the first-limiting amino acid. A further series of experiments was conducted in order to obtain more data relating intake of methionine to live-mass gain in broilers and the results for the starter and finisher periods were presented in Part 3 of this study.

In order to obtain a more general equation relating mass gain to methionine intake, the Reading model was fitted to the combined data from all the methionine experiments. The data used was confined to the first dilution series (initial dilution) described in Chapters 3 and 4, plus the combined data over all energy concentrations described in Chapters 9, 10 and 11. The parameters used in fitting the equation and the resulting coefficients are presented in Fig 11.13.

Optimum daily intakes of methionine calculated from the Reading model of the combined data in Fig 11.13 (a = 5,226; b = 0,0118), for chickens gaining between 10 and 60 g body mass per day and for marginal costs of broiler mass ranging from 100 c/kg to

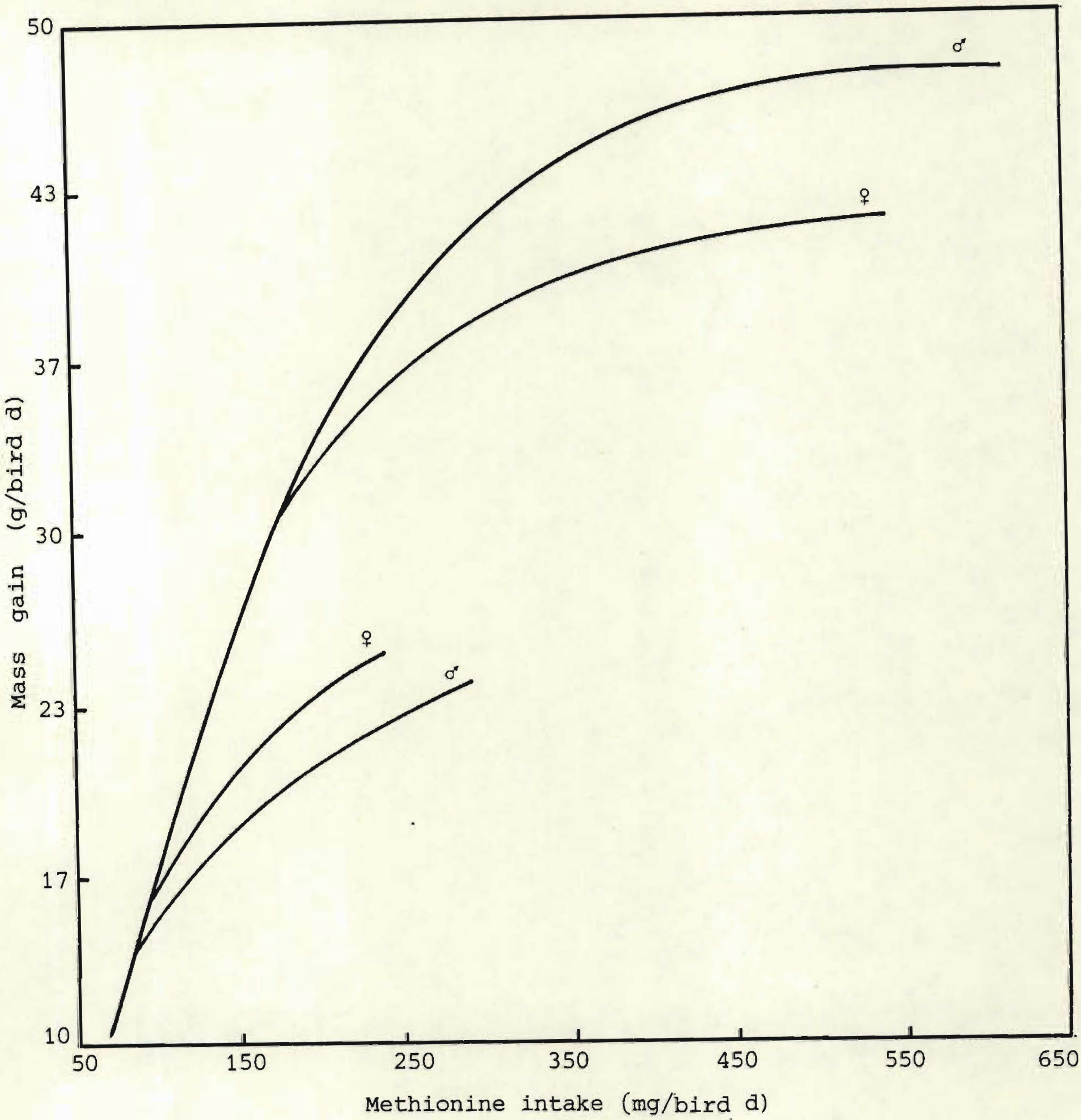

Figure 11.13 The relationship between mass gain and methionine intake using data from experiments reported in Chapters 3, 4, 9, 10 and 11. The fitted curves illustrate treatments that resulted in growth rates in the starter and finisher trials by male and female chickens respectively and are based on the following parameters: a = 5,023; b = 0,009; $\Delta\bar{W}$(♂F) 52 g/bird d; $\Delta\bar{W}$(♀F) 52 g/bird d; $\Delta\bar{W}$(♂S) 25 g/bird d; $\Delta\bar{W}$(♀S) 22 g/bird d; $\bar{W}$(starter) 200 g; $\bar{W}$(finisher) 800 g; r = 0,6; $\sigma\Delta\bar{W} = 0,3\ \Delta\bar{W}$; $\sigma\bar{W} = 0,12\ \bar{W}$

TABLE 11.13 Calculated methionine requirements as affected by body mass gain ($\Delta\bar{W}$) and the ratio between cost of methionine and value of broiler mass (k)

$\Delta\bar{W}$ (g/bird d)	Methionine requirements[1] (mg/bird d) k[2] 0,00280	0,00224	0,00186	0,00160	0,00140
10	89	90	91	92	93
15	132	134	136	137	138
20	176	179	181	182	184
25	219	223	225	227	229
30	266	270	273	276	278
35	309	314	318	321	324
40	353	358	362	366	369
45	399	406	410	415	418
50	443	450	455	460	463
55	486	494	500	505	509
60	529	538	544	550	554

[1] The following values were assumed in all cases:
a = 5,226; b = 0,0118; r $\bar{W}.\bar{W}$ = 0,6;
$\bar{W}$(for $\Delta\bar{W}$ from 10-20 g) = 250 g;
$\bar{W}$(for $\Delta\bar{W}$ from 25-40 g) = 500 g;
$\bar{W}$(for $\Delta\bar{W}$ from 45-60 g) = 800 g;
$\sigma\Delta\bar{W}$ = 0,3 $\Delta\bar{W}$; $\sigma\bar{W}$ = 0,12 $\bar{W}$

[2] The five values of k corresponding to broiler mass values of 100, 125, 150, 175 and 200 c/kg and a methionine cost of 280 c/kg

200 c/kg and of methionine at 280 c/kg, are shown in Table 11.13 The wide range of optimum intakes of methionine are an indication of the importance of this type of response curve analysis in determining optimal requirements of essential nutrients for broiler chickens.

From the results of the methionine trials in which dietary methionine and energy concentrations were tested, the optimum methionin/energy ratio for maximum growth rate appears to be 0,428 g methionine/MJ for the starter period and 0,395 g methionine/MJ for the finisher period. For maximum FCE this ratio is 0,453 g methionine/MJ and 0,408 g methionine/MJ for the starter and finisher periods respectively.

Unlike the lysine trials, no feather eating or cannibalism was evident in the methionine studies. The number of feathers appearing on the litter appeared to be normal in all treatments. The birds receiving the fifth dilution series (lowest methionine content) although severely stunted were well feathered. It therefore appears that a methionine deficiency will not elicit feather eating or cannibalism.

S U M M A R Y O N P A R T T H R E E

Quantitative estimates of the responses of broiler chickens to dietary methionine and energy concentrations were made using the dilution technique. Responses in livemass gain (ΔW), food intake (F), metabolisable energy intake (MED) and food conversion efficiency (FCE) were calculated. The Reading model was fitted to the data relating mass gain to methionine intake to determine the optimum daily methionine intake.

Linear regressions of ΔW, F, MED and FCE on methionine/DME ratio were calculated on the data recorded for the first and second dilution series. The optimum methionine/DME ratios for the variates ΔW and FCE were determined.

From further analyses of suitable estimates of response relationships between dietary methionine and energy levels and ΔW, F and FCE were defined for use in the practical formulation of broiler diets for marginal costs of dietary methionine and broiler mass.

PART FOUR

APPLICATION OF GENERAL ESTIMATES OF BROILER RESPONSE

CHAPTER 12

PRACTICAL CONSIDERATIONS IN FORMULATING BROILER DIETS

INTRODUCTION

It is an accepted principle that the amount of food consumed by chickens is regulated by the energy concentration of the diet, provided the diet is adequate in other essential nutrients. Chicks do not adjust their energy intake exactly, but will consume somewhat more energy as the energy content of the diet increases. Therefore the amino acids, vitamins and minerals must be present in the diet in a very definite ratio to energy so that the chickens will receive sufficient of all essential nutrients in satisfying their hunger for energy. The absolute amount consumed depends upon the needs of the bird which vary depending upon its size, its activity, its environmental temperature, whether it is growing or simply maintaining itself.

It is essential, therefore, that we accurately assess the energy requirements of broilers during each stage of their growth and development. With this information it is possible to closely predict the food consumption of any flock of chickens in a particular environment and thus to set the level of amino acids, vitamins and minerals such that all nutrients will be provided in adequate amounts for daily growth and performance.

The purpose of this study was to determine the food intake of broilers receiving practical diets, and to measure their relative performance on the various dietary energy concentrations. Food consumed per week would then be used as a basis for determining the optimum nutrient densities for commercial broiler diets.

MATERIALS AND METHODS

Day-old chicks of the Ross broiler strain were allocated at random to 40 pens, such that 20 pens each contained 188 male chicks, and 20 pens contained 188 female chicks. The stocking density was 20,2 birds/m^2. The pens were in an environmentally controlled broiler house of a commercial design and management procedures were adopted that conformed as closely as possible with commercial practice. Gas canopy brooders were used during the brooding period, and the photo-period was 23 hours/day.

Five practical broiler starter and finisher diets were formulated on a least-cost basis at DME levels of 12,55; 12,97; 13,39; 13,81 and 14,23 MJ/kg respectively. The amino acid levels were based on the recommendations of Thomas *et al.* (1978) and the available lysine and methionine figures quoted were converted to total requirements based on an availability of 90 percent. The composition and analysis of the experimental diets is presented in Tables 12.1 a, b. The 10 diets were mixed according to the procedure described in Chapter 8 to minimise any raw ingredient variation between diets. The treatments were allocated such that four pens of each sex were fed each diet. The food which was supplied as crumbles during the starter period, (0-21 days), and pellets during the finisher period (22-49 days), was provided *ad lib.* in tube feeders.

The mass of the birds was determined at seven day intervals and food consumption was recorded for the intervals corresponding to the mass recordings. The starter diets were fed from 0 to 21 days of age, and the finisher diets from 22 to 49 days of age, and the criteria studied were growth rate and food intake.

TABLE 12.1 a Composition (g/kg) of experimental starter diets

Ingredient	Dietary metabolisable energy (MJ/kg)				
	12,55	12,97	13,39	13,81	14,23
Yellow maize meal	649,3	691,5	696,5	685,5	685,5
Wheat bran	60,0				
Soyabean unextracted			50,0	100,0	100,0
Sunflower meal	100,0	108,0	39,0		
Fish meal	174,0	185,0	200,0	200,0	200,0
Monocalcium phosphate	2,0	2,0	2,0	2,0	2,0
Limestone flour	11,0	10,0	9,0	9,0	9,0
Sunflower oil					
Lysine HCl	0,2				
Vitamins and trace minerals*	3,0	3,0	3,0	3,0	3,0
Anti-coccidial	0,5	0,5	0,5	0,5	0,5
Calculated analysis					
Arginine	12,60	13,20	12,70	12,70	13,50
Histidine	5,10	5,30	5,20	5,30	5,50
Isoleucine	8,10	8,40	8,70	9,00	9,30
Leucine	18,90	19,60	20,00	20,30	20,50
Lysine	11,50	11,70	12,70	13,30	13,60
Methionine	5,10	5,20	5,20	5,00	5,20
Cystine	3,90	3,90	3,70	3,70	3,80
Phenylalanine	9,00	9,30	9,30	9,50	9,80
Tyrosine	7,10	7,30	7,80	8,00	8,00
Threonine	8,10	8,30	8,50	8,70	8,90
Tryptophan	2,60	2,60	2,70	2,70	2,90
Valine	11,00	11,20	11,40	11,50	11,80
Protein (gN × 6,25/kg))	220,00	226,00	227,00	229,00	236,00
Calcium	9,90	9,90	9,90	9,90	9,90
Phosphorus	6,90	6,90	6,90	6,90	6,90

*Supplies per kg of diet: Vit A 7 027 IU, Vit D_3 2 000 IU, Vit E 20 IU, Hetrazine 3 ppm, Thiamine hydrochloride 0,985 ppm, Riboflavin 8 ppm, Calcium pantothenate 7,846 ppm, Niacin 29,823 ppm, Folic acid 0,95 ppm, Biotin 0,08 ppm, Choline chloride 300 ppm

Copper 125 ppm, Iron 40 ppm, Zinc 28 ppm, Iodine 2 ppm, Manganese 78 ppm, Selenium 0,1 ppm

TABLE 12.1 b Composition (g/kg) of experimental finisher diets

Ingredient	Dietary metabolisable energy (MJ/kg)				
	12,55	12,97	13,39	13,81	14,23
Yellow maize meal	672,2	674,5	669,3	653,0	615,9
Sunflower meal	185,0	114,0	49,0	--	--
Fish meal	121,0	130,0	130,0	130,0	130,0
Soyabean unextracted	--	61,0	130,0	189,0	210,0
Monocalcium phosphate	6,0	6,0	7,0	8,0	7,0
Limestone flour	12,0	11,0	11,0	11,0	11,0
Sunflower oil	--	--	--	5,0	22,0
Lysine HCl	0,3	--	--	--	--
DL - Methionine	--	--	0,2	0,5	0,6
Vitamins and trace minerals*	3,0	3,0	3,0	3,0	3,0
Anti-coccidial	0,5	0,5	0,5	0,5	0,5
Calculated analysis					
Arginine	13,50	13,10	12,70	12,60	13,00
Histidine	5,00	5,00	5,00	5,00	5,10
Isoleucine	7,70	8,00	8,30	8,60	8,80
Leucine	18,10	18,50	18,80	19,00	19,20
Lysine	9,90	10,60	11,40	12,10	12,50
Methionine	4,80	4,70	4,70	4,80	4,90
Cystine	4,00	3,90	3,80	3,70	3,80
Phenylalanine	9,00	9,10	9,10	9,20	9,50
Tyrosine	6,40	6,90	7,30	7,60	7,70
Threonine	7,70	7,90	8,00	8,20	8,40
Tryptophan	2,50	2,60	2,60	2,70	2,70
Valine	10,40	10,50	10,50	10,60	10,80
Protein (gN x 6,25/kg)	214,00	214,00	213,00	214,00	219,00
Calcium	9,90	9,90	9,90	9,90	9,90
Phosphorus	6,90	6,90	6,90	6,90	6,90

*Supplies per kg of diet: Vit A 7 025 IU, Vit D_3 2 000 IU, Vit E 8,5 IU, Hetrazine 3 ppm, Thiamine hydrochloride 0,969 ppm, Riboflavin 8 ppm, Calcium pantothenate 7,837 ppm, Niacin 24, 42 ppm, Folic acid 0,95 ppm

Copper 125 ppm, Iron 40 ppm, Zinc 28 ppm, Iodine 2 ppm, Manganese 78 ppm, Selenium 0,1 ppm

TABLE 12.2 Weekly gain (g) in body mass of male and female broilers from day-old to 49 days of age

Period (days)	Dietary metabolisable energy (DME) (MJ/kg) 12,55	12,97	13,39	13,81	14,23
Males					
0- 7	88	89	91	90	91
8-14	193	191	189	196	199
15-21	285	279	284	285	287
22-28	355	361	353	363	370
29-35	383	389	387	402	400
36-42	414	427	438	415	416
43-49	350	348	366	359	379
Females					
0- 7	87	83	85	84	86
8-14	172	175	174	176	180
15-21	246	242	238	239	241
22-28	285	296	295	298	308
29-35	324	324	317	329	329
36-42	298	304	320	323	330
43-49	295	303	303	303	305

TABLE 12.3 Weekly food intake (g) of male and female broilers from day-old to 49 days of age

Period (days)	Dietary metabolisable energy (DME) (MJ/kg) 12,55	12,97	13,39	13,81	14,23
Males					
0- 7	114	107	109	110	110
8-14	287	288	288	279	274
15-21	445	441	430	423	425
22-28	699	678	655	649	631
29-35	858	837	860	822	803
36-42	976	969	947	921	881
43-49	979	985	981	917	983
Females					
0- 7	109	111	108	103	106
8-14	270	265	253	255	260
15-21	405	401	389	386	390
22-28	631	598	579	584	549
29-35	720	719	689	682	671
36-42	834	814	799	835	795
43-49	800	820	825	846	784

TABLE 12.4 Weekly food conversion efficiency (ΔW:F) of male and female broilers from day-old to 49 days of age

Period (days)	Dietary metabolisable energy (DME) (MJ/kg) 12,55	12,97	13,39	13,81	14,23
Males					
0- 7	0,775	0,834	0,830	0,824	0,831
8-14	0,673	0,664	0,657	0,700	0,724
15-21	0,641	0,632	0,660	0,674	0,677
22-28	0,507	0,534	0,539	0,559	0,587
29-35	0,446	0,464	0,450	0,489	0,498
36-42	0,424	0,440	0,462	0,450	0,472
43-49	0,358	0,354	0,373	0,392	0,385
Females					
0- 7	0,794	0,748	0,787	0,814	0,813
8-14	0,637	0,660	0,688	0,688	0,694
15-21	0,606	0,603	0,612	0,621	0,617
22-28	0,452	0,494	0,510	0,511	0,562
29-35	0,450	0,450	0,460	0,483	0,490
36-42	0,357	0,374	0,400	0,387	0,415
43-49	0,368	0,370	0,368	0,359	0,389

RESULTS

Weekly means of the five treatments for the variates mass gain, food intake and food conversion efficiency for the males and females are given in Tables 12.2 to 12.4 respectively.

DISCUSSION

The objective of this experiment was to obtain weekly values of body mass and food consumption in order to derive equations relating these variates to age. Such equations could then be used to determine the daily requirement of lysine and methionine throughout the growing period using the coefficients for maintenance and gain in body mass derived in earlier chapters.

The most satisfactory equation to describe growth and food intake in terms of age was found to be an exponential function of the form

$$y = \theta_1 + \theta_2[\exp(\theta_3.t)]$$

where y is either body mass or food intake at time t.

The derivative of this function allows an estimation to be made of the daily growth rate and food intake.

$$\frac{dy}{dt} = \theta_2.\theta_3[\exp(\theta_3.t)]$$

Equations were derived using the logarithm of weekly body mass and food consumption.

Values of θ_1, θ_2 and θ_3 for body mass of males and females fed diets containing the five different energy concentrations are given in Table 12.5. The exponential function fitted the data extremely well in all cases.

Coefficients of the exponential function describing food intake of male and female chickens with age when these chickens were fed diets differing in energy concentration, are given in Table 12.6.

Daily lysine and methionine requirements of broilers were then calculated using the Reading model and making use of the values of $\bar{W}$ and $\Delta\bar{W}$ obtained by means of the exponential function described above. The coefficients *a* and *b* for lysine were 16,530 and 0,0174 respectively, and were 5,226 and 0,0118 respectively in the case of methionine, these values being the coefficients derived from all the relevant data on these amino acids and presented in Parts 2 and 3 of this thesis.

TABLE 12.5 Coefficients of the exponential functions fitted to data relating mass gain ($\log_{10}$) in male and female chickens to age

Energy concentration (MJ/kg)	Coefficients θ_1	θ_2	θ_3
Males			
12,55	3,549	-1,941	-0,308
12,97	3,559	1,949	-0,305
13,39	3,563	-1,951	-0,304
13,81	3,558	-1,947	-0,308
14,23	3,560	-1,950	-0,310
Females			
12,55	3,434	-1,821	-0,319
12,97	3,449	-1,839	-0,314
13,39	3,450	-1,838	-0,313
13,81	3,461	-1,850	-0,311
14,23	3,461	-1,849	-0,315

TABLE 12.6 Coefficients of the exponential functions fitted to data relating food intake ($\log_{10}$) in male and female chickens to age

Energy concentration (MJ/kg)	Coefficients θ_1	θ_2	θ_3
Males			
12,55	3,892	-2,518	-0,323
12,97	3,868	-2,542	-0,332
13,39	3,875	-2,522	-0,326
13,81	3,860	-2,500	-0,325
14,23	3,859	-2,490	-0,322
Females			
12,55	3,801	-2,445	-0,334
12,97	3,806	-2,421	-0,326
13,39	3,804	-2,426	-0,321
13,81	3,806	-2,458	-0,324
14,23	3,771	-2,414	-0,332

The intake of lysine and methionine required to sustain the growth rate achieved in the four examples given in Figures 12.1 to 12.4 respectively can be seen to increase rapidly in the first 28 days of life and thereafter only marginally to a maximum at 35 days of age. In order to provide sufficient daily intakes of these amino acids in the first weeks of life, the concentration of lysine and of methionine in the starter diets should be around 13,60 g/kg and 6,34 g/kg assuming local costs of 350 c/kg and 280 c/kg for lysine and for methionine; a cost of 150 c/kg for broiler mass; and a daily food intake of 50 g/bird. Naturally the required concentration would vary depending on the daily intake of food, but for comparative purposes 50 g of feed/d was chosen.

The lysine requirement of 680 mg/bird d derived on this basis compares favourably with the expected intakes of 697 and 661 mg/bird d recommended by Thomas *et al.* (1978) and AEC (1978), based on an intake of 50 g/bird d for a broiler starter diet containing 14,23 MJ/kg. The methionine requirement of 314 mg/bird d is slightly higher than the recommendations of Thomas *et al.* (1978) and AEC (1978), who advocate 285 and 299 mg/bird d respectively for the same starter diet.

A more sophisticated approach to determining the optimum concentrations of each amino acid in the starter, finisher and post-finisher diets would be to minimise the theoretical excess intakes of these amino acids, on the basis of the equations presented, by adjusting both the concentration of each amino acid in the different diets and the length of time that each diet is fed. This would thus improve the efficiency of diets fed to broilers, enabling decisions to be made as ingredient costs and broiler prices change.

Determination of optimum energy concentrations in diets fed to broilers

The responses to energy concentrations measured in the preceding chapters were pooled in order to obtain linear equations relating body mass gain, food intake and FCE to DME in both the starter and the finisher periods. The results of these analyses are presented in Tables 12.7 and 12.8 and illustrated in Figures 12.5 and

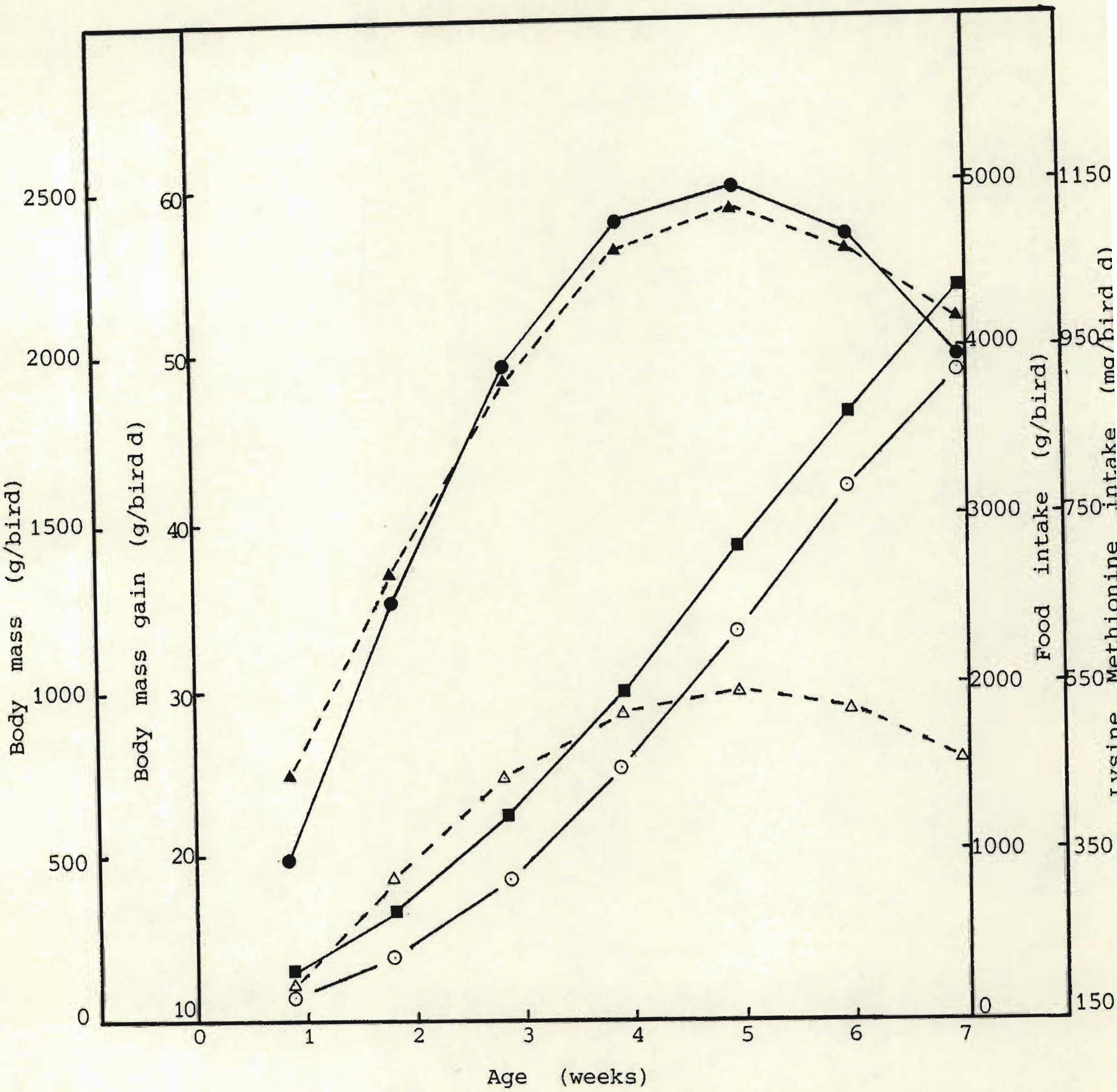

Figure 12.1 Theoretical cumulative body mass and food intake, and daily gain in body mass, and lysine and methionine requirement on a high energy diet (14,23 MJ/kg) of broiler males from day-old to 7 weeks of age

- ■ Cumulative body mass (g/bird)
- ● Body mass gain (g/bird d)
- ○ Cumulative food intake (g/bird)
- ▲ Daily lysine requirement (mg/bird d)
- △ Daily methionine requirement (mg/bird d)

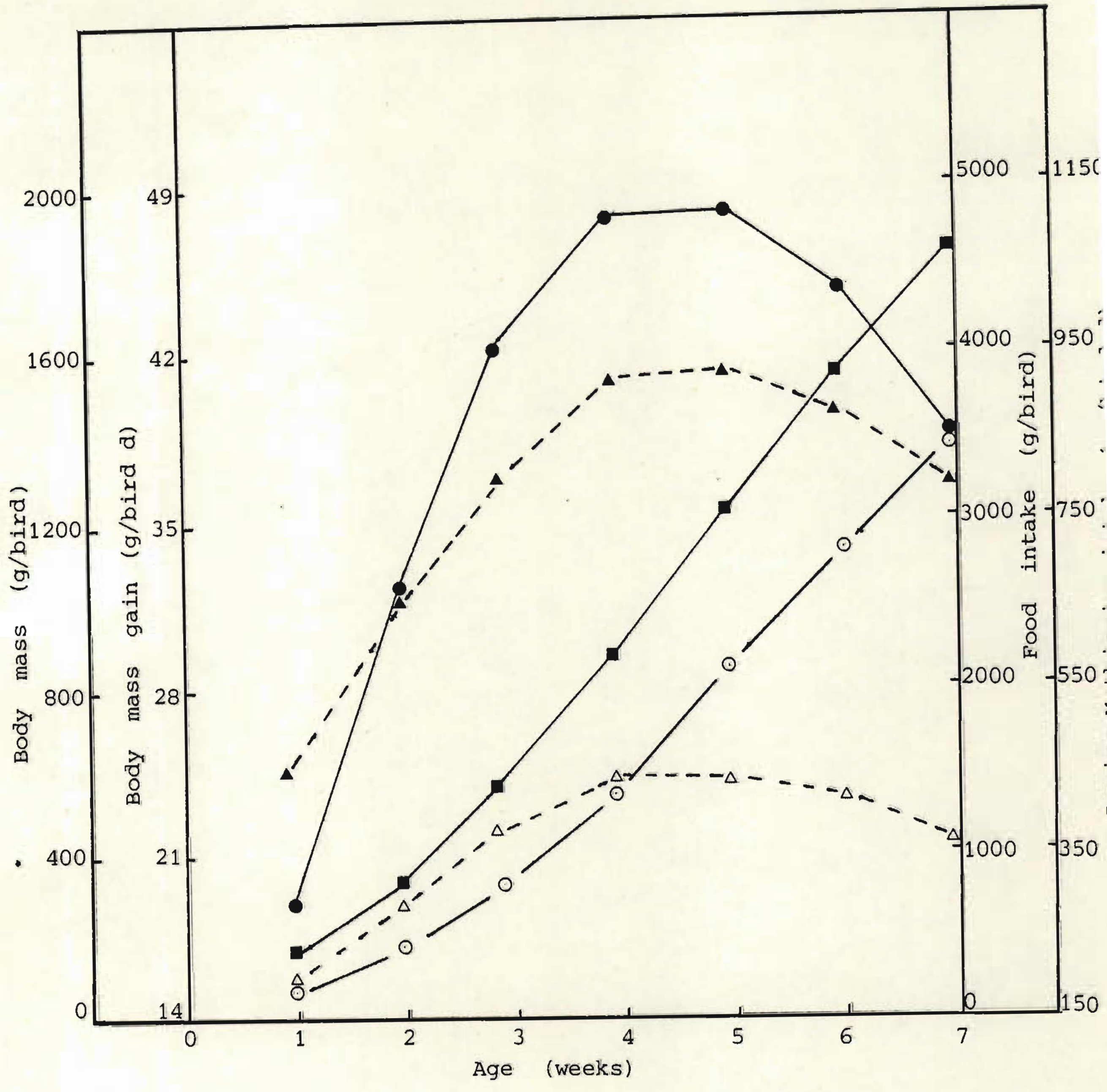

Figure 12.2 Theoretical cumulative body mass and food intake, and daily gain in body mass, and lysine and methionine requirement on a high energy diet (14,23 MJ/kg) of broiler females from day-old to 7 weeks of age

- ■ Cumulative body mass (g/bird)
- ● Body mass gain (g/bird d)
- ○ Cumulative food intake (g/bird)
- ▲ Daily lysine requirement (mg/bird d)
- △ Daily methionine requirement (mg/bird d)

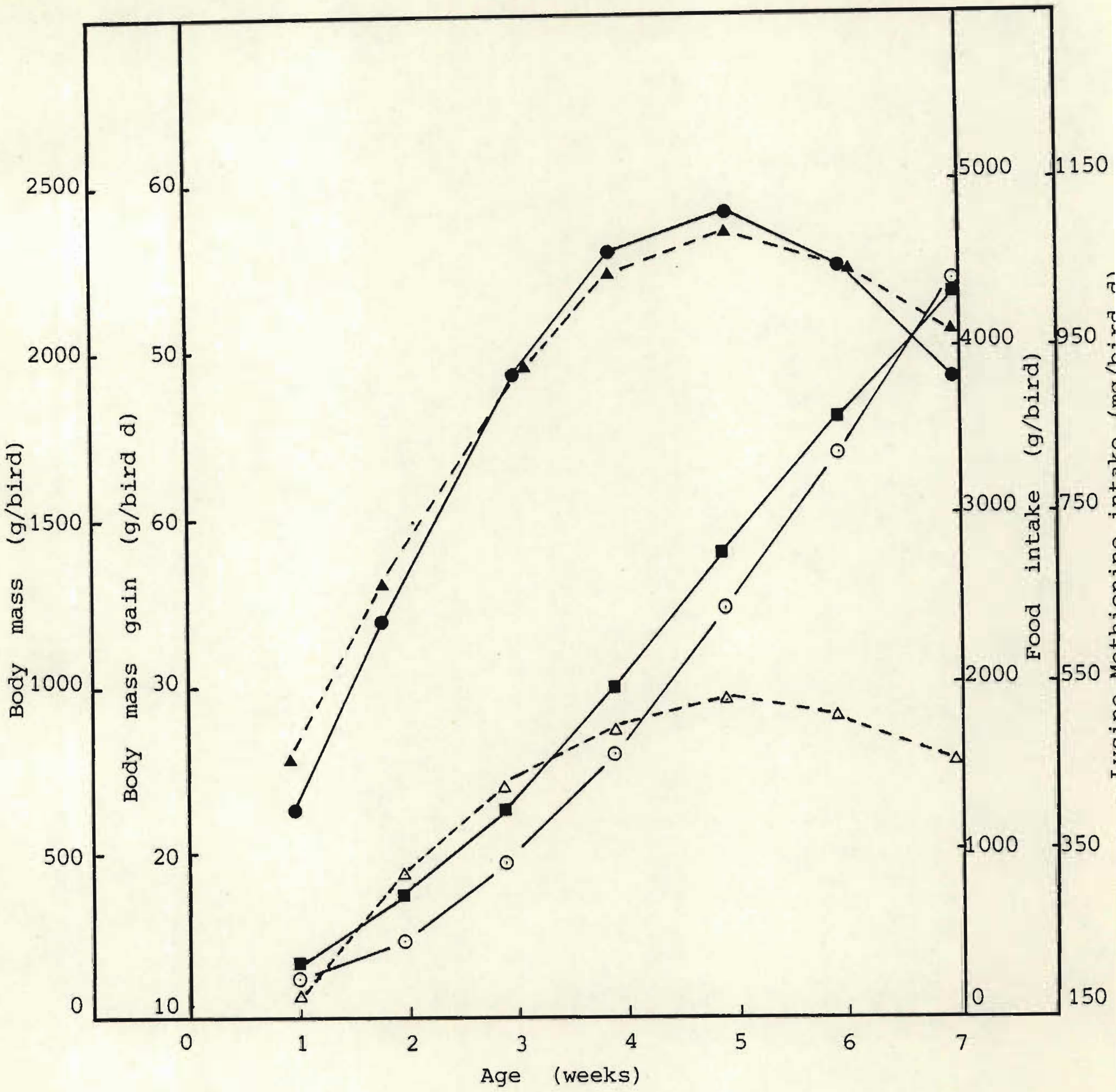

Figure 12.3 Theoretical cumulative body mass gain and food intake and daily gain in body mass, and lysine and methionine requirement on a low energy diet (12,55 MJ/kg) of broiler males from day-old to 7 weeks of age

■ Cumulative body mass (g/bird)

● Body mass gain (g/bird d)

○ Cumulative food intake (g/bird)

▲ Daily lysine requirement (mg/bird d)

△ Daily methionine requirement (mg/bird d)

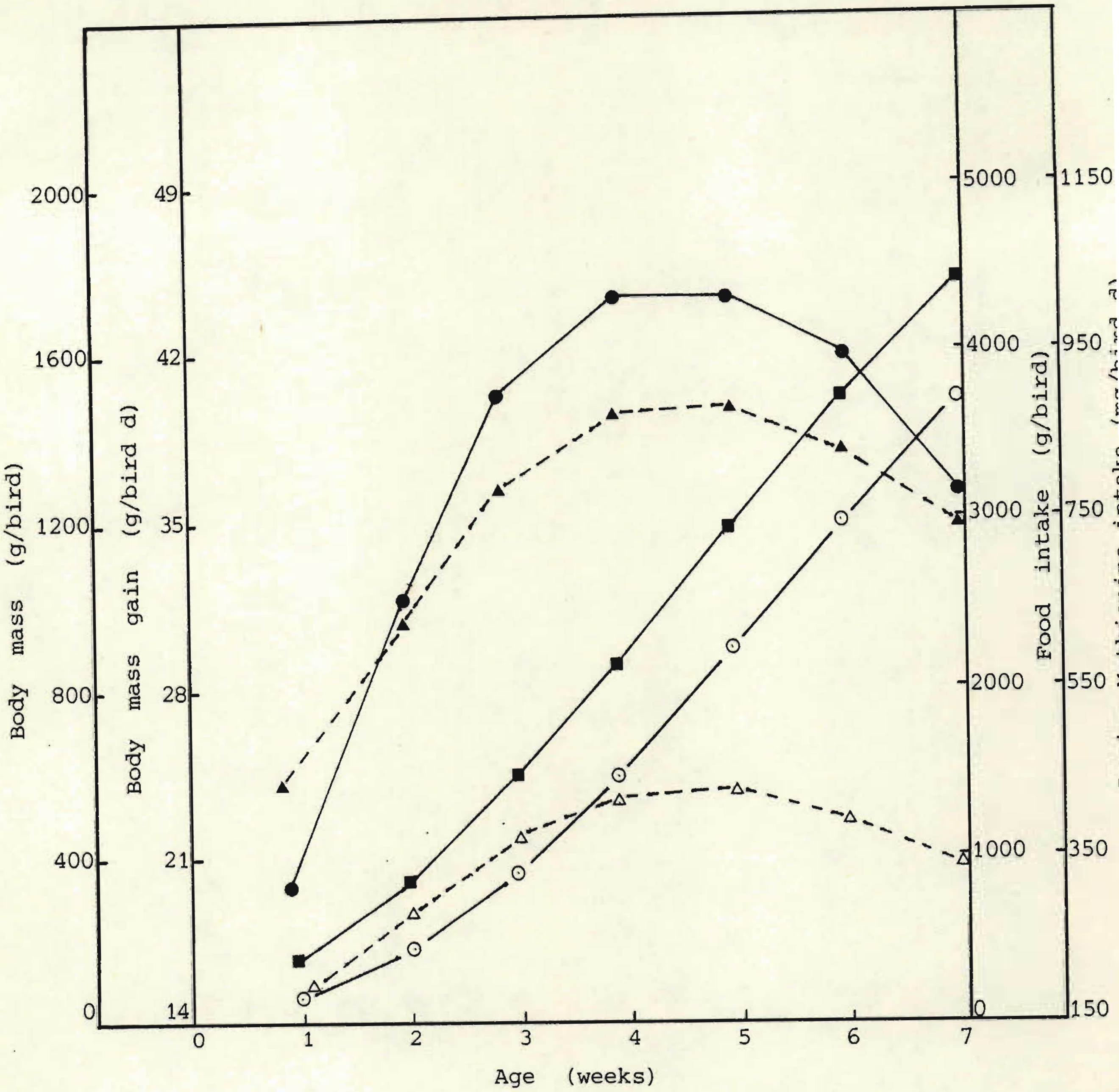

Figure 12.4 Theoretical cumulative body mass gain and food intake, and daily gain in body mass, and lysine and methionine requirement on a low energy diet (12,55 MJ/kg) of broiler females from day-old to 7 weeks of age

■ Cumulative body mass (g/bird)

● Body mass gain (g/bird d)

○ Cumulative food intake (g/bird)

▲ Daily lysine intake (mg/bird d)

△ Daily methionine intake (mg/bird d)

TABLE 12.7 Pooled responses of performance characteristics on dietary metabolisable energy concentration for males, females and for the sexes combined in the starter period (units are per MJ/kg)

Character regressed on DME	Constant term	Regression coefficient (b)	SE (b)
Livemass gain (ΔW, g/bird d)			
males	-35,563	4,778	0,930**
females	-15,297	3,058	0,700**
combined	-25,430	3,918	0,601**
Food intake (F, g/bird d)			
males	1,586	3,213	1,488*
females	-3,281	3,289	0,996**
combined	-0,847	3,251	0,934**
Energy intake (MED, MJ/bird d)			
males	-0,564	0,087	0,019**
females	-0,572	0,084	0,013**
combined	-0,557	0,085	0,012**
Food converseion efficiency (FCE, ΔW, g/F,g)			
males	-0,129	0,057	0,017**
females	0,318	0,023	0,009**
combined	0,094	0,040	0,010**

*, **, see footnote Table 6.4

TABLE 12.8 Pooled responses of performance characteristics on dietary metabolisable energy concentration for males, females and for the sexes combined in the finisher period (units are per MJ/kg)

Character regressed on DME	Constant term	Regression coefficient (b)	SE (b)
Livemass gain (ΔW, g/bird d)			
males	17,402	2,566	1,127*
females	19,869	1,740	0,447**
combined	18,636	2,153	0,997*
Food intake (F, g/bird d)			
males	135,508	-2,081	2,253^{NS}
females	138,301	-3,177	1,868^{NS}
combined	136,904	-2,629	1,820^{NS}
Energy intake (MED, MJ/bird d)			
males	0,308	0,084	0,031**
females	0,549	0,055	0,025*
combined	0,429	0,070	0,024**
Food conversion efficiency (FCE, ΔW, g/F,g)			
males	0,046	0,033	0,006**
females	0,012	0,033	0,009**
combined	0,029	0,033	0,006**

^{NS}, * etc see footnote Table 6.4

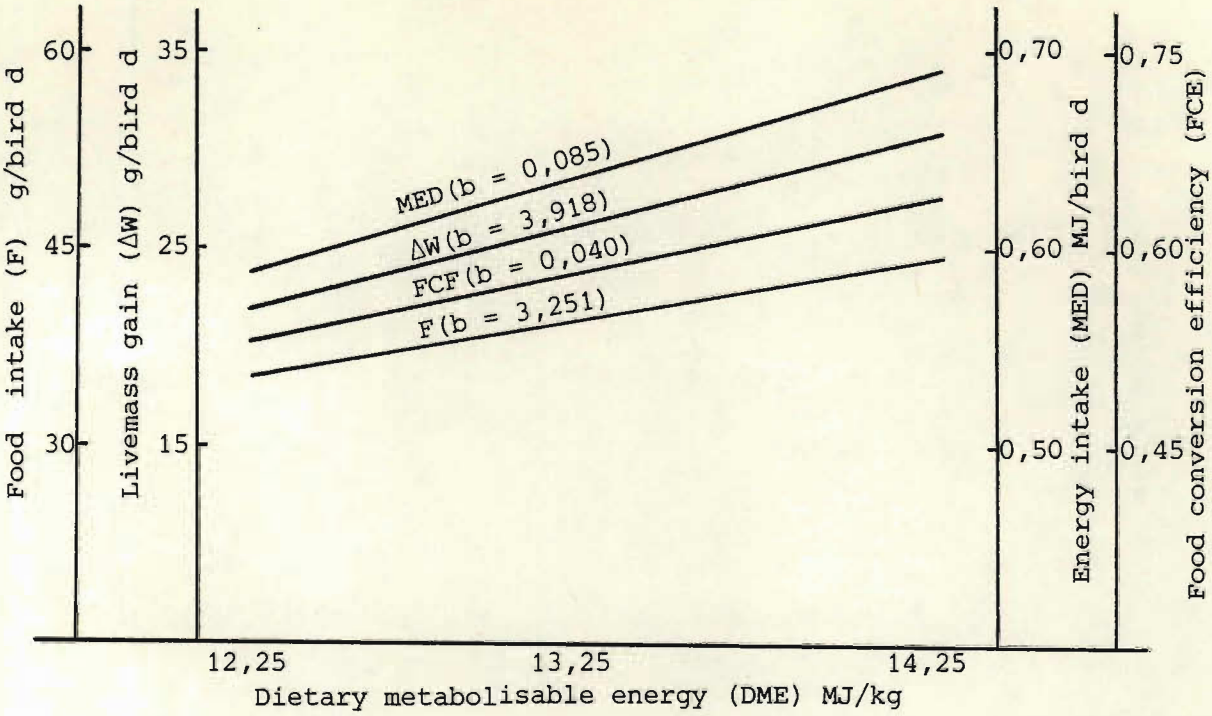

Figure 12.5 Pooled linear regressions of male and female broiler performance characteristics on dietary metabolisable energy levels for the starter period

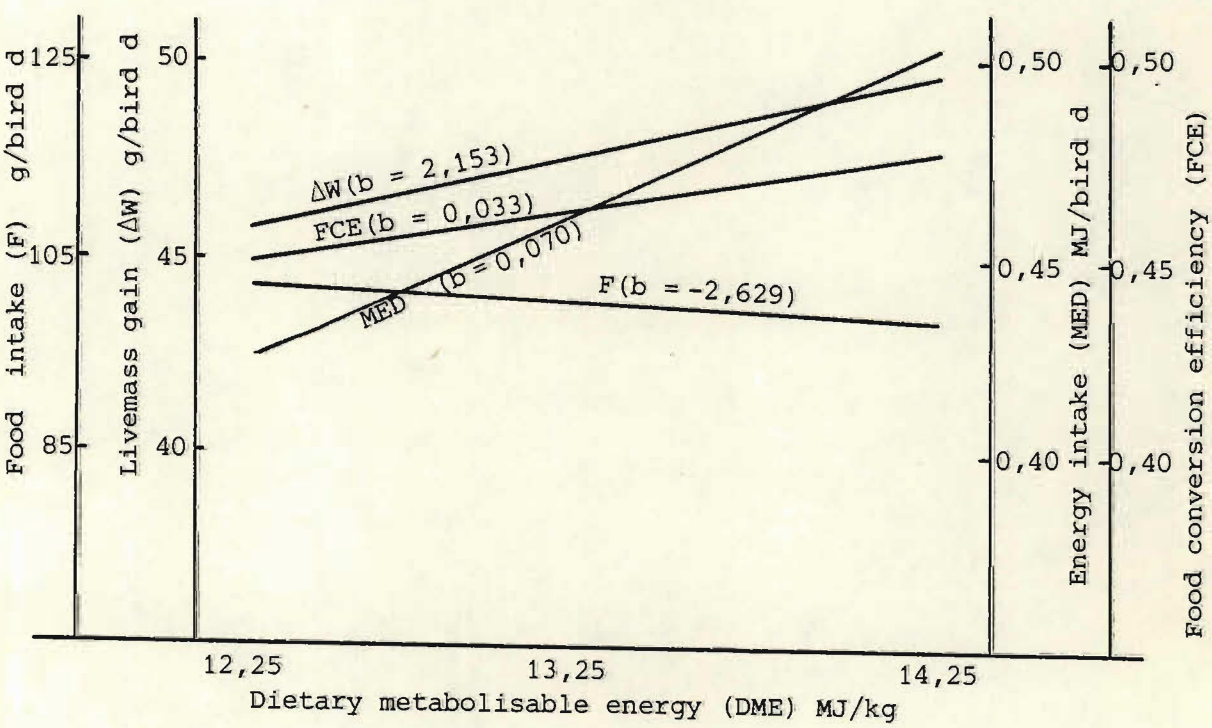

Figure 12.6 Pooled linear regressions of male and female broiler performance characteristics on dietary metbolisable energy levels for the finisher period

TABLE 12.9 Regression coefficients recommended for use in the formulation of practical broiler diets. The coefficients correspond to metabolisable energy changes of 1 k cal/g (Fisher and Wilson, 1974 b)

Response	Sex	Starter phase (1 - 28 d)	Finisher phase (29 - 56 d)
Livemass gain (ΔW, g/d)	male	5,11	6,06
	female	3,29	4,24
	combined	4,20	5,15
Food intake (F, g/d)	male	-1,64	-15,35
	female	-3,02	-16,73
	combined	-2,33	-16,04
Food conversion efficiency (FCE, $\Delta W/F$)	male	0,21	0,15
	female	0,17	0,12
	combined	0,19	0,13

12.6. The trends obtained by Fisher and Wilson (1974 b) are included in Table 12.9 to allow comparisons to be made between the trends obtained in this thesis with those based on the data collected by Fisher and Wilson (1974 a). The trends obtained compare favourably in many instances with the values obtained by Fisher and Wilson (1974 b). However, because many more sets of data covering a far greater range of DME concentrations were used by these authors than in the present study, the coefficients given by Fisher and Wilson (1974 b) should be regarded as being more universally applicable to broiler growth data.

The responses reported in this study can form the basis of an evaluation of dietary amino acid and energy levels in broiler diets in economic terms. In order to determine the optimum dietary nutrient concentrations a full analysis of marginal costs and returns in each circumstance must be completed. Fixed costs must be incorporated to calculate real margins for broiler production at different nutrient concentrations. A consequence of altering diet densities may be a change in killing age and hence in fixed costs as well as feed costs. It is therefore important that the choice of the optimum nutrient concentrations should be made on the basis of margin over total costs.

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APPENDIX

1.1 Raw ingredient matrix

During the course of this study no facilities were available for determining the amino acid composition of the experimental diets. The large volume and high cost of such analyses precluded their analysis at an independent laboratory. Representative samples of the feed ingredients used in the studies were however forwarded to Degussa in Germany and to the University of Natal for amino acid analysis. The results received from these two laboratories were in close agreement with one another and the raw ingredient matrix (Appendix Table 1.1) used in this study was constructed according to the amino acid analyses received from Degussa. Representative samples of summit and dilution mixtures were collected at the time each experiment was conducted, and these samples were stored in a refrigerator. The amino acid analyses of these samples was delayed due to the lack of sufficiently accurate equipment. The results in the thesis are therefore based on amino acid concentrations calculated from a knowledge of the respective concentrations in the feed ingredients used to prepare the diets. Although it is realised that this method is in general not as accurate as the method based on analyses of the final diets fed to the birds, it is doubtful that the latter method would have resulted in a more accurate estimation of lysine and methionine concentrations considering the equipment that was available for such analyses at that time.

The net energy (NE) values used in the thesis were calculated according to the method of De Groote (1974) who suggested the following formula :

$$NE = \frac{ME \times \left[(0{,}6 \times P) + (0{,}75 \times CHO) + (0{,}9 \times F) \right]}{P + C + F}$$

where P = crude protein (%)

C = crude starch and sugar (%)

F = crude fat (%)

and 0,6 : 0,75 and 0,9 are the respective utilisation coefficients for protein, carbohydrate and fat.

Appendix Table 1 Composition of feed ingredients (g/kg)

	Protein	Calcium	Phosphorus (total)	Phosphorus (available)	ME (MJ/kg)	NE (MJ/kg)
Blood meal	800,0	3,0	2,5	2,5	11,72	7,23
Bone meal	240,0	220,0	95,0	95,0	6,28	3,98
Feather meal	840,0	8,0	6,0	-	9,67	7,23
Fish meal	660,0	29,0	21,0	21,0	12,55	7,43
Gluten meal	600,0	-	4,0	2,0	16,11	9,28
Lucerne meal	160,0	12,0	2,0	2,0	3,68	3,22
Soyabean unextracted	380,0	2,5	6,0	2,5	14,69	10,46
Starch	6,0	-	-	-	15,27	11,46
Sugar	-	-	-	-	15,48	11,61
Sunflower husk	40,0	-	-	-	1,05	0,63
Sunflower oilcake	380,0	4,0	9,0	3,0	8,37	5,33
Sunflower oil	-	-	-	-	37,45	33,71
Wheat bran	150,0	1,3	9,0	3,0	5,98	3,87
Yellow maize meal	86,0	0,2	2,2	0,7	14,35	10,56
Mono calcium phosphate	-	210,0	210,0	210,0	-	-
Limestone flour	-	360,0	-	-	-	-
DL-Methionine	980,0	-	-	-	15,34	8,79
Lysine HCl	800,0	-	-	-	12,52	6,59

Appendix Table 1 (continued)

	Arginine	Lysine	Methionine	Cystine	Tryptophan	Histidine
Blood meal	34,5	69,0	9,0	14,0	10,5	42,0
Bone meal	16,0	8,9	1,8	-	1,6	-
Feather meal	56,0	15,0	5,0	30,0	5,0	-
Fish meal	37,4	50,0	18,2	7,7	7,9	15,0
Gluten meal	22,0	14,0	16,0	9,0	3,0	16,0
Groundnut oilcake	50,0	15,5	4,5	6,5	4,8	9,5
Lucerne meal	5,8	7,7	2,0	2,0	2,8	3,0
Soyabean unextracted	28,0	24,0	5,1	6,4	5,5	8,9
Starch	-	-	-	-	-	-
Sugar	-	-	-	-	-	-
Sunflower husk	-	-	-	-	-	-
Sunflower oilcake	35,9	15,4	8,7	6,7	5,2	10,0
Sunflower oil	-	-	-	-	-	-
Wheat bran	10,0	6,2	2,3	3,3	2,1	3,0
Yellow maize meal	4,3	2,7	2,2	2,4	0,9	2,0
Mono calcium phosphate	-	-	-	-	-	-
Limestone flour	-	-	-	-	-	-
DL-Methionine	-	-	980,0	-	-	-
Lysine H Cl	-	784,0	-	-	-	-

Appendix Table 1 (continued)

	Leucine	Isoleucine	Phenylalanine	Tyrosine	Threonine	Valine
Blood meal	103,0	10,0	61,0	18,0	37,0	65,0
Bone meal	-	-	-	-	7,8	-
Feather meal	-	-	-	-	-	-
Fish meal	50,0	35,0	27,0	20,0	26,6	34,0
Gluten meal	94,0	29,0	45,0	24,0	25,0	37,0
Groundnut oilcake	27,0	17,0	21,0	15,0	12,2	18,0
Lucerne meal	11,0	7,5	7,0	5,0	6,4	7,0
Soyabean unextracted	28,0	20,0	18,0	12,0	15,0	18,0
Starch	-	-	-	-	-	-
Sugar	-	-	-	-	-	-
Sunflower husk	-	-	-	-	-	-
Sunflower oilcake	26,0	21,0	22,0	-	15,0	23,0
Sunflower oil	-	-	-	-	-	-
Wheat bran	9,0	6,0	4,5	4,0	4,9	7,0
Yellow maize meal	11,0	4,0	5,0	4,1	3,4	4,0
Mono calcium phosphate	-	-	-	-	-	-
DL-Methionine	-	-	-	-	-	-
Lysine H Cl	-	-	-	-	-	-

1.2 Mixing of experimental diets

1.2.1 Starter experiments

The experimental diets fed in the starter period were mixed in a one metric ton vertical auger mixer and fed in the form of meal. In the balanced : unbalanced series of trials (Chapters 1 and 2) the summit and dilution diets were mixed separately, after which the intermediate diets were blended accordingly. The mixing procedure adopted in the main lysine and methionine starter trials (Chapters 6, 7, 9 and 10) was to prepare the summit and dilution diets at the extreme energy levels and then to blend these accordingly to make up the diets of intermediate dilution.

1.2.2 Finisher experiments

The experimental diets fed in the finisher period were mixed in a two metric ton horizontal batch mixer after which they were pelleted through a five millimetre die. The large volume of food required for the finisher trials (Chapters 2, 4, 8, 11 and 12) made it impractical to prepare the total summit and dilution requirements and then to blend the intermediate diets proportionally. These diets were therefore mixed according to the procedure described in Chapter 2. The summit and dilution diets were formulated at each energy level and the intermediate diets were calculated proportionally. after which all the diets were mixed individually. Great care was taken to minimise any raw ingredient variation by blending the total requirements of each of the major ingredients prior to mixing each experimental diet.

2. The Reading Model

2.1 Theory

Fisher, Morris and Jennings (1973) proposed a model to determine the response of laying hens to graded levels of amino acid intake.

They assumed that each individual bird has a characteristic maximum level of egg output (E max), and that for each bird,

when E is less than E max.

then $A = \underline{a} E + \underline{b} W$ - - - (1)

where A = amino acid intake (mg/bird d)

E = maximum egg output (g/bird d)

W = body mass (kg/bird)

$\underline{a}$ = amount of amino acid required/unit of output (mg/gE)

$\underline{b}$ = amount of amino acid required/unit of body mass (mg/kg W)

They assumed that when A was less than $\underline{b} W$, E was 0, thereby excluding negative egg production. These relationships are illustrated in Appendix Fig. 2(a). This model defines the response for an individual bird by means of the factorial curve illustrated, from which the flock response is determined by averaging out the individual responses, taking into account variations in maximum egg output (E max) and maintenance requirements ($^{\sigma}W$) in the flock. For the purposes of this model the variates egg output and body mass are assumed to be normally distributed.

Curnow (1973) expressed the equation for the curve for the average individual in a flock as

$$A = \underline{a} E + \underline{b} W$$

and for determining the economic optimum requirement of the flock as

$$A = \underline{a} \bar{E} \max + \underline{b} \bar{W} + x\sqrt{(\underline{a}^2 \sigma^2 E + \underline{b}^2 \sigma^2 \bar{W} - 2 \underline{a} \underline{b} r_{EW} \sigma E \sigma W)}$$

where A = amino acid intake (mg/bird d)

$\bar{E}$ max = mean maximum level of egg output (g/bird d)

$\bar{W}$ = mean body mass (kg/bird)

$^{\sigma}E$ = standard deviation of E

σ_W = standard deviation of W

r_{EW} = the correlation between E and W

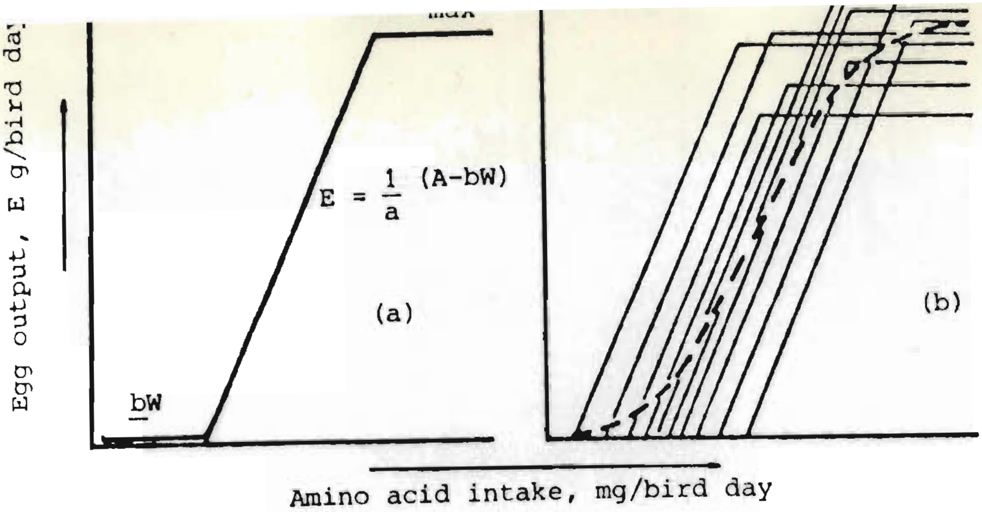
a and b = as defined above

x = the deviation from the mean, of a standard normal distribution, which is exceeded with probability ak in one tail

k = cost per unit amino acid/value per unit egg.

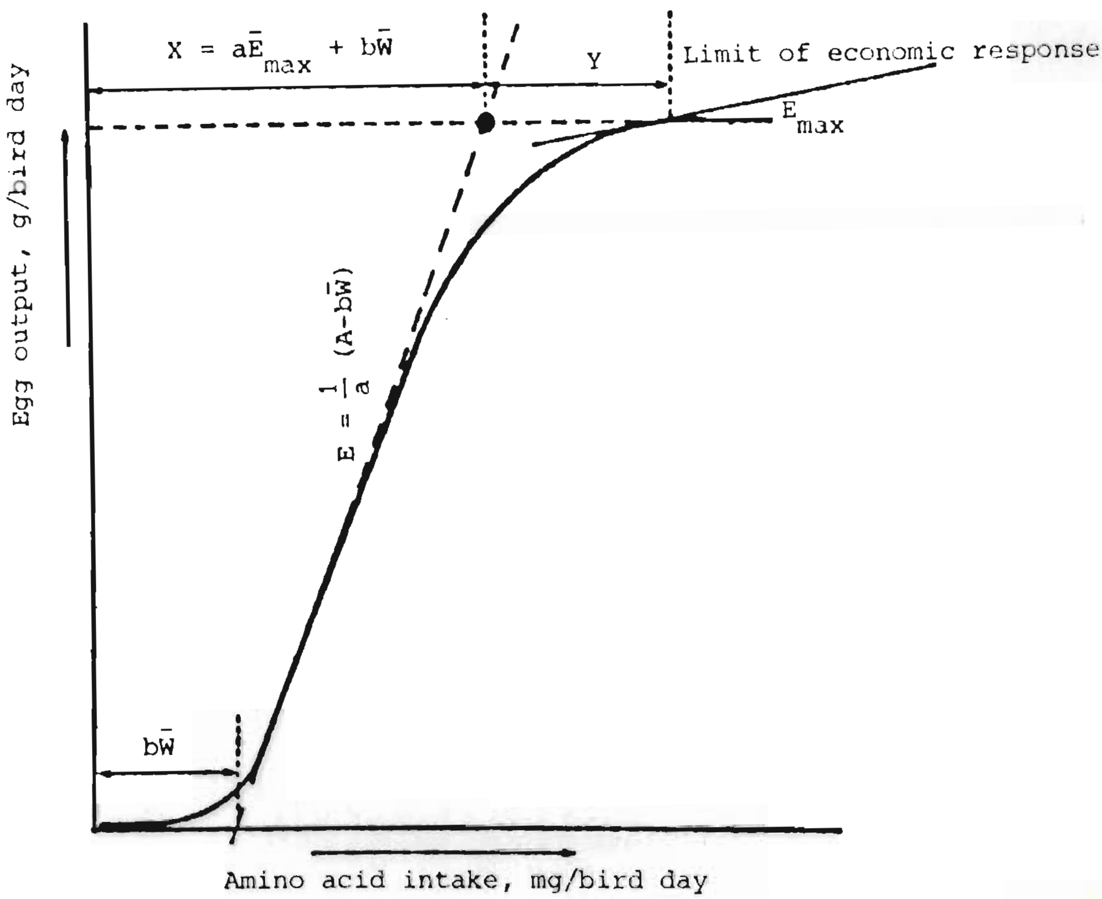
The response curve presented in Appendix Fig. 3 shows that the optimum amino acid intake is made up of the sum of two parts. The first part represents the requirement of the average bird, or alternatively the average requirement of the flock and would be the optimum if all birds in a flock were identical, which is not the case in practise due to the genetic variation within a flock. The average requirement is determined directly from equation (1) using appropriate values for mean body mass ($\bar{W}$) and egg output ($\bar{E}$ max) and is shown as X in Appendix Fig. 3. The second part of the expression represents the extra amount worth feeding to those individuals in the flock whose requirements are above average and are economically worth satisfying. The value of Y is defined by X and by a point on the response curve where the slope of the tangent at that point equals the cost ratio, i.e. when k = cost of input/cost of output. The value of Y is governed by economic factors and the slope of the response curve which is dependent on the variates a, $\sigma_{E\max}$, σ_W and r_{EW} (Fisher *et al.*, 1973)

The above authors emphasise that the rate of increase in egg output for increasing intakes of amino acid is less in flocks than for individual birds, therefore when a least squares regression line is fitted directly to experimental data (eg. Combs, 1960, 1968 ; Bray, 1965) the regression coefficient obtained is not an estimate of a, as defined in this discussion, i.e. the requirement based on the "bent-stick" method would be lower than the value of X in appendix Fig. 3.



Appendix Fig. 2 The model proposed for the response of laying hens to amino acid intake (a) the response of an individual bird (b) individual (————) and mean (——— ———) responses for a small group of birds (from Fisher *et al.*, 1973)

MODEL FOR AMINO ACID RESPONSES



Appendix Fig. 3 The relationship between the calculated amino acid requirement for the average bird in the flock (indicated thus O) and the "requirement" of the whole flock in economic terms. The line representing the limit of economic response has a slope which reflects the optimum ratio between the cost of the input and the value of the output (from Fisher *et al.*, 1973)

Given that we wish to increase nutrient input until the increased value of the output is just equal to the increased cost of the input, we need to determine at what point on the response curve the slope reaches the current value of k (cost of input/value of output).

All birds are assumed to respond in output at a rate of $^1/_{\underline{a}}$, until they reach their individual maximum output. The slope of the curve at any point to the right of that at which all individuals have satisfied their maintenance requirement, will thus be the proportion (q) of birds responding multiplied by the rate of response ($^1/_{\underline{a}}$). The optimum input dose is reached when this slope equals the cost ratio, i.e. when $k = q\ (^1/_{\underline{a}})$.

From a knowledge of a and the current k value therefore, the proportion (q) of the flock which do not have their requirement fully satisfied can be calculated. The corresponding value of x for substitution into the amino acid intake requirement equation, is then obtained from statistical tables, and the optimum nutrient intake is then determined. Together with the expected food intake calculated on the basis described in Chapter 12 it is a simple matter to determine the level of the nutrient in the diet which will maximise profit.

2.2 Fitting the Reading model to experimental data

2.2.1 Computation of the equation

The computer programme used to fit the Reading model to experimental data was obtained from Dr Colin Fisher of the Poultry Research Centre, Edinburgh. For this programme three assumptions are made :

a) The threshold and plateau values for individuals are jointly normally distributed.

b) The slope of the linear part of the response curve is the same for all individuals.

c) The threshold for response is linearly related to some available measurement on each individual.

The computer programme uses an iterative procedure (Nelder and Mead, 1965) to determine values of a, b and the maximum production response that minimises the sum of squares of observed values of production at given intake levels about the theoretical population curve defined by the parameters of the model.

The derivatives of the curve are printed out together with observed and expected values of intake, as well as the minimised sum of squares.

2.2.2 Choosing parameters of the Model

It is possible to use theory or data to provide values of $\overline{W}$, σ_W, σ_{max} and $r_{max.w.}$ Also, the programme requires inputs of initial values of a, b and the maximum output response.

In order to obtain some information on the magnitude of the parameters σ_W, $\sigma_{\Delta W}$ and r, 640 chickens involved in a trial were wingbanded, and the parameters were estimated from their growth and body mass during a two-week period starting at one week of age. The estimated values of the three parameters were as follows :

$$\sigma_W = 0{,}10 \times \overline{W}$$

$$\sigma_{\Delta W} = 0{,}12 \times \Delta\overline{W}$$

$$r_{w.\Delta w} = 0{,}88$$

The effects of varying these values on the sum of squared deviations was tested and it was found that the value of σ_w made very little difference to the "goodness of fit", nor did the correlation between W and Δ W. The values of these two parameters used throughout the thesis were, for σ_W, 0,10 (or slight variations around 0,10) x $\overline{W}$, and for $r_{w.\Delta w}$ a value of 0,6 was chosen. It was felt that the value of 0,88 was higher than would be found in large populations where there was a greater diversity among individuals in the population.

It was found that $\sigma_{\Delta W}$ had a significant effect on the coefficients a and b of the model, the derivatives obtained and the "goodness of fit". In all instances an initial value of 0,12 x $\Delta \bar{W}$ was used as an estimate of $\sigma_{\Delta W}$, but in many cases no feasible solution was obtained with a value as low as this (for example, derivatives would be zero for all observed input values, or derivatives would all have the same high value, or SS deviations would be very large). In order to obtain a more feasible solution, either the initial "guesses" of a and b were altered (see later) or the value of $\sigma_{\Delta W}$ was increased or decreased. Manipulation of $\sigma_{\Delta W}$ was often resorted to as a means of improving the fit of the model and this accounts for the large differences in this parameter throughout the thesis.

The value of $\sigma_{\Delta W}$ should in theory not be manipulated. Indeed, when using larger volumes of data, i.e. more observations within an experiment, or grouping together data from more than one experiment, it was not necessary to alter the value of $\sigma_{\Delta W}$. It was, however, found to be necessary in certain instances especially where the number of observations was small or when the observations in the area of maximum response were found to be scattered rather than being positioned along a horizontal line. Sample size is therefore apparently of importance in fitting the Reading model to data.

The initial "guesses" of a and b for the Model were found to influence the result to a marked extent in certain cases. This is presumably due to the presence on the response surface of local minima which would be chosen as giving the best fit to the data for a given value of a and b, but which would not necessarily give the best possible fit to the data. Consequently, initial guesses were varied until the best fit was obtained as indicated by the lowest SS deviations.

The value used for $\overline{W}$ was calculated as the arithmetic mean of the mean initial and final body mass of the chickens in the trial.

The maximum growth rate accomplished by the fastest-growing chickens was used for the initial value of $\Delta\overline{W}max$. The values of $\Delta\overline{W}max$ published in the thesis were those values calculated by means of the computer programme.

2.2.3 Comparison of a and b values with external evidence.

One of the major advantages of the Reading model over previous methods of expressing nutrient requirements of poultry, is that the coefficients a and b of the Model are meaningful, i.e. they represent the amount of ingested amino acid associated with a unit of production and maintenance respectively. This was demonstrated by Fisher et al. (1978) and by Pilbrow and Morris (1974), who showed that the coefficients of the equations describing responses of laying hens to methionine and to lysine were similar to the amounts of methionine and lysine in egg material and in body tissue respectively.

A similar exercise was carried out with the combined data of all trials involving lysine and those where methionine was fed. The coefficients presented in Appendix Table 2.1 represent the values of a and b estimated for the combined sexes over both experimental periods. These values indicate that the apparent utilization of lysine and methionine by broilers for tissue growth and feathering (the a values) compare

Appendix Table 2.1 Recommended values for the coefficients a and b and comparison with data for carcass composition and maintenance

Amino acid	a (mg/g W)	Percentage lysine and methionine in carcass protein *	Lysine and methionine in total carcass (mg/g)	Apparent percentage utilisation
Lysine	16,53	7,46	14,17	85,7
Methionine	5,23	1,76	3,34	63,9

	b (mg/g W)	Protein required for maintenance * (mg/g W)	Lysine and methionine content of food protein * (%)	Lysine and methionine required for maintenance (mg/g W)
Lysine	0,0174	1,56	5	0,078
Methionine	0,0118	1,56	2	0,031

* Scott *et al*. (1976)

favourably with the corresponding values quoted by Scott et al. (1976). These coefficients indicate that dietary lysine and methionine are utilized with an efficiency of between 65 and 85 percent, a not unreasonable estimate. This would indicate therefore that the values of a for lysine and methionine are reasonably accurate estimates of the requirement for tissue growth.

The estimates of maintenance requirements for lysine and methionine suggested by Scott et al. (1976) vary markedly from the equivalent values estimated in this thesis. It is clear that one or the other approach is incorrect as the different estimates vary by several orders of magnitude. Further research is needed on this issue. One possibility is that the maintenance requirements for amino acids, like energy, are related more closely to metabolic body size than to the actual body mass of the birds.

In spite of the discrepancies found between the observed and the theoretical values of a and b it is nevertheless clear that the coefficients obtained with the use of the Reading model are meaningful and hence can be analysed, checked and compared with theoretical values. There is thus a considerable advantage in using the Reading model to describe responses of poultry to the intake of amino acids.

2.2.4 The Reading model as it applies to the growing bird.

The Reading model was originally proposed as being a means of expressing the response of a population of animals or plants to varying levels of a stimulus (Curnow, 1973) although it was designed specifically to describe the response of laying hens to increasing intakes of an amino acid. Fisher et al. (1973) critically evaluated the model in relation to the nutrition of laying hens (such birds being in a relatively steady state with regard to daily food intake and body mass) and they concluded that the model is an accurate predictor of nutrient intake when the birds are not gaining body mass.

In the case of broilers, the productive response being measured is, of course, daily gain in body mass, this variate thus replacing that of egg production in the case of laying hens. A problem arises here, however, in that the "maintenance" body mass (W) in the equation is increasing daily as is the food (nutrient) intake. The broilers are therefore not in a steady state, and this consequently casts a certain amount of doubt on the validity of the Reading model as applied to growing birds. There would, however, be an advantage in using a model which describes nutrient intake in terms of maintenance body mass and daily body mass gain, as this would allow for the calculation of the daily requirement of amino acids throughout the growing period from a knowledge of the growth curve of the broiler.

The strategy applied in this thesis was therefore to determine responses to the intake of lysine and of methionine during the early part of the growing period and again near the time of slaughter, when the composition of gain would be expected to differ from that of the earlier period of growth. The results indicated that the coefficients a and b were not significantly altered with age and consequently the same equation could be used to describe amino acid intake requirements throughout the growing period. In order to make use of such an equation in the case of growing birds, therefore, an equation describing growth in terms of food intake (such as the Parks (1970) equation which characterises the growth of the birds being used) can provide information on daily body mass and body mass gain, the two variates associated with intake of nutrients in the model. The daily requirement of each amino acid could then be estimated, from which a feeding strategy can be determined which would provide the broilers with the required amounts of nutrients each day. Whether the nutrient concentrations in the diet should be altered only three times (as is the practice in South Africa at present, where a starter diet is fed for the first 21 days, a finisher is fed to 42 days and a post-finisher diet is fed thereafter), whether more diets should be fed, or whether the amount of each diet supplied should be scheduled on a quantitative rather than a time basis, could be determined by means of a linear programming technique. Such a technique would minimise excesses as well as deficiencies and provide an optimal feeding programme for broilers throughout the growing period.

One of the assumptions made with regard to laying hens, that might limit the accuracy of the model, is that the distribution of ^{E}max is normal, this being a prerequisite when applying the model (Curnow, 1973). This is not likely to be a problem with broilers, as the maximum growth rate among individuals in a population would be expected to be normally distributed.

There is, however, a difficulty which arises when calculating the concentration of amino acids that have to be included in diets in order to meet the optimal requirements of the flock. In so doing, the average food intake of the flock is used to determine the required concentrations of nutrients, but it would be expected that the faster-growing individuals in the flock would exhibit an above-average food intake. Nutrient concentrations in diets for such birds would thus be overestimated. This is not a problem solely with the Reading model, but applies to any method which determines optimal nutrient intakes which then have to be converted to nutrient concentrations. The nutrient concentrations in the feed for slower-growing birds might similarly be underestimated, although a slight deficiency of nutrients in the diet has been shown to increase food intake (Lee, Gulliver and Morris, 1971). On average, then, the flock is supplied with sufficient nutrients in the diet to meet their requirements for these nutrients if they consume an average amount of feed each day. Variation in intake about this mean can be reduced by rearing sexes separately, thereby meeting more precisely the requirements of all birds in the flock.

From the above discussion it is apparent that the Reading Model, although developed to describe the response of laying hens to nutrient intake, can also be successfully used to describe the response of growing birds to nutrient intakes. There was no evidence in this thesis to suggest that the coefficients a and b of the Reading Model should be altered as the birds aged, indicating that the same coefficients could be used throughout the growing period of the broiler.

Because the Reading Model allows for the separate estimation of the requirement for maintenance and for growth, this Model overcomes one of the major problems in the nutrition of growing birds, namely, the calculation of daily nutrient requirements.

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