

T BIOLOGICAL AND ECONOMIC RESPONSE  
OF LAYING HENS TO DIETARY  
ISOLEUCINE, METHIONINE, LYSINE  
AND ENERGY /

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D  
- 1980. -

I HEREBY CERTIFY THAT THIS RESEARCH IS THE RESULT  
OF MY OWN INVESTIGATION.

A handwritten signature in blue ink, appearing to read 'M. Griessel', with a horizontal line drawn underneath the name.

M. GRIESSEL

"The results of an experiment are never  
wrong, only our interpretation".

Leonardo Da Vinci,

1452 - 1519.



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## INTRODUCTION

The study of laying hen nutrition involves ascertaining the concentration of nutrients in the diet that will allow the hen, when consuming this food ad libitum, to meet her daily nutrient requirements for maintenance, growth and egg production. The definition of these nutrient requirements involves a study of nutrient inputs and the outputs (growth and egg production) corresponding to such inputs, followed by an economic analysis of such responses in order to ascertain the optimal daily intake of each nutrient.

Although a vast number of studies have in the past been conducted on laying hens in an attempt to ascertain the "requirement" of the hen for each of the essential nutrients, the majority of these reports have attempted to present a single value (usually some percentage of the diet) as the requirement of the bird for that nutrient. Such a single value is unacceptable when formulating diets for breeds varying in body mass and daily egg output in different environments, as the daily intake by these breeds will vary thereby causing the hen to consume either too little or too much of each nutrient, leading to uneconomical laying hen nutrition.

This problem was recognised by Fisher and Morris (1970) and a more logical approach to the problem was devised which led to the definition of response curves relating nutrient input to egg mass output which were independent of food intake. Such response curves allow the optimum nutrient intake to be estimated under changing economic conditions, and it is relatively simple to then formulate a diet for laying hens that will provide the birds with this optimal daily nutrient intake.

Although it is possible to use the results of previous research to determine such response curves many factors that are now recognised to be of importance in determining such curves were not considered in much of the earlier work. Such factors will be discussed at



length in this thesis. Also, a response curve of this nature could have universal application, and consequently should be a response curve derived from a number of well-planned experiments in order to prove that it has universal applicability.

For these reasons the work reported in this thesis was aimed at providing additional data to that already collected by Fisher and Morris (1970) on the response of laying hens to methionine intake, and by Pilbrow and Morris (1974) who studied the response of laying hens to lysine intake. Because isoleucine has on occasions been found to be limiting in laying diets in South Africa this amino acid was used in the third laying trial reported here. It is also important to demonstrate that no interaction exists between those factors that determine food intake and the utilization of nutrients by the laying hen. To this end, in each of the three experiments reported here, the effect of energy concentration (as a modifier of food intake) on the utilization of the three amino acids was studied.

## CHAPTER 1

REVIEW OF FACTORS AFFECTING THE PROTEIN  
AND AMINO ACID REQUIREMENTS OF LAYING HENS

Of the economic factors affecting egg production, feeding costs are by far the most important and are now responsible for between 80 percent and 85 percent of the total costs. It is therefore not surprising that in recent years increasing emphasis has been placed on the need to specify economic levels of essential amino acids rather than levels that will maximise production. Next to energy, greater amounts of the dietary costs are spent on meeting amino acid needs than on any other nutritive component.

Much of the laying hen nutrition research effort in the past has been directed towards ascertaining the "requirement" of a laying hen for various nutrients. Such a value is of little use outside the limits of the conditions set by the researcher, as the "requirement" thus established would not necessarily apply under different environmental conditions, for different breeds, and, most important, under different economic conditions.

Such research is therefore expected to yield very different "requirements", thereby confusing nutritionists formulating diets for a specific breed with its characteristic daily egg output, in a different environment to that used in the experiment.

Fisher and Morris (1970) recognised this problem and were among the first workers to obtain a response curve to increasing intakes of a nutrient (methionine). Such a curve can be used to determine the optimum intake of the specific nutrient under the economic conditions prevailing at the time, and then, with a prior knowledge of the amount of food consumed by a specific laying flock during peak egg mass output, the correct concentration of the nutrient in the diet of

the laying flock can be ascertained. This approach is a major step forward in our understanding of laying hen nutrition, and negates the earlier approach of determining a universal "requirement" for each nutrient.

In spite of the above, many of the experiments conducted on this subject prior to the pioneering work of Fisher and Morris (1970) could nevertheless be used to confirm the response curves obtained by Morris and his co-workers (Fisher and Morris, 1970; Pilbrow and Morris, 1974; Wethli and Morris, 1978). The conditions under which such results could be used are: that the amino acid under test was shown to be first-limiting; that the amino acid balance was not drastically altered with each further dilution of the diet; that adequate feed intake and egg mass data was published, and that the diets fed were shown.

Before summarising such results, the factors that would be expected to influence the "requirement" of an amino acid, and hence the factors that preclude the use of "a value" to indicate the optimum concentration of a dietary amino acid, are discussed. These factors will be seen to lead to inconsistencies and contradictions, mainly as a result of the incorrect manner in which the data is interpreted.

Throughout this thesis, the term "requirement" will refer to the concentration of the nutrient in the diet that was proposed by the respective authors to give optimal yields. The term is used purely in its historical sense thus allowing comparisons to be made between various techniques, breeds, environments etc.

# 1. METHODS USED IN DETERMINING AMINO ACID REQUIREMENT OF LAYERS.

## a. *Dietary treatments.*

Several dietary procedures have been adopted in the last two decades to determine the optimal dietary amino acid concentrations for layers. In some instances a purely empirical approach has been adopted, namely to analyse a diet known to



support a good level of production and to deem the amino acid content to be the recommended allowance. This approach is too rigid since it is impossible to seek improvement by altering the level of any one individual amino acid. Altering the proportions of the protein-containing ingredients will change the levels of all the amino acids in the diet.

Another approach was based on the empirical determination of the requirement of a single amino acid and the calculation of the requirement of the others by their ratio to the former in whole egg protein (Johnson and Fisher, 1958). Using the N-balance technique these workers determined the lysine requirement of layers. The N-balance appeared to be stabilized at a level of 0,5 percent lysine in the diet. The results of this study are presented in Table 1.1.

TABLE 1.1 Amino-acid requirements of laying hens based on the composition of whole-egg protein and the lysine requirement

Amino Acid	Whole-egg		Calculated requirement (percentage of diet)
	( g/16 gN*	Ratio )	
Cystine	2,3	0,33	0,16
Histidine	2,4	0,35	0,18
Isoleucine	6,9	1,00	0,50
Leucine	9,4	1,36	0,68
Lysine	6,9	(1,00)	(0,50)
Methionine	3,3	0,48	0,24
Phenylalanine	5,8	0,84	0,42
Threonine	5,0	0,72	0,36
Tryptophan	1,6	0,23	0,12
Tyrosine	4,1	0,59	0,30
Valine	7,4	1,07	0,54

\* Block and Weiss (1956) cited by Johnson and Fisher (1958)

Lysine taken as 1,00

Based on the lysine requirement of 0,5 percent

It must be remembered however that with this approach all the amino acid levels were calculated from the determined lysine requirement for egg production. This requirement includes

both the requirements for egg production and for maintenance. If, for example, the maintenance requirement for any one amino acid as a proportion of the total requirement should be greater than the proportion of lysine required for maintenance, the calculated total requirement would be too low. Bray (1969) pointed out that the requirement for lysine may increase at a more rapid rate than the requirement for isoleucine as the level of egg output increases. This is due to the relatively low maintenance requirement for lysine compared to isoleucine and tryptophan.

Studies on nutritional allowances for the chick have been relatively successful with an approach based upon the use of free amino acids i.e. semi-purified diets. However, such studies have been less successful when applied to determining the allowances for the laying hen. Johnson and Fisher (1959) were able to devise a diet that could support egg production, but egg mass and body mass of the birds were not maintained. Adkins, Harper and Sunde (1961) found that their diets supported body mass but only a poor level of production. Since the amino acids in such diets must be supplied as free amino acids and not as proteins, dietary ingredients tend to be atypical and secondary effects can influence the results. Further, the cost of free amino acids imposes a limitation upon the magnitude of duration of such feeding trials.

Askelson and Balloun (1964) pointed out that differences in availability of amino acids for absorption from the gastrointestinal tract, between a purified diet and a diet which contains intact proteins, may also affect the apparent requirement for a given amino acid. In the former diet, the amino acids are readily available for absorption without aid of certain enzymatic digestive processes. In contrast, a diet containing intact proteins must be subjected to a selective degradative process, which results in the release of individual amino acids in a relatively slow and orderly fashion. For

a period of time immediately following ingestion of a purified diet, it would be anticipated that there would be an excess of amino acids present in the gastrointestinal tract. This excess of amino acids would probably result in a lower efficiency of absorption than that observed when an intact protein is fed. If there was an excessive quantity of amino acids in the gastrointestinal tract, a high blood plasma amino acid concentration would also be expected. Thus when a purified diet is fed, the concentration of a given amino acid in the blood plasma may be greater than the body tissue's ability to efficiently utilize that amino acid. This would result in a further loss of the amino acid through deamination and/or excretion in the urine.

Another widely adopted approach involves adding graded supplements of an amino acid to a basal ration deficient in that particular amino acid. The disadvantages of this method are that it only allows the study of a narrow range of input levels, that the balance between the amino acids changes with each addition of the synthetic amino acid, and that at high levels of supplementation the amino acid under study may no longer be first-limiting.

Bray (1969) overcame this problem to a certain extent by using the egg protein ratio requirement standard (Johnson and Fisher, 1958) and making the basal diet 125 percent adequate by adding graded supplements of crystalline amino acids. The basal diet used consisted of 8.5 percent protein and was produced by diluting a 12 percent protein corn-soya diet with starch (corn provided 60 percent and soyabean meal 40 percent of the protein in the 12 percent protein corn-soya diet). The abovementioned author produced egg yields and body mass changes similar to those supported by an 18 percent protein corn-soya diet when using this 125 percent adequate diet brought up to 11.97 percent protein with glutamic acid. The synthetic portion of the amino acid under study could then be omitted from the



fortified basal diet and then included in graded levels. This enables an assessment to be made of which level gives optimum response. This procedure enables the attainment of high performance as opposed to the performance achieved on free amino-acid diets developed by Adkins et al. (1961). The technique, although very expensive, enables more replicates to be used over slightly longer periods compared to the Adkins et al. (1961) method.

Fisher and Morris (1970) suggested that to maximise profit margins attention should possibly be given to the adjustment of level of each nutrient rather than to achieve maximum levels of production. This implied abandoning the idea of a "fixed" requirement for a nutrient and replacing it by data relating rates of output to levels of input.

They developed a method for the determination of amino acid requirements of laying hens which involved the dilution of a high protein "summit" diet with an isocaloric nitrogen-free mixture. The undiluted or "summit" ration supplied an excess level of all amino acids but was unbalanced so that the excess of the amino acid being studied (145 percent of requirement) was smaller than that of the other amino acids (185 percent of requirement). When the summit ration was diluted with a protein-free energy source, the amino acid studied would obviously be first-limiting at all levels of dilution if the requirement standards used are accurate.

The method therefore rests on the interpretation of the response to different levels of dilution as a response to the first limiting amino acid. The requirements standards used were those derived by Johnson and Fisher (1958).

Pilbrow and Morris (1974) in comparing the lysine requirements of eight stocks of laying fowls formulated "summit" and "dilution" diets. Specific protein contents were not used in formulation but the minimum contents of all essential amino

acids except lysine were set at 175 and 70 percent of requirement for the "summit" and "dilution" diets respectively. The extremes of the range of lysine contents used were 150 percent and 60 percent of requirement in the "summit" and "dilution" diets respectively.

This formulation procedure is not a strict dilution procedure, as described by Fisher and Morris (1970), but is very similar in principle. In this case the pattern of surplus amino acids above those specified will not be identical in both rations but this is probably of little practical importance.

The advantage of this method is that it makes unnecessary the use of costly protein-free feedstuffs in the diets of low protein content and so makes a large scale experiment possible. Furthermore it overcomes the possibility that, at high levels of dilution, a proportion of birds will refuse to eat rations containing high levels of starch.

The desirable features of this procedure are:-

- (i) That the maximum response to the amino acid being studied is not limited by the level of other amino acids in the diet.
- (ii) The experimental rations are inexpensive and permit feeding of large numbers of birds for periods of many months.
- (iii) Maximum levels of performance can be obtained and the ingredients used in the rations reflect closely the commercial conditions under which the results will be applied.
- (iv) A wide range of the amino acid being studied can be used.
- (v) It could be extended to determine the most economic

intake under a given set of conditions (Fisher, Morris and Jennings, 1973).

- (vi) The balance between the amino acids remains the same in all diets.

b. *Using equations to determine the amino acid requirements of layers.*

Scott (1962) described a method for estimating the daily protein and amino acid needs of the laying chicken by calculating the output in the egg and in 3 g of body tissue protein needed to replace "wear and tear" and by assuming 85 percent digestibility of dietary protein. Experimental evidence to support this approach was given.

Moran, Summers and Pepper (1967) refined this method by using the maintenance needs for the adult rooster as reported by Leveille, Shapiro and Fisher (1960) in place of the body tissue protein figures of Scott. Subsequently Moran (1969) and Moran and Chiah (1971) pointed out that the hen must use her amino acid pool, presumably by breakdown of tissue protein, to accomplish the very rapid formation of egg white glycoproteins and shell membranes. Hurwitz and Bornstein (1973) applied this approach in two models. Model A assumed the need to breakdown 2 g of tissue protein to form 1 g of albumen protein. Model B assumed the need to breakdown 2,2 g and 4 g of tissue protein for the synthesis of 1 g of ovomucoid and shell membrane proteins, respectively. The resultant values were adjusted on the assumption of 85 percent absorption of dietary protein and amino acids. Hurwitz and Bornstein (1973) showed that the requirements of laying chickens by their Model B fell within the range of published values for amino acids somewhat better than those by Model A.

Their amino acid requirement (Model B) equation is as follows:-

$$\text{A A. (grams per day)} = 1,85 \text{ W.Am} + 0,21 \text{ G.At} + \text{EM} \\ (62 \text{ Ay} + 59 \text{ Ao} + 52 \text{ At.})$$

where      W = bodymass (kg)  
               G = mass gain (g/day)  
               EM = egg mass (g/day)

and        Am, At, Ay and Ao are the fraction of amino acid in protein of maintenance, tissue, egg yolk and ovalbumin respectively.

The author considered the examination of the tabled amino acid composition of various protein fractions together with the required calculation from the Hurwitz and Bornstein equations very laborious. He thus simplified the procedure by tabulating the amino acids requirements per gram and kg egg mass, mass increment and body maintenance respectively. (Table 1.2.)

TABLE 1.2      The amino acid requirements for maintenance, growth and egg production (modified from Hurwitz and Bornstein, 1978)

	Maintenance mg/kg day	Growth mg/g day	Egg Production (Model A      Model B)	
			mg/g day	
Arginine	125,8	14,1	15,19	11,55
Histidine	0,0	4,2	4,29	3,51
Isoleucine	75,9	8,6	10,76	10,48
Leucine	131,4	13,9	15,78	14,01
Lysine	31,5	15,8	15,32	11,09
Methionine	75,9	3,8	4,61	5,50
Methionine + Cystine	94,4	7,6	8,71	8,80
Phenylalanine	28,0	8,4	9,22	9,06
Phenylalanine + Tyrosine	62,9	13,4	16,22	15,89
Threonine	77,7	8,4	9,66	7,84
Tryptophan	20,35	1,7	2,21	2,17
Valine	50,1 *	14,1	15,19	13,03

\* Valine maintenance requirement found to be 2,71 percent of the maintenance protein instead of 3,5 percent. (Hurwitz and Bornstein 1978).



Wilgus (1976) applied Model B to laying chickens with some modifications.

1. The amino acid composition of body tissue protein of Scott, Nesheim and Young (1969) for chickens was modified by utilising those of Robel and Menge (1973). Arginine and cystine were thereby raised slightly, lysine raised one-third, phenylalanine and threonine reduced slightly and valine reduced one-third.
2. The maintenance values of Leveille et al. (1960) for adult chicken males were used. However, the protein was adjusted from full body mass basis ( $W^1$ ) to metabolic body size basis ( $W^{0,75}$ ). This was done because Wilgus (1973) found that use of  $W^1$  for both growing and adult turkeys resulted in excessively high sulfur amino acid estimations. This adjustment had little effect on the calculated maintenance requirements of a small bird but a marked effect on large turkeys.

The methionine content of the maintenance protein, given as 4,1 percent or 80 percent of total sulfur amino acids (T.S.A.A.), was reduced to 1,3 percent or 25 percent of T.S.A.A. This modification is based on the report of Graber, Scott and Baker (1971) that the T.S.A.A. requirements of chickens can be met by increasing proportions of cystine, being about 55 percent at two weeks of age and about 70 percent at 8 weeks. They refer to evidence that this proportion approaches 90 percent in mature animals. The methionine level is about 50 percent greater than the minimum maintenance level of Leveille et al. (1960). Applying it to Model B results in a decrease from 350 mg methionine per day by the 1,85 kg laying hen given by Hurwitz and Bornstein (1973) to 310 mg.

As with the Hurwitz and Bornstein equation the author similarly simplified the Wilgus (1976) equation. (Table 1.3).

TABLE 1.3 Model B

Amino acid requirements for mass gain and egg mass (Wilgus, 1976, modified)

Amino Acid	Body mass gain mg/g body mass gain/bird d.	Egg mass mg/gm egg mass prod. per bird d.
Arginine	15,50	12,09
Histidine	4,23	3,58
Isoleucine	8,45	10,69
Leucine	14,09	14,31
Lysine	21,13	12,71
Methionine	3,52	5,60
Cystine + Methionine	8,45	9,20
Phenylalanine	7,75	9,02
Phenyl + Tyrosine	14,09	16,27
Threonine	7,75	7,71
Tryptophan	2,11	2,33
Valine	9,16	12,04

To this must be added the maintenance requirement which can be calculated as follows:-

$$\frac{W^{0,75} \times 2 \times AM \times 10}{0,85}$$

where W = live body mass (kg)

and the amino acid composition of the protein for maintenance (AM) is:-

Arginine 6,9; Histidine 0; Isoleucine 4,1; Leucine 7,1; Lysine 1,7; Methionine 1,3; Methionine + Cystine 5,1; Phenylalanine 1,50; Phenylalanine + Tyrosine 3,40; Threonine 4,20; Tryptophan 1,10; Valine 3,50.

Smith (1978), based on more recent information on amino acid analysis of egg components, also suggested some refinements to the models of Hurwitz and Bornstein (1973) and produced two models to calculate the amino acid requirements of the laying hen.

The first of the suggested models was based on the assumption that only the formation of ovomucoids required the breakdown of albumin, whereas the second model postulated that serum albumin was also needed to supply the amino acids for the shell and membranes.

The recommendations of Smith (1978) are presented in Table 1.4.

TABLE 1.4 The amino acid requirements for maintenance, growth and egg production. (Smith 1978)

	Maintenance mg/kg/day	Growth mg/g/day	Egg Production	
			Model 1 mg/g/day	Model 2 mg/g/day
Arginine	126,4	14,2	8,0	9,9
Histidine	0,2	4,2	3,1	3,9
Isoleucine	76,2	8,7	8,0	8,4
Leucine	132,0	14,0	11,9	14,9
Lysine	31,6	15,9	9,0	12,3
Methionine	76,2	3,8	4,3	4,4
Methionine + Cystine	94,8	7,6	7,5	8,1
Phenylalanine	27,9	8,5	7,0	9,1
Phenylalanine + Tyrosine	63,2	13,6	12,8	16,2
Threonine	78,1	8,5	6,9	8,0
Tryptophan	20,4	1,7	2,1	2,1
Valine	65,1	14,2	10,5	11,6

The protein and amino acid requirement of laying hens can vary considerably depending largely on differences in egg output and body size. There is thus much merit in using an equation such as that proposed by Hurwitz and Bornstein (1973) in which these determinants are taken into consideration. It must however be noted that this method of amino acid determination together with the modifications proposed by Wilgus (1976) and Smith (1978) is still based on certain assumptions because of a lack of direct evidence. It must also be pointed out that Hurwitz and Bornstein's recommendations for maintenance vary considerably from those determined by Kandatsu and Ishibashi (1966) cited by A.R.C. (1975). Furthermore their equations assume a linear response to amino acid intake which is not tenable with results of biological experiments. For this reason especially, this approach should not be recommended.

c. *Using blood analysis to determine amino acid requirements of layers.*

Numerous studies have attempted to relate the plasma amino acid

concentrations with dietary amino acid adequacy. Zimmerman and Scott (1965) found that a definite relationship existed between plasma amino acid concentrations in chicks and the amino acid adequacy of the diet. The first-limiting amino acid remained at a very low concentration in the blood, irrespective of the amino acid deficiency. They suggested that this helps to explain why, in some instances, supplementing diets with the first-limiting amino acid has failed to increase the concentration of this amino acid in the plasma. According to Zimmerman and Scott (1965) no increase would be anticipated until the dietary concentration exceeded that needed for maximum growth. It is of interest that severe deficiencies of either lysine or arginine markedly increased plasma threonine. According to the authors this did not appear to be unique for threonine since many of the other amino acids behaved in this manner, but to a lesser degree.

Chi and Speers (1976) pointed out that in studies of the amino acid requirements of the laying hen, egg production, feed efficiency, egg mass and maintenance body mass are commonly used as criteria. Although these criteria are measurable end points, long feeding experiments are required to measure them and the results vary widely amongst investigators.

Salter, Coates and Westgarth (1971) demonstrated that the amino acids required for synthesis of egg proteins and for body maintenance in the laying hen were derived from the free amino acid pool, represented by the plasma pool. Chi and Speers (1976) therefore felt that plasma free amino acids would be a useful parameter for assessing dietary requirements of amino acids in laying hens, as this probably reflected the balance between dietary supply and the utilisation of amino acids in metabolism. The authors therefore fed a diet containing varying amounts of lysine to layers. They found that the plasma lysine remained at relatively low levels until dietary lysine was increased to a level where egg production and nitrogen retention were



maximised, and thereafter increased rapidly. The dietary lysine requirement of laying hens determined by plasma free lysine, nitrogen retention and egg production were 677, 664 and 687 mg/hen day, respectively.

It must however be pointed out that some investigators have failed to establish a relationship between dietary concentrations of amino acids and increases in plasma concentrations during the absorption period (Eggum, 1976). There may be several reasons for this discrepancy, one of them being the differences in the availabilities of the amino acids. Another reason may be the differences in the rate of absorption. It has also been reported by some workers that excitement of experimental animals markedly affects plasma concentrations of amino acids.

It can therefore be concluded that convincing evidence has not as yet been presented to indicate unique advantages in using plasma amino acid concentrations as indicators of protein adequacy.

## 2. NUTRITIONAL FACTORS AFFECTING AMINO ACID REQUIREMENTS OF LAYERS.

### a. *Availability of amino acids in different raw materials.*

Efforts to formulate diets in order to maximally utilise the amino acids in feedstuffs are often frustrated by the absence of consistent estimates of amino acid availability. Data for the total amino acid content of feedingstuffs are accumulating, but these amino acids may not be available for a number of reasons. The physical nature of the feedingstuffs, the physico-chemical nature of the protein, and the extent of chemical interactions between the amino acids and carbohydrates in the feedingstuff can all affect availability. These characteristics can be modified both advantageously and adversely by the processing of the feedingstuff. A mild heat treatment improves the digestibility by a denaturation of the native proteins and an inactivation of certain protease

inhibitors. Extensive damage arises if higher temperatures are used for a longer period of time. In this case interactions between functional groups within the protein or with other food components like reducing sugars may occur.

The most important interaction making an amino acid unavailable is the so-called Maillard reaction. It has been well known for several years that in the case of proteins mainly lysine is involved in these "browning reactions" since lysine can react with its free and reactive E-amino groups thus forming first an unavailable lysine-sugar complex and then leading in further stages to a total destruction of the lysine molecule.

The standard method of measuring availability is by biological assay, using either growth rate or the feed conversion efficiency of chicks. The time, cost and variability involved in such assays preclude their routine use and so microbiological and chemical methods have been sought which provide effective substitutes. Of these latter methods, only the chemical estimation of available lysine, originally developed by Carpenter, Ellinger, Munro and Rolfe (1957), has found consistent use. The problem of providing reliable routine estimates of amino acid availability for specific batches of ingredients therefore remains largely unsolved.

The work of Coser, Jokl and Vieira (1976) showed that a milder processing in the preparation of bloodmeal markedly improves its nutritive value when supplemented with isoleucine. Blood meal is deficient in isoleucine, since haemoglobin, its main protein, does not contain this amino acid.

Experimental and commercial blood meal, without supplementation, showed negative values for protein efficiency ratio and food efficiency, with no significant difference between them. Isoleucine supplementation improved the nutritive value of both products. However, the results were more dramatic for the



experimental blood meal. In the latter case, the nutritional value was nearly the same as casein. The workers pointed out that the temperature of processing they used does not exclude the possibility of contamination by microorganisms, since spores resist boiling for 30 minutes. This work shows, however, that a milder temperature may be found so that the resulting product will be more nutritious and at the same time bacteriologically safe.

When diets are calculated based on feed composition and analysis information, the assumption is usually made that amino acids are 80 - 90 percent available. This assumption is not necessarily valid. By-products such as feathers or blood are either indigestible in native form or made indigestible by overheating in processing, respectively. In such cases, the product must either be demonstrated to contain highly available amino acids, or availability coefficients should be used as formulations modifiers. Certain other feedstuffs such as carcass meal and oilcakes are also affected by treatment during normal manufacturing processes. As more research is done in this area, it may be possible to reduce our estimates of amino acid requirement levels and use amino acid tables that consider the availability factor. The results of amino acid determinations on the same feedstuff between different laboratories, unfortunately, differ considerably. When this occurs, a small difference in availability (between 80 or 90 percent for a particular amino acid) in a feedstuff becomes relatively unimportant.

b. *Amino acid imbalance.*

The usual approach to assuring both adequate and economic amino acid nutrition for layers has been to combine energy and protein concentrates and supplemental amino acids in such a way as to meet the requirements for essential dietary amino acids on a least-cost basis. In so doing, it has been assumed that the excesses of some amino acids programmed into the diet are



deaminated and used as sources of energy to a varying degree depending upon the amino acid in question.

Since amino acids in general enter into a variety of metabolic pathways it has been widely assumed that any surplus ingested and not subsequently used for protein synthesis exerts no adverse effects. It should be recognised, however, that surpluses of amino acids necessarily reduce the efficiency of utilisation of the total protein content of a diet, the magnitude of this effect depending upon the extent of the deviation from an ideal balance of amino acids. There are moreover, certain instances in which a dietary excess of an amino acid, or of a mixture of amino acids, is known to precipitate an ill-effect which is totally disproportionate to the degree of imbalance (Harper, Benevenga and Wohlueter, 1970). Following the consumption of such an imbalanced diet there are reductions both in the rate of growth and in feed intake. There has been some controversy as to which of these features is the primary consequence of the consumption of the imbalanced diet but convincing information is now available showing a marked depression in feed intake after three to six hours. This implies that the depression in feed intake is the primary effect, which in turn is responsible for the retardation in growth. (Harper and Rogers, 1965; D'Mello and Lewis, 1971).

Nesheim (1974) points out that growth depressions can be caused in animals by feeding a diet low in protein to which an amino acid mixture devoid of one amino acid is added. This growth depression can be alleviated by providing a small amount of the amino acid missing in the mixture. The depressed growth rate is not caused by impaired utilization of the limiting amino acid.

There is a very rapid and marked reduction in food intake by animals fed imbalanced diets. However, if the diet is force-fed or if food intake is stimulated by other means there are no apparent detrimental effects to the animal (Harper, 1970).



The addition of an amino acid mixture lacking one amino acid to a low-protein diet seems to stimulate protein synthesis. This in turn causes a reduction in the quantity of the most limiting amino acid in the plasma and other body pools. Apparently the alteration of the body amino acid pool is detected by some receptors, most likely in the brain, that respond and cause a reduction in food intake. Rogers and Leung (1973) cited by Nesheim (1974) have reported that depressed food consumption resulting from the feeding of imbalanced diets could be overcome by destroying certain sites in the brain of rats.

(i) Arginine and Lysine

D'Mello and Lewis (1970) found a linear relationship between the arginine requirement of chicks and dietary lysine levels while Allen, Baker, Scott and Norton (1972) showed that efficiency of arginine utilization decreased to 59 percent of control values as the level of dietary lysine increased to 1,84 percent of the diet.

Nesheim (1968) cited by Nesheim (1974) interpreted data from several sources as indicating that when the ratio, percentage of dietary lysine/percentage of dietary arginine, was greater than 1,2 to 1,3 less than maximum growth was likely to be observed. This conclusion is supported by a similar analysis of the data of D'Mello and Lewis (1970). The relationship seems to hold over a wide range of dietary arginine and lysine levels.

The mechanism of the arginine-lysine interaction is related to the influence of lysine on the level of the enzyme, arginase, in chick kidney. This enzyme splits arginine to urea and ornithine. Ornithine can be further metabolised while the urea is excreted in the urine.

When chicks are fed excesses of dietary lysine, the

activity of kidney arginase is increased and arginine breakdown is also increased as shown by increased excretion of urea in the urine (Austic and Nesheim, 1970).

(ii) Arginine and Methionine

An interesting relationship between arginine and methionine was reported by Keshavarz and Fuller (1971). When dietary methionine levels are marginal, excesses of arginine depress growth rate of chicks. The depressed growth can be overcome by small amounts of added dietary methionine. This interaction could be demonstrated when a corn-soyabean meal type diet was fed to growing chicks to which was added 1 percent L-arginine. This apparently increased the methionine required for use in creatine synthesis, since the extra arginine increased creatine excretion. Since high levels of added arginine are not likely to be added to diets for growing chickens, this interaction probably has little practical significance. It is conceivable, however, that chicks fed diets in which peanut meal is used in large quantities, might have a slightly higher methionine requirement since peanut meal is very high in arginine.

(iii) Leucine, Isoleucine and Valine

These three amino acids have very similar structures and their interrelationship is fully discussed in the chapter dealing with the isoleucine requirements of layers. (Chapter 3).

(iv) Arginine and other amino acids

It is quite clear that the mechanism of the arginine-lysine interaction is not unique. Other amino acids will cause elevated arginase activity in chick kidney when fed in excess and cause an increase in arginine requirement (Austic and Nesheim (1970). These



amino acids include histidine, isoleucine, ornithine, tyrosine and phenylalanine. With the exception of phenylalanine, much higher dietary levels of these amino acids are required to cause a reduction in growth rate and elevated arginase activity compared to lysine. These amino acids do not seem to accumulate as readily in plasma as lysine, possibly because they are degraded at a more rapid rate. When they are present in large amounts, these amino acids elevate kidney arginase and cause increased breakdown of arginine. Phenylalanine increases the arginine requirement when fed in relatively small excess.

(v) Threonine - Tryptophan

The tryptophan requirement is increased if excesses of threonine are fed to chicks. According to D'Mello and Lewis (1970), an increase of 0,5 percent in the threonine content of the diet for young chicks increases the tryptophan requirement by 0,01 percent of the diet. This was true for a range of threonine levels from 0,8 to 2,3 percent of the diet. No mechanism for this interaction was suggested. This is unlikely to be a practical problem, since threonine is not present in excessive quantities in most proteins.

The methods used in the present study to identify amino acid response curves assume that surpluses of amino acids in the diet have no affect on performance. This assumption is probably valid for the great majority of diets, but the mounting evidence that amino acid imbalance can be an issue of practical importance, must not be ignored. More research should be directed towards quantifying the effects of amino acid surpluses so that suitable conditions can be incorporated in linear programme matrices to prevent the formulation of imbalanced diets.

Jensen, Chang and Falen (1974) summarised a number of reports which indicated that the estimated lysine requirement of laying hens was usually greater if wheat had been used as the major component of the diet rather than maize. In their trial the requirement was found to be about 100 mg lower if the diet was based on corn rather than wheat.

Morris (1980, unpublished) produced summit and dilution mixtures based on wheat and maize. By blending appropriate quantities of these mixtures, five wheat diets and five maize diets were obtained with descending protein levels, supplying total lysine concentrations from 7,50 down to 3,54 g/kg. (Table 1.5).

TABLE 1.5 Comparing the responses of laying pullets to lysine in wheat-based diets with the responses to lysine in maize-based diets. (Morris, 1980)

Diet	Lysine content (g/kg)	Feed intake g/hen d	Eggs per 100 bird days	Mean egg mass g	Mean egg output g/hen d
W1	7,50	132,3	71,91	68,06	48,94
W2	6,51	137,4	70,47	67,83	47,80
W3	5,52	139,5	67,98	67,61	45,95
W4	4,53	135,7	58,63	65,90	38,64
W5	3,54	106,0	34,64	60,90	21,15
W6	4,54	119,8	45,85	64,03	29,37
M7	7,50	128,4	69,68	68,70	47,85
M8	6,51	133,2	70,15	68,57	48,11
M9	5,52	129,1	71,75	67,45	48,39
M10	4,53	123,8	67,52	66,36	44,81
M11	3,54	123,5	56,57	65,37	37,01
M12	4,54	127,9	63,05	66,22	41,77
S.E.		2,99	1,663	0,414	1,167

The lowest maize protein M11 and lowest wheat protein diets (W5) were also supplemented with L-lysine HCl to obtain diets M12 and W6. These provided a test of the assumption that the mixtures used were first limiting in lysine. Warren S.S.L.F. pullets which were



60 weeks old at the start of the trial were used to compare output from the 12 diets.

There was a clear response to supplementary lysine (Table 1.5 and Figure 1.1) and the protein response curves obtained were regarded as responses to dietary lysine intake. There was a significant separation of the response curves for the wheat and maize-based diets, confirming the conclusion of Jensen *et al.* (1974) that the effective requirement for lysine is higher when wheat is used as the major dietary component.

Morris (1980) speculated that the lower egg outputs obtained from diets W4 and W5 compared with M10 and M11, in spite of similar total lysine intakes, might be due to: (a) deficiencies of amino acids other than lysine; (b) some anti-nutritive property of the wheat; (c) low availability of lysine in the wheat or (d) the effects of surplus amino acids supplied by the wheat.

Effects due to second limiting amino acids seem unlikely in view of the response obtained to lysine supplementation (see figure 1.1). Wheat used at high levels in diet W1 and W2 supported egg output equal to the best of maize diets and this seems to rule out any hypothesis that the wheat contains substances which depress egg production in any general sense. The most obvious interpretation of Figure 1.1 is that the lysine in the wheat had low availability; yet Morris (1980) points out that the digestibility of lysine in wheat has been shown to be high, as judged by examination of ileal contents in the chick. In a suitably designed chick bioassay, the Reading workers (Morris 1980) have furthermore shown that the availability of lysine in wheat is about 90 percent.

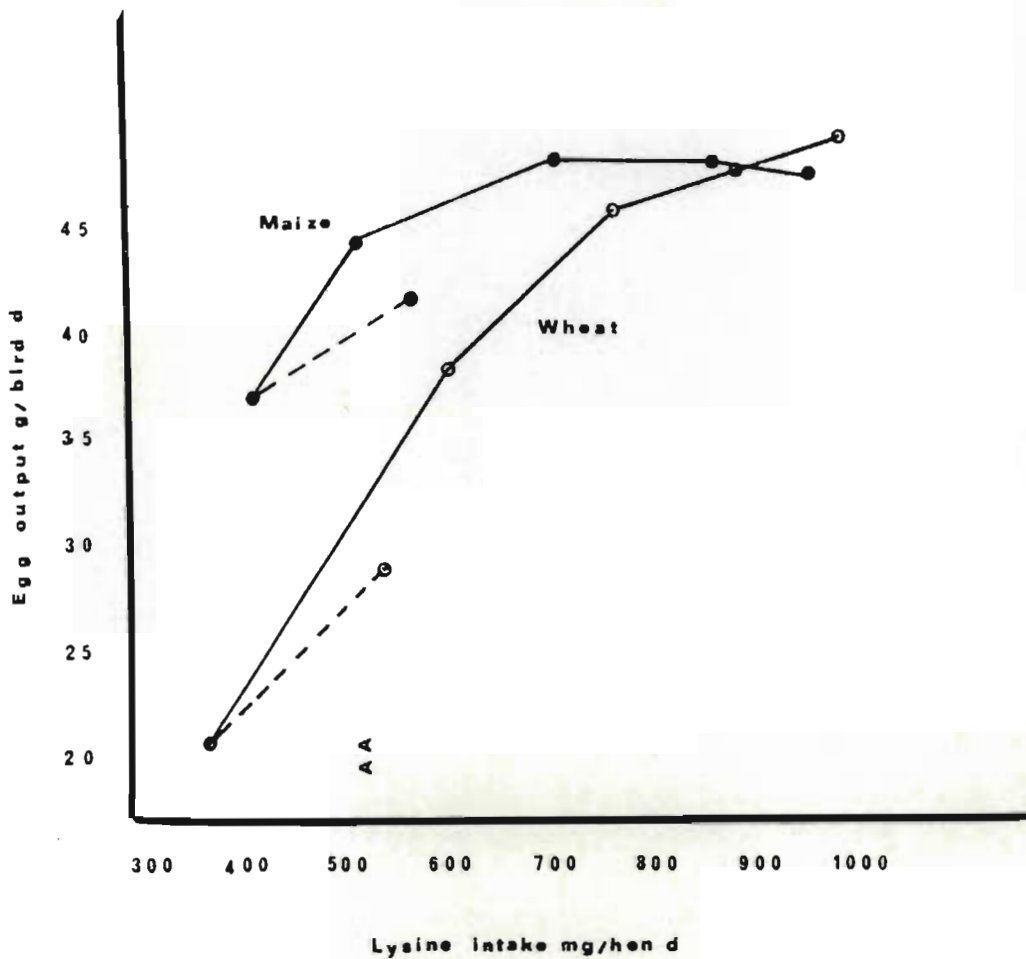


FIGURE 1.1 Response of laying pullets to lysine supplied from wheat diets - ; and maize diets - . Broken lines indicate the response to adding purified L-lysine to the bottom diet in each dilution series (Morris, 1980)

This left these workers with the fourth hypothesis - that we are dealing with a problem of amino acid imbalance. According to Morris (1980), a series of studies conducted with chicks at Reading has shown that amino acid imbalance is responsible for the apparent unavailability of lysine in wheat, as judged by chick bioassays of the simple substitution type.



c. *Amino acid assigned to feedstuff.*

The results of amino acid determinations on the same feedstuff between different laboratories differ considerably.

Factors which could affect the values ascribed to a particular amino acid on chemical analyses of a raw material fall under two main categories, namely the equipment and technique used for the separation and the method of hydrolysis. Those which play a role in the method of hydrolysis will be elaborated on as an example to point out the differences that could be allotted to a particular optimum amino acid level. However just as important in determining this optimum amino acid level could be the equipment and technique used by a particular analysing laboratory.

Davies and Thomas (1973) comprehensively surveyed the effect of hydrolytic methods on amino acid determinations and in summary they are:-

(i) Protein source.

In a pure protein source lysine appeared to be released more slowly than in one containing impurities like carbohydrates and different minerals, this being more than 60 hours and 20 hours respectively in 6 N HCl at 137°C for a pure protein (casein) and a potential food protein of microfungal origin (Fusarium graminearum). Of importance here are the proteins per se and the presence of carbohydrates and minerals in Fusarium graminearum, resulting in errors as high as 30 percent or more if the optima are not known.

(ii) A particular peptide bond.

Amino acids differ in the ease with which they are liberated from peptides during hydrolysis. Davies and Thomas (1973) cited the work of Synge (1945) and Christensen (1944) who showed that in a series of

dipeptides the resistance to hydrolysis with 10 N HCl at 37°C is greatly increased when the carboxyl groups of valine and leucine are involved.

- (iii) The length of hydrolysis of a particular protein

The optimum time of hydrolysis for complete liberation of amino acids depends on the nature of the linkage in each particular protein. Although some amino acids need longer hydrolysis times to be released some on the other hand become destroyed when longer times are used.

- (iv) The hydrolytic agent used and its concentration

There are many hydrolytic agents which are better suited for different amino acids. For example, with casein, optimum values for aspartic acid are obtained in 16 hours using 12 N H<sub>2</sub>SO<sub>4</sub>. On the other hand with Fusarium graminearum many different hydrolytic agents at varying concentration and hydrolysis times will give the same value for aspartic acid.

In the past, lysine has been the amino acid under consideration in most instances. The hydrolysate for lysine is normally prepared by using 6 N HCl for 22 - 24 hours. If other amino acids are required the same hydrolysate is normally used. Under these circumstances the values for isoleucine, leucine and valine will be substantially lower than if the optimum time or hydrolysis method is used. For example, with these amino acids and using 6 N HCl the optimum time for hydrolysis appears to be nearer to 60 hours.

From the graphs presented by Davies and Thomas (1973) it would appear that an increased value of 15 percent for isoleucine was obtained by extending the period of casein hydrolysis from 20 to 60 hours. Similarly



isoleucine values increased by 28 percent when hydrolysing Fusarium graminearum for 60 hours instead of 16 hours.

d. *Specific amino acid relationships and conversion of amino acids to vitamins.*

The requirement for methionine can be met only by methionine, while the requirement for cystine may be met by cystine or methionine. This is because methionine is readily converted to cystine metabolically, while the reverse is not possible. If sulphate is deficient, a portion of the cystine that is normally converted to sulphate metabolically may be spared by the addition of sulphates to the diet.

Similarly, the requirement for phenylalanine may be met only by phenylalanine, while the requirement for tyrosine may be met by tyrosine or phenylalanine.

It has also been shown that high levels of methionine may partly compensate for a deficiency of choline or vitamin B12 by providing needed methyl groups. High levels of tryptophan may alleviate a niacin deficiency through metabolic conversion to niacin. Incorrect conclusions may therefore be drawn if consideration is not given to adequate nutrient levels in the diet other than the parameter investigated. For example, a higher methionine level will be required in cases where metabolites of methionine such as cystine, choline and vitamin B12 are lacking.

e. *Toxic or harmful compounds in certain protein feedstuffs.*

Another problem with some protein feedstuffs is that they may contain toxic compounds. Examples are cottonseed meal and peanut meal, which may contain gossypol and aflatoxin respectively.

Even soyabean meal, the most widely used protein supplement, contains harmful substances, such as a trypsin inhibitor, but

these are destroyed by proper heating. Heat treatment improves the utilization of the protein in this excellent feedstuff.

Of the various chemical tests performed on soyabean meal to determine its quality, the urease test is by far the most useful; this test is used as a guide to proper cooking of the meal, to ensure maximal nutritional value and trypsin inhibitor destruction. One of the most complete experiments on the effects of cooking on protein efficiency of soyabean products was conducted by Hayward (1959). His results showed that the quality of the protein is increased with protein denaturation. However, excessive cooking resulted in a lower protein efficiency due to decreased amino acid availability. It is suggested that a pH rise of 0,2 represents adequate cooking.

As the cooking time increases the meal will eventually record a pH rise of 0,0. Since it is impossible to know whether an 0,0 rise in pH indicates a meal that is perfectly cooked to the point of destroying the trypsin inhibitor or whether it was overcooked and the proteins damaged, the lower value of 0,05 is included as a check against overcooking.

Similarly, histamine in fishmeal has at times been incriminated as the causative agent of gizzard erosion in poultry. It is a well known fact that pelagic species such as herring, mackerel and tuna contain higher quantities of the amino acid histidine than other species Harry, Tucker and Lauvsen-Jones (1975). Should fishmeal be allowed to decompose prior to processing, microbial action decarboxylates histidine to histamine. Serious attention should therefore be given to expedite processing after catching.

Furthermore, Wessels (1975) concluded from trials that contact of fish with 0,2 percent formaldehyde in the form of 40 percent solution in most instances depressed available lysine. It is a well known fact that fishmeal manufacturing plants use up



to 0,02 percent formaldehyde to "firm up" fish before processing. Consideration should therefore also be given to the harmful effects of excessive formaldehyde on protein quality in fishmeal.

Because the abovementioned factors could have a significant effect on the performance of layers, the question arises as to whether researchers investigating amino acid requirements have in fact established whether the raw materials used in their diets were adequately processed and free from toxic compounds.

f. *Feed intake (kilojoule intake).*

If the requirement for an amino acid is expressed as a proportion of the diet, that requirement will be altered by all the factors which modify voluntary feed intake by the hen. Thus the concentration of protein required in the diet will increase when the energy content of the diet is increased or when the hen is kept in a warmer environment. These effects can be fully explained by the change in feed intake which occurs. There is no change in the absolute protein requirement of the bird in these situations, and the daily intakes of essential amino acid required to maximise production remain the same (Bray and Gesell, 1961; Frank and Waibel, 1960).

The importance of feed consumption and its relationship to temperature have been discussed in the chapter dealing with energy (Chapter 2). The influence of energy concentration on feed consumption is certainly the major factor to be considered at moderate temperatures. Both low and high protein diets can also affect feed consumption. These relationships have increased interest in other means of expressing protein and amino acid needs, rather than as a percentage of the diet.

In an experiment by Latshaw (1976) an attempt was made to compare the lysine requirement of laying hens fed diets containing

different energy concentrations. Intake of the higher energy diet, as compared to the lower energy diet, was not decreased as would have been predicted on the basis of a nine percent difference in metabolisable energy. Based on feed intake, and using egg mass as the criterion, the lysine requirement was 657 mg per hen day for the lower and 636 mg for the higher energy diet.

g. *Total versus available amino acids.*

Some researchers use an amino acid analyser to determine the amino acid composition of diets whilst others use microbiological or chemical assays to determine available amino acids. When their publications are studied it becomes clear which method was employed. However, when tabulated recommendations by various workers are presented no indication is normally given as to whether the figures reflect total or available concentrations. This method of presentation can cause confusion and it is therefore necessary to refer to the text to establish whether the discrepancies can be attributed to the amino acid assay method used.

3. INTERPRETATION OF DATA.

a. *Production criterion.*

Latshaw (1976) pointed out that the requirements for lysine will depend on the production criteria used. If egg production is used, the lysine requirement is relatively low. He referred to one of their trials where calculated intakes of 534,558 and 588 mg of lysine per hen day supported egg production rates of 73,7, 74,3 and 70,4 percent respectively. The amount of lysine required for maximum egg mass in the above-mentioned diets however, were 607, 657 and 636 mg per hen per day respectively. Predictions based on body mass paralleled those based on egg mass. The author concluded that if the lysine shortage was severe enough to decrease egg mass it would also decrease body mass. Feed consumption in the



above trial was of no use in determining the requirement, because the levels of lysine used in their study did not change feed consumption.

Similarly Thornton, Blaylock and Moreng (1957), Biely and March (1964) and many others indicated that size of egg is more sensitive than rate of production to a dietary amino acid deficiency.

Janssen (1974) and Schutte *et al.* (1978) also found that the total sulfur amino acid requirement for maximum efficiency of food utilisation is distinctly higher than that for maximum egg yield. They indicated that the low methionine requirement found by Fisher and Morris (1970) and Jensen *et al.* (1974) could be explained by the fact that they used mainly egg production criteria, rather than food conversion efficiency in their assessments.

b. *Statistical procedure.*

Bray (1965) concluded that layers require 224 mg methionine per day whilst Fisher and Morris (1970) recommended 275 mg/hen day. The latter authors indicated that the discrepancy between their finding and that of Bray (1965) could be attributed to different statistical methods used.

Fisher and Morris (1970) fitted several response curves to the data of Bray (1965). Comparing these results with those of other studies they concluded that the higher estimate derived from a quadratic or exponential curve might be a better interpretation of the data obtained. The estimate of 223 mg methionine per day suggested by Bray (1965) was clearly inadequate when considering the results of other studies. They indicated that the method of fitting two straight lines used by Bray (1965) will always underestimate the requirement for maximum output if the true response line is a continuous curve.

Several of the response curves fitted to the data of Bray (1965) are presented in Figure 1.2



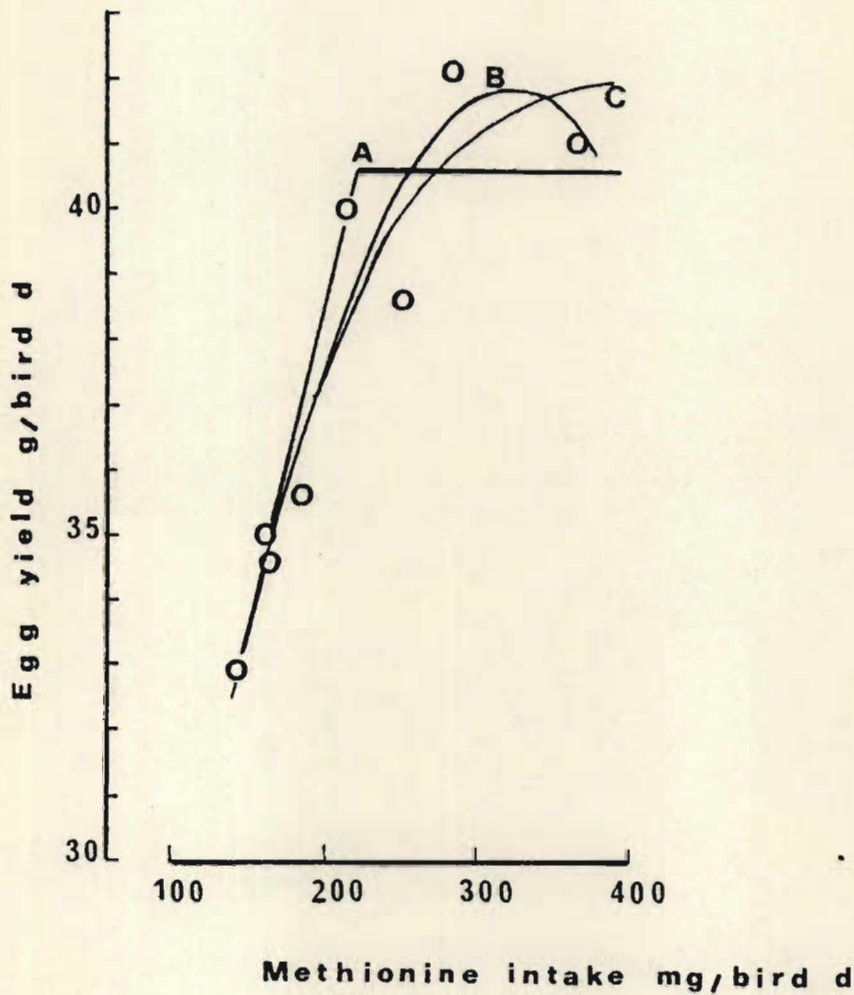


FIGURE 1.2 Several response curves fitted to the data of Bray (1965)

- A. Taken from Bray's paper.  $y = 40,58$  for  $x \geq 2,235$ , for  $x < 2,235$ ,  $y = 40,58 + 10,04 (x - 2,235)$  Residual ssy = 8,5.
- B.  $y = 12,66 + 18,1101x - 2,8441x^2$ . Residual ssy = 7,3.
- C.  $y = 43,20 - 43,2190e^{-x}$ . Residual ssy = 8,5,  
 where, in each case,  $y$  = egg yield, g per bird day  
 $x$  = daily methionine intake,  $\text{mg} \times 10^{-2}$ .

Fisher et al. (1973) pointed out that the use of a linear response up to a maximum level usually leads to the underestimation of requirements. On the other hand use of the better known curves, based on quadratic, exponential or inverse polynomial equations, frequently leads to overestimation of the intake required for a given level of output in the area of response in which practical decisions are made. These authors presented a model which describes the response of groups of laying hens to different levels of amino acid intake. The model is based on the assumption of simple linear relationships between amino acid intake and the output characteristics, egg production and maintenance, for individual birds. The response for a group of birds is then derived as the average of the individual responses. In this model the group response is described by a line, the shape and position of which are a function of the following seven parameters: the mean maximum egg output ( $E_{max}$ ); variation in  $E_{max}$ ;  $\bar{W}$ , the mean body mass; variation in  $W$ ;  $r_{EW}$ , the correlation between  $E$  and  $W$ ;  $a$  and  $b$ , the quantities of amino acid associated with a unit of  $E$  and a unit of  $W$  respectively.

Curnow, cited by Fisher et al. (1973) showed that the principle used in this model to define an approximate equation for the requirement of a flock could be extended to give a completely general statement for economic intake under a given set of conditions.

This method has been used successfully by Fisher and Morris (1970) to determine the response of laying hens to methionine; by Pilbrow and Morris (1974) for lysine responses and by Wethli and Morris (1978) for describing the response of laying hens to tryptophan. Because of the logical basis of the equation both with regard to the meaningful coefficients produced and to the calculation of the optimum intake of the nutrient concerned, this "Reading Model" has been used throughout this thesis to describe the response of laying hens to dietary amino acid intakes.



#### 4. AGE AND RATE OF LAY.

In a well-designed experiment to study protein requirements of pullets throughout the laying year Fisher and Morris (1967) found no indication that the amount of protein needed for maximum egg-output was any less in one period than in another.

The data of March and Biely (1972) and Latshaw (1976) regarding the daily lysine requirement of layers also indicate the impracticability of attempting to adjust the dietary level of lysine downward in relation to the decline in the rate of lay during the laying year.

In all cases in which responses to protein have been measured independently in flocks at different stages of the first laying year, nutrient utilisation has been lower in the older birds (Fisher 1976). The small differences in body mass over such a period do not provide a satisfactory explanation for this observation. When responses at similar stages of the first and second years of lay are compared, the older birds are again less efficient if they are laying at a lower rate (Fisher 1970, Fisher 1976; Wethli and Morris, 1978).

The effect of stage of lay on the protein requirement of a pullet flock does not show the same relationship between egg-output, body mass and the amount required of each amino acid. Egg output (measured in g/day) normally reaches a peak between 30 and 40 weeks of age and thereafter declines, but the amino acid requirement of a flock does not decline correspondingly.

Figure 1.3 is an example showing the decline in utilization of protein with age (Jennings, Fisher and Morris, 1972).



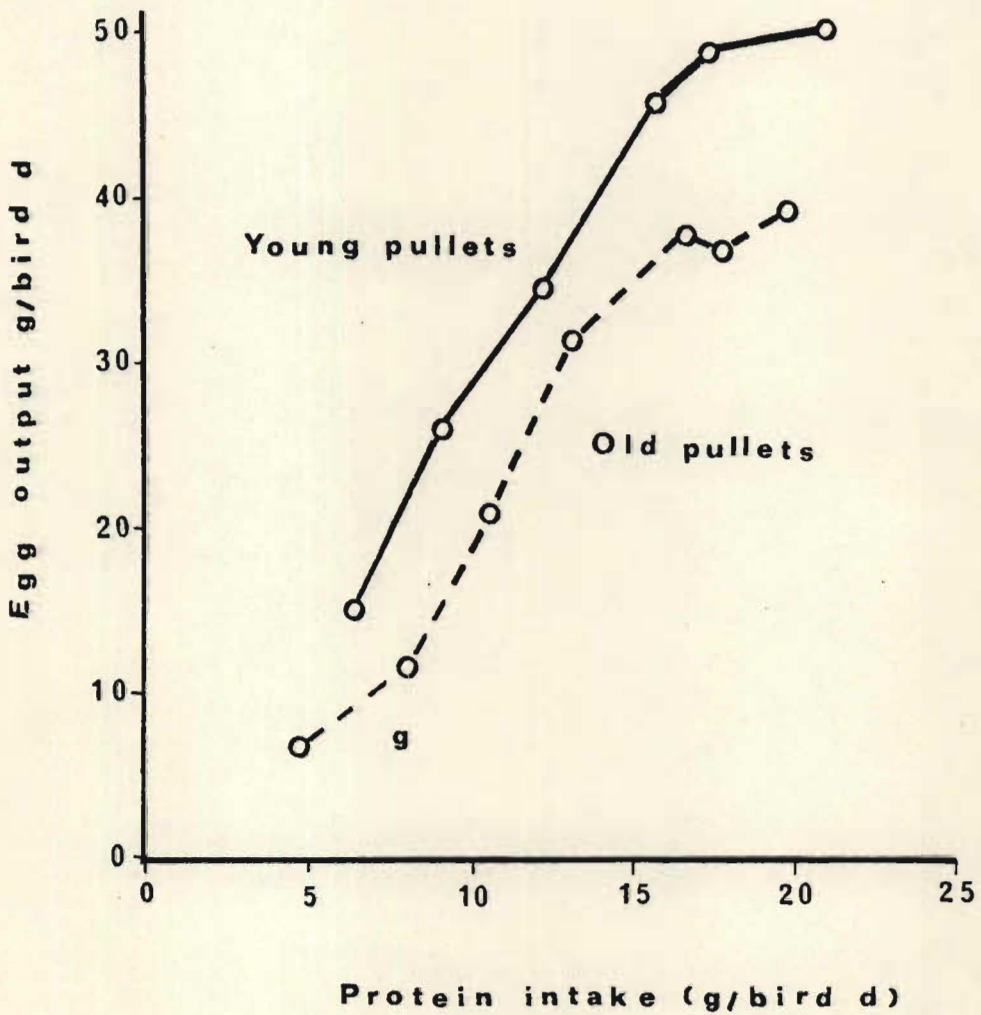


FIGURE 1.3

The response to graded levels of dietary protein at two different ages. (Jennings, Fisher and Morris, 1972).

A part of the decline in apparent efficiency of amino acid utilisation with age can be accounted for by the presence in the flock of pullets which are not laying eggs. These birds are eating a high protein layer diet in quantities determined by their maintenance requirement for energy. The inevitable result is that they take in more protein than is needed for maintenance and so their amino acid intake is largely wasted and the average utilisation of amino acids by the flock is depressed. Fisher (1970) cited by A R C (1975) has shown that about half the decline in efficiency of methionine utilisation which occurs during the laying year is due to the presence of poor-producing birds in the flock. The other half of the decline presumably reflects some real change in metabolic efficiency in the aging bird. The available evidence indicates that, for practical purposes, a laying diet should be designed to support the maximum egg output which the flock is expected to reach. The protein level should not be adjusted after peak production has passed, except to allow for real or anticipated changes in feed intake.

Some data for calculated methionine utilisation and rate of lay for individual birds of three ages are shown in Figure 1.4. (Fisher, 1976).

Although the method of calculating methionine utilisation is comparatively crude the effect of rate of lay is shown quite clearly, with a rapid decline in utilisation occurring, as expected, below 50 percent lay. The regression lines shown in the figure suggest that this effect does not provide a complete explanation for the effects of age on methionine utilisation since there is a significant age effect which is distinct from that of rate of lay. Fisher (1976) considered differences in feather growth the most likely explanation for this observation.

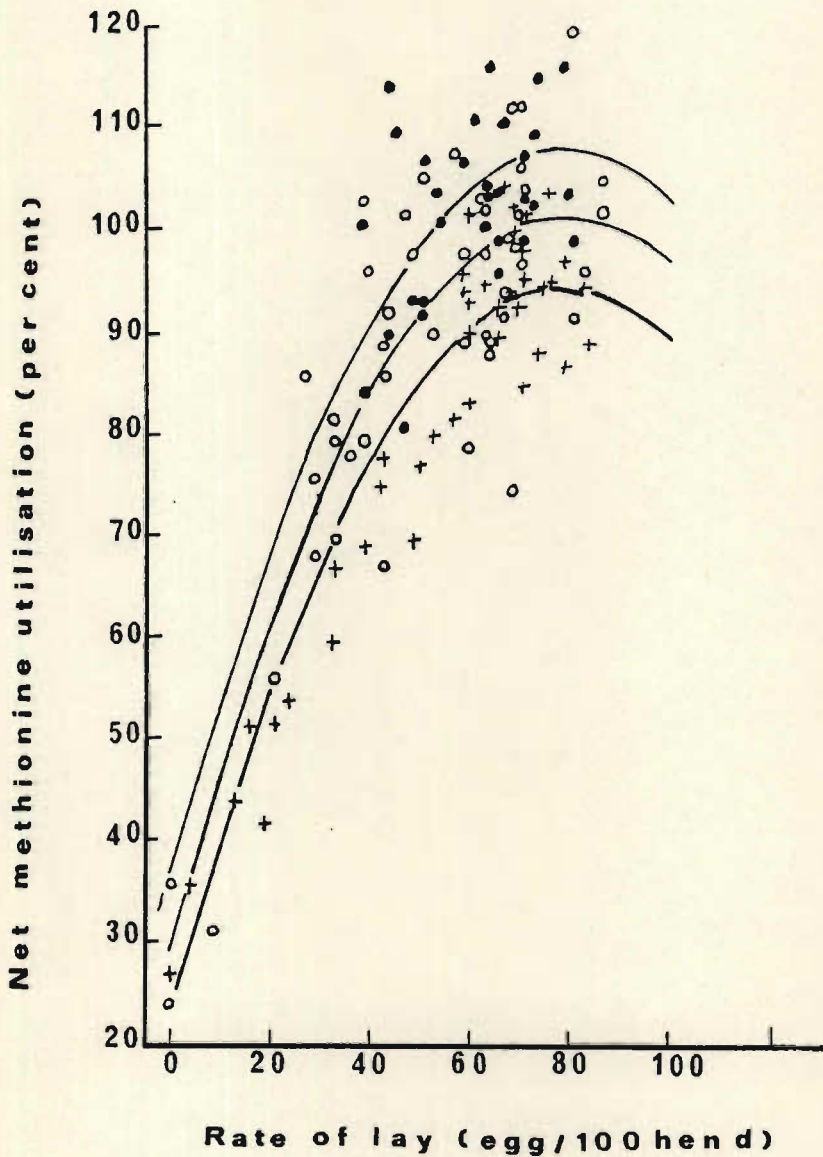


FIGURE 1.4

The relationship between net methionine utilisation and rate of lay in individual laying pullets of three ages:- 30 to 40 weeks (●), 45 to 55 weeks (○), 60 to 70 weeks (+). All birds received a diet containing 0,156 percent methionine which was shown to be the first-limiting nutrient. Net methionine utilisation = methionine utilised/methionine intake where methionine utilised =  $4E + 25W + 3,0 \Delta W$ . The curves are parallel quadratic regressions (From Fisher, 1970)



Wethli and Morris (1978) conducted a trial where egg output in response to different intakes of tryptophan was measured in young pullets, older pullets and moulted hens. From Figure 1.5 it is clear that the response to tryptophan intake was almost identical in the force moulted hens and younger pullets. The egg output on similar tryptophan intakes was considerably lower from the older pullets. This work clearly indicates that the reason for the poorer utilisation must not be sought in age. They pointed out that some of the older birds were moulting and an allowance for feather growth was therefore appropriate. This allowance however seemed unlikely to exceed 2 mg/hen d. Because maintenance requirements per kg body mass had increased from 4 to 17 mg/hen d another explanation for the higher requirement is needed.

It could possibly be concluded from the work of Wethli and Morris (1978) that since the efficiency of the force-moulted birds improved as a result of a rest period that the oviduct requires a restorative period to return to its optimal physiological efficiency.

It is interesting that adverse lighting is the only other situation in which protein utilisation is decreased (Bray, 1968), and that an increase in the number of pausing birds also occurs in this case (Morris, Fox and Jennings, 1964).

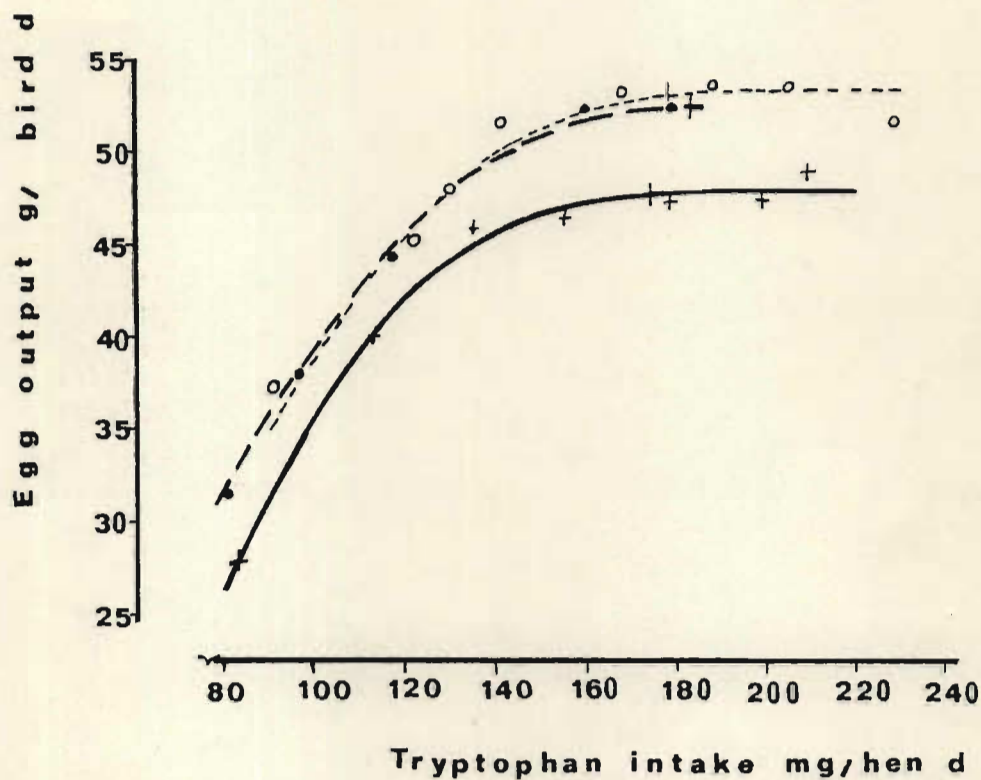


FIGURE 1.5

Responses to diets supplying different concentrations of tryptophan in three experiments. Young pullets (experiment 2), ● --- ●; older pullets (experiment 3), + —+; moulted hens (experiment 4), ○ --- ○. Vertical marks on the response curve represent optimal tryptophan intakes calculated by methods described in the text (Wethli and Morris, 1978)



## 5. STRAIN OF LAYERS.

Harms and Walldroup (1962) report a significant strain X protein level interaction for egg production when two similar strains of White Leghorn pullets were fed 13, 15 or 17 percent crude protein. The similarity of the response curves suggests that the response to different protein intakes was identical for these strains and the interaction arose because of different responses in food intake. Examination of the points shows that protein consumption is identical for the two strains at 17 percent protein but is higher in Strain A at lower levels of protein. This leads to a lower percentage protein requirement in Strain A.

Sharpe and Morris (1965) compared responses in a Rhode Island Red X Light Sussex strain and a small White Leghorn-type hybrid. These strains differed in egg output and also very substantially in body mass. The data indicate that the response curves are quite separate but the exact nature of the difference cannot be determined. For an output of 35 g egg material per day the larger bird appears to need 4 - 5 g extra protein. Part of this at least will be required to maintain the 1,1 kg of extra body mass and to cover extra growth requirements (3,95 g and 1,78 g live mass gain per day). Whether this is a quantitatively sufficient explanation of the difference cannot be decided.

Tolan and Morris (1969) found that after correction for output and maintenance the methionine requirements of individual birds still varied phenotypically by more than  $\pm 100$  mg/day. There was not direct evidence for a genetic component to such variation.

Pilbrow and Morris (1974) studied the response of eight commercial strains of laying hen to lysine. From the results it was clear that there were differences between the percentages of lysine which the stocks required in the diet for maximum output. For practical purposes, it would be necessary



to formulate diets of different protein contents if each of these stocks were to be fed at minimal cost for maximum egg production. But further inspection of the data shows that the differences between percentage requirements can be related to differences in food intake and in the associated yield and body mass characteristics of the stocks.

Table 1.6 shows the estimated lysine requirement of an individual from each stock for a mean body mass of 2 kg and a mean daily egg output of 44 g.

TABLE 1.6 Estimates of the coefficients of lysine utilisation per g egg produced a and per kg body mass maintained b for each stock, and for all stocks combined (Pilbrow and Morris, 1974)

	a (mg/gE)	b (mg/kg W)	Estimates of lysine required (mg/d) for a 2 kg bird produc- ing 44 g egg/d
Thorner 606	10,2	73	595
Hyline 934	5,0	190	600
Shaver 288	8,5	120	614
Babcock B <sub>300</sub>	7,25	140	599
Thorner 404	9,1	95	590
Sykes H <sub>4</sub>	10,0	70	584
Harco Sex Link	8,25	105	573
Alexander and Angell <sub>3</sub>	7,0	120	548
All stocks combined	9,5	90	598

From this it can be seen that these requirements, when the effects of different body sizes and different egg outputs are eliminated, are within about 10 percent of the mean. If this is an accurate estimate of the range in efficiencies of lysine utilisation it may be questioned whether the information about lysine in feedstuffs is sufficiently accurate to

justify using different coefficients for different stocks. For purposes of formulating diets for the range of stocks used in this experiment, the average values (at the foot of Table 1.6) might as well be used as the individual values.

More recent experiments with tryptophan led to an identical conclusion (Morris and Wethli, 1978) but the authors again conclude "there is thus no basis on which one could reliably conclude from the present evidence that the stocks differ in their tryptophan requirements per unit of maintenance or egg output". They also make the interesting observation that the differences between one stock in two successive years are as great as those between stocks.

In the case of amino acid inputs to the laying hen there is therefore strong theoretical and experimental evidence that stocks do not vary in their response characteristics.

The amount of lysine, methionine or tryptophan needed per gram of egg output or per kilogram bodymass does not differ to any measurable extent between stocks and therefore the optimum dietary concentrations for these amino acids can be calculated for any existing (or future) stock for which the egg output, body mass and feed intake characteristics can be predicted.

Any single estimate of the coefficient for egg output  $\underline{a}$  and for body mass gain  $\underline{b}$  is subject to a number of variable influences, especially if the number of experimental observations is small. On the basis of available evidence however it seems reasonable to conclude that  $\underline{a}$  and  $\underline{b}$  are invariate. Fisher (1976) has listed the best available estimates of the coefficients for methionine, lysine and tryptophan and these are shown in Table 1.7. Also shown are values for the composition of egg and direct estimates of maintenance requirements.



TABLE 1.7 Recommended values for the coefficients a and b; comparison with data for egg composition and maintenance requirements determined by direct experimentation (Fisher, 1976)

Amino acid	<u>a</u> mg/gE	<u>b</u> mg/kgW	Egg composition mg/gE	Maintenance requirements mg/kgW
Methionine	4	25	3,52	25 - 71
Lysine	9,5	90	7,90	29 - 60
Tryptophan	2,25	10,25	1,84	10 - 19

The value of a is expected to exceed slightly the corresponding value for egg composition, and this is found to be the case. The net efficiency of utilisation of the amino acids for egg production can be calculated, and is found to be 88, 83 and 82 percent for methionine, lysine and tryptophan respectively. The similarity of these figures for different amino acids is interesting. The direct estimates of maintenance requirements have all been made with adult roosters and are very variable (Leveille and Fisher, 1959; 1960, Leveille, Shapiro and Fisher, 1960; and Kandatsu and Ishibashi, 1966 cited by A R C, 1975. In spite of this variability, they tend to be in broad agreement with the suggested values of b.



## CHAPTER 2

REVIEW OF FACTORS AFFECTING ENERGY UTILIZATION  
AND REQUIREMENTS OF LAYING HENS

Lavoisier discovered over 200 years ago that the metabolic process to which we refer as 'respiration' actually involves the oxidation of dietary carbon and hydrogen sources to form carbon dioxide and water. He showed that food supplies energy in the form of calories to the animal body in a manner similar to the production of heat from the food by burning it. It was much later, however, that the need for energy for growth of body tissues, production of eggs, carrying out of vital physical activities, and maintenance of normal body temperatures was fully appreciated. The discovery that energy concentration of the diet is of primary importance in determining the amount of feed ingested by chickens and other species is the key which has unlocked the door to a scientific understanding of the proper relationships among all nutrients in the diet. Animals such as the chicken eat food primarily to satisfy an inner craving or hunger for energy. When this hunger is satisfied the animal stops eating.

In order to closely predict feed consumption for a flock of chickens and thus formulate a diet so that it will provide the amount of amino-acids, vitamins and trace elements required for optimum performance, one needs to know rather precisely the energy requirements of the bird.

It is well known that there are many factors which influence the energy requirements and energy intake of laying hens. It was thus considered necessary to give a brief review of the more important factors that can influence these two characteristics.

A laying hen can be considered to partition her metabolisable energy intake between three functions: maintenance, growth and

egg production. The factors affecting the energy requirements for these functions will be briefly discussed.

#### FACTORS AFFECTING ENERGY REQUIREMENTS FOR MAINTENANCE.

Maintenance energy can be defined as the difference between metabolisable energy intake and the energy needed for production.

Whether expressed per  $\text{kg}^{0.75}$  or per kg, heavier strains which typically lay brown-shelled eggs ("brown strains"), have lower maintenance energy requirements than do lighter strains which typically lay white-shelled eggs ("white strains") (Waring and Brown, 1965, 1967; Marsden, Wethli, Kinread and Morris, 1973; Morris 1968a,b; Grimbergen, 1970; Jackson, 1972). Most of this evidence is indirect but convincing.

Waring and Brown (1965) described experiments using a respiration chamber to determine the energy requirements for maintenance of a heavy-type hybrid layer (Thornber 404). The mean body mass over the first laying year of this type of bird (brown strain) was 2,5 kg. It was found that the maintenance ME requirement was 377 KJ/kg of body mass per day and the efficiency of ME utilisation for maintenance and production was 84 percent.

Following this work similar experiments (Waring and Brown, 1967) were carried out using birds of a high-producing strain of the White Leghorn breed (white strain). From the regression obtained it was calculated that the maintenance requirement of this bird was 481 KJ/kg day. The efficiency of ME utilisation for maintenance and production was 86 percent.

Various researchers have since attempted to determine the maintenance requirement of laying hens. Table 2.1 gives estimates of the maintenance ME requirement of the laying hen as computed from the experimental work of various authors.



TABLE 2.1: Daily maintenance ME requirement of laying hens, derived either from regression analysis (regr.) or respiration calorimetry (cal.)

Reference	Body mass (kg)	Method	Daily Maintenance requirement per $\text{kg}^{0.75}$	
			kcal ME	KJ ME
Titus (1928)	1,60	regr	119	498
Brody, Funk and Kempster (1938)	1,8 - 2,6	regr	122	510
Bird and Sinclair (1939)	2,90	regr	128	536
Byerly (1941)	0,68 - 3,29	regr	133	556
Reid et al. (1978)	1,8	regr	111,1	465
Waring and Brown (1965)	2,00	cal	106	444
Waring and Brown (1967)	1,70	cal	131	548
Grimbergen (1970)	2,00	cal	102	427
Leeson and Porter-Smith (1970)	2,25	cal	92	385
Burlacu and Baltac (1971)	1,72	cal	126	527
Farrel (1975)	1,73	cal	121	507
Farrel (1975)	2,38	cal	110	461
Farrel (1975)	2,62	cal	106	443

When estimates of energy requirements from calorimetric work are compared with estimates of ME derived from laying trials it becomes obvious that there are wide discrepancies between data from the two sources. This may be related to a number of factors including environmental temperature, feathering, activity etc.

(a) *The effect of Temperature on maintenance energy requirements.*

It is clear that the effect of temperature on maintenance energy will depend on degree of feather cover and size of wattles and comb and these factors may account for at least some of the variation present in the estimates given above.

Emmans (1974) used the data of several authors to estimate maintenance energy from ME consumption by assuming that energy



for production was given by  $8,37 E + 20,92 \Delta W$  KJ ME/bird d. Maintenance energy declined by 2,5 KJ/kg day °C (0,6 kcal) over the range 7 and 35 °C (Davis, Hassan and Sykes, 1973) by 3,8 KJ/kg d °C (0,9 kcal) over the same temperature range (Hassan, 1969 cited by Emmans, 1974); by 14,6 KJ/kg d °C (3,5 kcal) between 26,5 and 32 °C (Smith and Oliver, 1972); by 14,6 KJ/kg °C (3,5 kcal) in a white strain and 11,3 KJ/kg day °C (2,7 kcal) in a brown strain between 15 and 32 °C (Marsden et al., 1973); and by 10,9 KJ/kg day °C (2,6 kcal) between 18 and 24 °C (Emmans and Dun, 1973). Shannon and Brown (1969) found a decline in starving heat production of 7,1 KJ/kg d °C (1,7 kcal) between 22 and 28 °C in hens, which with an efficiency of ME for maintenance of 80 percent gives an estimate of 8,8 KJ/kg d °C (2,1 kcal) for maintenance energy.

Sykes (1977), in his review of nine studies of the effect of ambient temperature on energy requirement of egg strain layers, concluded that at 20 °C the average energy intake is 1,297 MJ per day, with a change of 20,9 KJ/day per degree Celsius. This corresponds to a change of 1,6 percent per degree Celsius, in excellent agreement with the suggestion of Payne (1968).

Byerly, Kessler, Thomas and Gous (1978), fed five different strains of 40 pullets each individually a diet containing 12,11 MJ ME/kg for ten 28 day periods. They concluded that the energy requirement declined as follows for each 1 °C increase in environmental temperature for the different strains used:

- (a) Small leghorns (1 426 gms) used 5,86 KJ per bird less for each 1 °C increase.
- (b) White-egg hybrid layers (1 809 gms) used about 6,7 KJ/bird d less for each 1 °C increase.
- (c) Brown-egg, sex-linked layers (2 610 gms) used 8,4 KJ/bird d less 1 °C increase.

- (d) Broiler breeders (4 197 gms) used 12,55 KJ per bird/d less for each 1 °C increase.
- (e) Broiler cross (4 158 gms) used 14,2 KJ/bird d less for each 1 °C increase.

The calculated values for white-egg layers for change in ME/°C are lower than Emmans' (1974) estimates but substantially higher than the estimates of Davis et al. (1973). Byerly et al. (1978) noted that their birds were fully feathered and subjective observations during colder periods indicated that the birds were comfortable.

Reid (1979) summarised the results of several of his experiments (Table 2.2) to show the effects of housing temperature on the maintenance energy requirement of laying hens. Constant temperatures ranging from 18 to 35 °C, maintained within 1 °C, were used in these studies. The maintenance requirement of the laying hen was estimated at 858 KJ of metabolisable energy/day when housed at 18,3 °C and this was decreased to 653 KJ at 35 °C. Regression analyses of these data indicated that for each 1 °C change in housing temperature, there was a decrease of 3,8 KJ of metabolisable energy required per kg of physiological body mass per day.

Table 2.2: Effect of Temperature on Maintenance Energy for Laying Hens (Reid, 1979)

Temperature °C	Maintenance, ME	
	/kg $BM^{0.75}$	KJ/day
18,3	498	858
21,1	481	724
32,2	439	682
35,0	431	653

b. *The effect of Feathering on Maintenance Energy requirements.*

Feathers provide the main form of insulation for the bird and within the thermoneutral zone they provide the major means of controlling the rate of heat loss from the body surface.



Wilson, Hillerman and Edwards (1952) observed that the temperature at the skin surface is higher than at the feather surface as a result of feathers trapping the warm air at the body surface and preventing a rapid heat loss. However, at environmental temperatures of 32 to 35 °C the temperature of the air and feathers is similar so that hens are unable to lose heat from the surface of their feathers at these temperatures and the major routes of heat loss are from the respiratory tract or from unfeathered areas of the body.

The degree of insulation can be adjusted by altering the spatial arrangement of the feathers and thus the depth of the insulating layer of air around the body.

Breed differences exist with regard to the closeness of plumage and Hutchinson (1954) has noted that although Leghorns have a closer plumage than that of Rhode Island Reds, feather cover in the latter breed is thicker so that the insulation should be greater at low wind velocities.

The importance of feather cover on heat production, is further illustrated by the results of O'Neill, Balnave and Jackson (1971) who used temperature-acclimatised feathered and defeathered cockerels of similar body mass. These data indicate that the starving heat production of the defeathered cockerels only approached those of the feathered cockerels at environmental temperatures above 34 °C while the differences increased rapidly as the temperature was reduced below 34 °C. At 22 °C the mean starving heat production of the defeathered cockerels was 2,5 times that of the feathered cockerels. The other point of note in this work was the fact that there was no indication of an increased heat output at 38 °C in the case of the defeathered cockerels and the heat production curves of the two defeathered birds appeared to level out when ambient temperature approached body temperature.



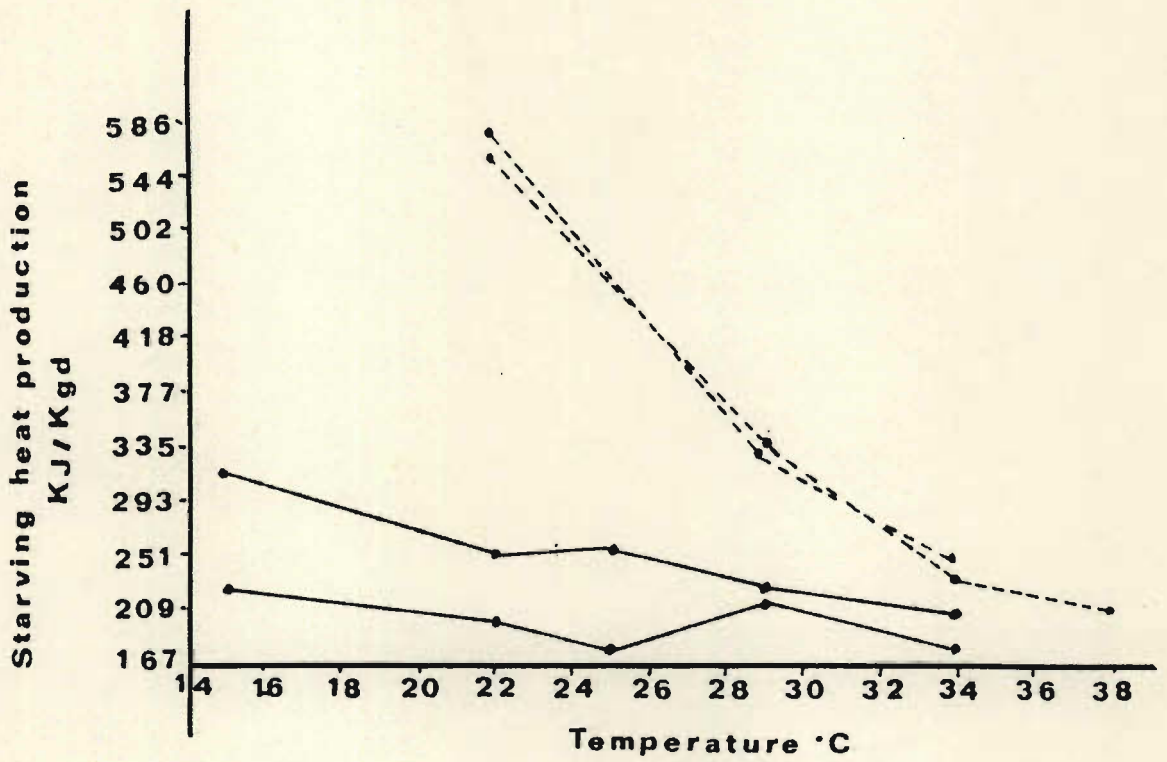


Figure 2.1: Starving heat production of feathered and defeathered cockerels at various environmental temperatures between 15 and 38 °C. (O'Neill et al., 1971)

c. *The effect of Activity on maintenance energy requirements.*

It is evident that any movement which disturbs the feather cover of the bird will increase the heat loss through the escape of some of the warm insulating air between the skin and feathers. The magnitude of this loss will be dependent on the temperature difference between the environment and the insulating layer.

Deighton and Hutchinson (1940) reported that heat dissipation varies continuously except when the bird is completely motionless. Activities involving little exercise but some disturbance of feathers caused a large evolution of heat and even small movements of the head and neck had considerable effects on metabolism as a result of feather separation.

Rising from the sitting to the standing position momentarily increased metabolism approximately 100 percent but over 24 hours this movement increased metabolism only 0,6 percent above that of the sitting position. However, the heat production in the standing position was approximately 42 percent above that in the sitting position and as observations indicated that birds in cages stood on average for more than 50 percent each 24 hours an increase in daily heat production of 24 percent above that in the sitting position was recorded.

De Shazer, Jordan and Suggs (1970) have similarly reported a 20 to 40 percent increase in sensible heat loss in the standing compared with the sitting position.

d. *Effect of Comb and Wattle size on maintenance energy requirements.*

Deighton and Hutchinson (1940) observed a 12 percent decrease in heat dissipation from fowls when they tucked their heads under their wings. These data suggest an important function of the comb in total heat expenditure.



Siegel and Beane (1961) observed a decreased tendency for hens to huddle together in the cold after their combs have been removed.

Van Kampen (1974) points out that the total surface area of the comb and the wattles of the WL chicken is about 7 or 8 percent of the total body surface. This suggests that these structures have an important role in heat dissipation.

The latter author also pointed out that the heat loss from the comb and wattles between 5 and 30 °C is linearly related to environmental temperature. However, at ambient temperatures above 30 °C there is an increase in heat loss, indicating an increase in blood flow through the head appendages. This overshoot in heat loss shows a rhythmic activity with a 6-minute periodicity of extra heat loss during 2 minutes (van Kampen, 1971). In the cold (below 5 °C) the heat dissipation of the head appendages is lower than would be expected from physical laws. In cold environments therefore, vasoconstriction seems to play an important role in the protection against excessive heat loss. An important vasomotor regulation in the feathered skin could not be detected. The heat loss by radiation, conduction and convection from the head appendages ranged from 9,3 to 25,6 percent at environmental temperatures from -5 °C up to +40 °C.

e. *Effect of Sex and Rate of Lay on energy requirements.*

Mitchell and Haines (1927) and Mitchell, Card and Haines (1927) reported the first detailed experiments carried out to investigate the effects of sex on the starving heat production of poultry. The first of these reports indicates a sex difference in starving metabolism between mature non-laying hens and cockerels. When the results are recalculated on a metabolic body size basis the mean starving heat production of the cockerels can be shown to be approximately 10 percent greater than that of the hens.



Although the studies of Mitchell and Haines (1927) were not carried out to investigate the influence of egg laying on the starving heat production of hens there was some indication that the starving heat production of laying hens was greater than that of the non-laying bird.

Waring and Brown (1965) also obtained evidence to suggest that the starving metabolic rate of the laying hen was higher than that of the non-laying hen. In this work there was a 20 per-cent increase in the metabolic rate of the laying hen as compared with the non-laying hen.

Tasaki and Sasa (1970) have also reported that the starving heat production of the laying hen is increased compared with the non-laying hen and their results indicate a difference of approximately 26 percent during the first 3 days of fast.

While there appears to be substantial evidence that the starving heat production of laying hens is somewhat greater than that of non-laying hens little evidence is available concerning possible variations in metabolic rate associated with the rate of egg production in laying hens.

Ota and McNally (1961) by regression analysis of caged hens failed to observe any significant relationship between egg and heat production. However, differences in the rate of transfer of nutrients to the ovary and oviduct and active synthesis in ovarian and oviduct tissue would be expected depending on the rate of egg production. Therefore, variations in heat production would be expected because work must be involved in transporting nutrients to these sites and energy expenditure is involved in the sythetic processes.

Data analysed by Balnave (1974) on laying hens which failed to lay on the day of measurement implied that only 25 KJ(6 kcal) are required for ovulation, mechanical movement and secretions within the oviduct and the final expelling of the completed

egg from the body.

#### FACTORS AFFECTING ENERGY REQUIREMENTS FOR GROWTH AND EGG PRODUCTION.

The metabolisable energy required for growth and egg production depends on the energy contents of the mass gain and eggs produced and on the efficiency with which dietary metabolisable energy is converted to carcass and egg energy.

Hassan (1969) cited by Emmans (1974) and Smith (1972) estimated the energy content for both carcass gains and losses to be 17 KJ/g (4 kcal). Davis et al. (1972, 1973) carried out calorimetric trials by means of the comparative slaughter method. Calculations based on their figures show that a change in body mass of 1 g is roughly comparable to 21 KJ (5 kcal). Reid (1979) referred to a rather extensive experiment with laying hens in their laboratory where the total hen minus the digestive tract contents was ground and analysed for protein, fat and energy. As a result of this study they calculated the energy per gram body mass change to be 21 KJ.

Emmans (1974) expresses the opinion that in most situations the energy stored in the carcass is less than 10 percent of that stored in eggs and no great precision in the estimate is needed for practical purposes.

Eggs vary in their relative amounts of yolk, white and shell and, since yolk contains considerably more energy than either white or shell (Romanoff and Romanoff, 1949), the energy content of eggs will vary. However, eggs typically have an energy content of 6,7 KJ/g (1,6 kcal) (Brody, 1945; Romanoff and Romanoff, 1949; Grimbergen, 1970; Reid, 1979).

The efficiency with which dietary ME is converted to egg or carcass energy could depend on the source of the ME, i.e. on whether it comes from carbohydrate, fat or protein (Weiss and Fisher, 1957; Donaldson, 1962, Waring et al., 1968; Jackson et al., 1969).



It is apparent from a number of calorimetric studies indicated in Table 2.3 that the ME of conventional diets is utilised by mature hens with an efficiency ranging between 64 and 86 percent. (A.R.C., 1975 and Reid et al., 1978).

TABLE 2.3: Calorimetric estimates of the net availability of metabolisable energy from conventional and high-fat rations fed to laying hens.

Source	Bird Type	Temperature	Mean net availability of ME %	Type of diet
Waring and Brown (1965)	Thornber 404	23	83,7	conventional
Waring and Brown (1967)	White Leghorn	22	86,2	conventional
Porter-Smith and Shrimpton (1968)	Warren SSL	-	81,3	maize-soya
Grimbergen (1970)	White Leghorn	20	64,2	conventional
Burlacu and Baltac (1971)	White Leghorn	25	78,5	conventional
O'Neill (1971)	White Leghorn	16	78,0	high fat
		23	77,9	high fat
		27	83,8	high fat
		33	87,6	high fat
Reid <u>et al.</u> , (1978)	White Leghorn	21,3-26,5	62,35	conventional

Taking the energy contents of mass gain and of eggs to be 16,7 KJ/g (4 kcal) and 6,7 KJ/g (1,6 kcal) respectively and assuming an efficiency of converting dietary ME of 80 percent, Emmans (1974) estimated the ME needed for production as:

$$8,37E + 20,9\Delta W \text{ KJ ME/bird d}$$

where E = egg output in g/bird d and

$\Delta W$  = mass change in g/bird d.

Mass gain and egg output (except for relatively minor changes in egg mass) in birds fed equal amounts of nutrients are virtually unaffected by temperature over a wide range. It is also unlikely that the composition of the eggs and mass gains are affected.



Emmans (1974) therefore pointed out that the above expression for the metabolisable energy needed for production was likely to be accurate for most purposes and to be unaffected by temperature.

Reid, (1979) estimated the requirements of laying hens for metabolisable energy at three levels of egg output (0,40 and 50 g/hend), at two different body masses (1,75 and 2,0 kg) and at temperatures of 18, 24 and 30 °C. His results are presented in Table 2.4.

TABLE 2.4: Estimated ME (KJ/bird d) Requirements for Laying Hens\* (Reid, 1979).

Body Mass kg	Egg output g/day	Temperature		
		18 °C	24 °C	30 °C
1,75	0	753	720	682
	40	1 163	1 130	1 092
	50	1 268	1 234	1 197
2,00	0	833	795	753
	40	1 243	1 205	1 163
	50	1 347	1 310	1 268

\* Estimates based on 65 percent efficiency for ME conversion to NE for production and maintenance.

It will be noted that the maintenance requirement at nil egg output decreases with increased environmental temperature. The energy requirements for egg production were based on several experiments with an average efficiency of 65 percent for the conversion of metabolizable energy to net energy of production and maintenance.

#### FACTORS AFFECTING ENERGY INTAKE

##### a. *Effect of dietary energy.*

It is known that ME intake can be increased by raising the energy concentration of the diet or feeding the diet in the form of crumbs or pellets. Although there have been some reports indicating that both growing and laying hens maintain

constant energy intake when diets of widely differing energy levels are fed (Hill, 1962) the reduction in feed intake which occurs at high dietary concentrations is generally insufficient to maintain a constant energy consumption and so energy intake and body fat deposition tend to be greater at higher dietary energy concentrations (Morris, 1968; Jackson et al., 1969).

Morris (1968) has shown that heavy breeds of bird are less able to control their energy intakes than light breeds. The extra energy is mainly converted to body fat. There may be some increase in egg mass but there is usually no increase in rate of lay (Morris 1969).

The extent to which energy intake can be expected to change for a given change in dietary concentration can be estimated in a general formula derived from a survey of published experiments by Morris (1968).

$$Y = Y_2\ 700 + (0,0005465\ Y_2\ 700 - 0,1466)(X - 2\ 700)$$

where  $Y$  = kcal ME intake/bird d

$X$  = kcal ME/kg diet

$Y\ 2\ 700$  = characteristic energy intake of layer.

Some representative values derived from this equation are set out in Table 2.5. This shows that for most strains of White Leghorns, which have daily metabolisable energy intakes in the region of 1 339 KJ, metabolizable energy intake can be expected to increase by 2 or 3 percent for each 10 percent increase in dietary energy. Heavier strains, eating approximately 1 674 KJ/bird d can be expected to increase their metabolisable energy intake by 4 or 5 percent for each 10 percent increase in dietary energy. The principle that birds adjust their food intake to maintain a constant metabolisable energy intake is thus not strictly tenable and this in turn will affect the formulation of a diet designed to minimise the cost of feeding.



TABLE 2.5: Predicted\* Caloric Intakes (Y = kcal/bird d) at Different Levels of Dietary Energy

Dietary energy × kcal ME/kg	Characteristic energy intake (Y 2 700), kcal/bird d					
	( 280	310	340	370	400	430 )
2 400	278	303	328	353	378	403
2 600	279	308	336	364	393	421
2 800	281	312	344	376	407	439
3 000	282	317	352	387	422	457
3 200	283	321	360	398	436	474

\* Predicted from the equation:

$$Y = Y\ 2\ 700 + (0,0005465\ Y\ 2\ 700 - 0,1466)(X - 2\ 700)$$

(Morris, 1968)

Since chickens tend to increase feed consumption as the energy content of the diet is reduced, a deficiency of energy can be produced by using low energy diets which contain so much indigestible bulk as to surpass the capacity of the crop and digestive system of the chicken to hold sufficient feed to obtain an adequate daily amount of energy. Thus it is possible to designate a quantitative level of energy per kilogram of diet below which chickens under practical conditions usually would have difficulty in increasing their feed intake sufficiently to obtain an adequate amount of energy per day for optimum growth or egg production. This lower limit of energy is approximately 10,88 MJ per kg of diet under cool or moderate environmental conditions and about 10,04 MJ in a warm environment, (Scott et al., 1976). According to the latter authors the minimum feed density which will allow birds to obtain adequate energy is approximately 6,28 KJ metabolisable energy per cubic centimeter.

b. *The effect of dietary fat.*

When fats are included in diets for growing animals, the efficiency of utilisation of energy consumed is improved compared



with that of animals fed low fat diets. This was demonstrated in work by Carew, Hopkins and Nesheim, (1964) who showed that 10 to 15 percent more energy was deposited in the carcass of chickens receiving diets containing 5 - 10 percent fat compared with similar diets low in fat. This was true even when metabolisable energy intake from the diets was the same.

Forbes and Swift (1944) had observed this phenomenon in rats, and termed it the associative dynamic action of fats. The improvement in energetic efficiency can be attributed to a lower heat increment with the diets containing fats.

Reid (1979), fed diets with and without tallow to layers at various housing temperatures. He expressed the metabolisable energy consumption as KJ above maintenance in order to arrive at an estimate of the potential production of the birds. (Table 2.6).

At 12,8 °C, the birds fed the basal diet consumed 565 KJ (135 kcal)/day above maintenance, while with the supplemental tallow the birds consumed 707 KJ (169 kcal) with the feeding of tallow in the diet.

TABLE 2.6: Effect of Added Tallow on ME Consumption Above Maintenance (Reid, 1979).

Constant Temperature °C	ME KJ/day		
	Without Tallow	With Tallow	Increase with Tallow
12,8	565	707	142
18,3	569	703	134
23,9	582	665	83
29,4	364	531	167
35,0	21	142	121

There is a rather rapid decrease in energy consumption above maintenance once the temperature is increased above 23,9 °C. Birds at this temperature consumed 582 KJ of metabolisable energy/day above maintenance, while at 29,4 °C they consumed

only 364 KJ. This was further decreased to only 21 KJ above maintenance when a constant temperature of 35 °C was maintained in the house. When one considers that only the metabolisable energy above maintenance is available for production over an extended period of time, it becomes obvious that birds housed at the higher temperatures would not be able to sustain any degree of egg production compatible with economic performance. The addition of tallow to the diet uniformly increased metabolisable energy consumption rates and would be expected to improve egg production.

It must be emphasised that from a production point of view, only energy remaining after the maintenance requirement has been met is of economic value.

Egg mass usually falls as temperature increases and this may be an indication that less energy is available for production as the environmental temperature rises.

Figure 2.2 shows the effects of increased environmental temperature on energy intake and the energy requirements for maintenance. The difference is energy available for production and it will be seen that it decreases as the temperature increases (Teter et al. (1973) cited by Filmer (1974).

Charles (1974) quoted by Filmer (1974) has pointed out that the elevation and slope for intake is affected by dietary energy level, body mass, egg output and heat loss. The elevation and slope for maintenance is affected by feathering, body mass, diet, energy intake, specific dynamic action and acclimatisation, including body mass changes.

The effects of food restriction under a varying temperature are of particular interest. With normal temperatures, restriction of food and thus daily energy intake, reduces egg mass even if daily protein intakes are maintained. If the same level of



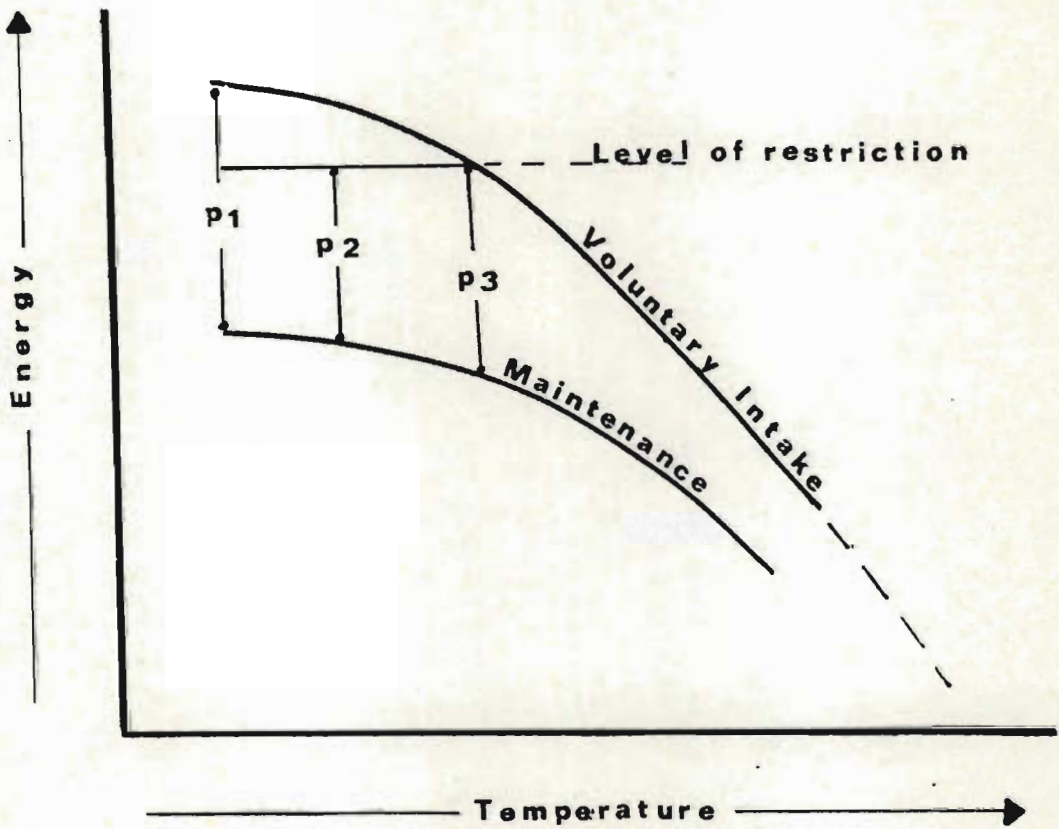


Figure 2.2: The effects of increased environmental temperature on energy intake and the energy requirements for maintenance. (Teter et al., 1973.)

food restriction is maintained and temperature is increased, maintenance energy level is reduced and more energy is available for production. Egg mass under these circumstances should increase.

c. *Restriction of energy intake.*

Dietary energy considerations and energy metabolism continue to play a major role in attempts to improve efficiency of egg production in laying hens. A laying hen allowed to eat ad libitum consumes more feed than necessary to produce eggs, and gains body mass during the laying year. Much of the increased body mass results from increased deposition of fat, which has little monetary return to the egg producer when the hen is sold even though it is a concentrated form of energy. Early work at Harper Adams College, inspired by the need to make the best possible use of war-time shortages of feeding-stuffs (Temperton and Dudley, 1948), showed that food restriction of up to 10 percent of the voluntary intake could be imposed without any loss of production, but the majority of reports on egg-laying strains, as distinct from broiler parents, indicate that production invariably suffers when food restrictions are imposed (Heywang, 1940; Sherwood and Milby, 1961; Walter and Aitkin 1961; McGinnis and Dronowat, 1967; Jackson, 1970).

Clearly food restriction reduces the intake of all nutrients and, since egg production is particularly sensitive to the level of protein and calcium intake, it is surprising that until recently there appears to have been no attempt to maintain an adequate daily consumption of all nutrients other than energy. Jackson (1970) adopted this method in his work on energy restriction but, for reasons which are not clear, he was not able to achieve the same levels of egg production as shown by his control birds allowed ad libitum intake.



From the evidence reviewed, Balnave, Farrell and Cumming (1978) concluded that for restricted feeding to be utilised satisfactorily during the laying period careful consideration must be given to accurate assessment of the mature body mass of the birds so that over-restriction does not result in the birds being seriously underfed.

Balnave et al. (1978) suggested that the minimum metabolisable energy requirement, (KJ/d) can be estimated from the following two equations:

i) Egg producing strain -

$$ME \text{ min} = 388 W^{0,75} + 0,027 \times 388 W^{0,75} \times [(22-T)] + 8,67 E$$

ii) Meat-producing strain -

$$ME \text{ min} = 450 W^{0,75} + 0,027 \times 450 W^{0,75} \times [(22-T)] + 8,67 E$$

where ME min = minimum ME requirement, KJ/d

W = body mass, kg

E = egg product, g/d

T = environmental temperature, °C

These estimates do not include any requirement for body mass gain. After peak-lay this requirement need be small since only a slight gain in body mass is necessary (Balnave, 1976) but prior to peak-lay this ME requirement will depend on the necessary daily rate of gain required to attain peak-lay body mass and will, therefore depend on the level of restriction, if any, applied during growth. However, this can be calculated knowing the relevant body mass and the relevant composition of mass gain. To a first approximation this may be calculated assuming 8 KJ/g of body mass gain.

Sykes (1972) points out that under conditions of energy restriction there is likely to be competition for the available supply and it appears that the immediate demands of egg production are met at the expense of reserves previously accumulated in response to endocrine stimuli. Thus body mass falls,

particularly during the first three weeks of the restriction. Sykes (1972) used three diets designed to provide 17,7 g of protein per day when fed at 120, 105 and 90 g/bird d respectively. The 120 g diet was also fed ad lib. Results are shown in Table 2.7.

TABLE 2.7: Egg Production in Relation to Energy Intake.  
Medium Hybrid Layers\* for 8 Weeks from 55  
Weeks of Age (Sykes, 1972)

Food intake (g/d)	Energy intake ME/d	Protein intake (g/d)	Egg production (%)	Egg mass (g)	Body mass change (g/d)	Energetic** efficiency (%)
143	1 841	25,3	68,3	66,9	+1,61	17,5
120	1 548	17,7	69,0	66,4	+1,07	20,5
105	1 347	17,9	69,9	64,7	-0,1	23,3
90	1 142	17,7	66,5	63,9	-6,2	25,7

\* Initial body mass 2,31 to 2,38 kg.

\*\* Gross energetic efficiency =  $\frac{\text{Egg mass/day} \times 6,95}{\text{ME intake/day}} \times 100$

From Table 2.7 it is clear that energy restriction has resulted in a considerable improvement in the energetic efficiency of egg production. Obviously egg production has a high priority for energy when calories are in short supply. It would appear that the bird makes economies elsewhere with little or no reduction in the energy used for egg production. Sykes (1972) suggests that the economic requirement for energy is appreciably lower than that found under ad libitum feeding systems.

Jaluludin (1969) cited by Sykes (1972) found that not only had body mass decreased following energy restriction, but the proportion of body fat was considerably reduced, this being partially replaced by water and protein.

Petersen (1971) has shown that daily ME intake can be reduced to 1 004 KJ/bird d without detracting from egg output, provided daily intakes of other nutrients are adequate. Birds were fed



measured quantities of food in floor pens at controlled temperatures. (Tables 2.8 and 2.9.) An intake of 1 004 KJ ME/bird d was the minimum level sustaining satisfactory performance at 27 °C. This temperature is much higher of course than in commercial units.

TABLE 2.8: Egg Production in Relation to Energy Intake.  
White Leghorn Layers for 28 Weeks from 24 Weeks  
of Age (Petersen, 1971)

Energy intake (KJ ME/d)	Egg Production (%)	Egg mass (g)	Body mass change (g/d)	Energetic efficiency (%)
1 314	79,3	56,4	+1,9	22,6
1 172	78,1	56,4	+2,0	25,0
1 105	78,1	56,0	+1,2	26,5
1 013	80,5	55,2	+1,2	29,4

TABLE 2.9: Egg Production in Relation to Energy Intake.  
White Leghorn Layers for 20 Weeks from 44 Weeks  
of Age (Petersen, 1971)

Energy intake (KJ ME/d)	Egg Production (%)	Egg mass (g)	Body mass change (g/d)	Energetic efficiency (%)
1 088	65,7	59,4	+0,9	24,2
1 004	65,3	59,9	+1,3	26,0
920	60,6	58,1	+1,4	25,5
837	46,5	58,3	+1,5	21,5

A consistent observation is that restriction of energy intake leads to improved viability. In some cases the mortality rate of fully fed hens has been 5 percent higher for a given productive cycle (Snetsinger and Zimmerman, 1974), though 1 to 3 percent is more usual. Pope (1971) observed that viability was improved by 4 percent in hens allowed 7 percent less food. Bell (1972) observed a 5 percent improvement in viability in one test but none in another. Both Sherwood and Milby (1961) and Hannagan and Wills (1973) have noted significantly higher viability in limited-fed hens.

The basis for this improved viability could be ascribed to several causes. There is an indication that a reduction in energy intake reduces tumour development in rats (Ross and Bras, 1971). Polin and Wolford (1973) suggests that limited feeding may reduce the incidence of fatty liver haemorrhagic syndrome although no direct evidence was presented to support this suggestion. Snetsinger and Zimmerman (1974) observed a reduction of the fat content of livers of medium-weight layers when limited in energy intake. However, no examination of mortalities was made to determine whether more hens died of fatty liver syndrome when fully fed than when limited.

d. *Effect of dietary calcium.*

Calcium levels influence feed/energy intake. A high level of calcium tends to inhibit feed intake of hens (Hurwitz, Bornstein and Bar, 1969), while a deficiency of calcium also significantly reduced their feed intake (Davidson and Boyne, 1970; Roland, Sloan and Harms, 1973). Hughes and Wood-Gush (1971) concluded that laying hens could regulate their intake of calcium. Hughes (1972) and Holcombe, Roland and Harms (1974) further concluded that the bird consumed calcium in response to depletion and anticipated need in relationship to hormonal changes associated with oviposition.

Morris and Taylor (1967) showed when hens are given a high calcium (30 g/kg) laying pellet, voluntary food intake is about 25 percent greater on egg-forming than on non egg-forming days. Further experiments demonstrated that this large difference did not occur when low calcium diets were fed and calcium was provided as calcareous grit (Taylor, 1970; Mongin and Sauveur, 1974; Sauveur and Mongin, 1974). It seems clear that when the diet is complete for calcium higher consumption on egg-forming days is a reflection of short term calcium requirements. On such diets this is evidence that hens will eat for calcium, and Taylor (1972) suggests that this is a major factor controlling food/energy intake on laying days.



e. *Effect of dietary amino acid content.*

Filmer (1974) stated that food or energy intake is not independent of dietary amino acid content.

The response is comprised of two main components:

- i) With marginally sub-optimal levels of amino acid in the diet, food intake increases and production is not restricted.

This observation has been made by numerous other research workers (Fisher and Morris, 1967; Harms, Damron and Waldroup, 1967; and Pilbrow and Morris, 1974).

- ii) With severely deficient levels in the diet, increased food intake can no longer supply the daily nutrients necessary to sustain egg output. Egg output therefore falls and consequently the demand for all nutrients diminishes. Food intake therefore falls in consequence.

Picard (1979) pointed out that the effect of amino acid balance on feed intake is more likely to be a "direct action" rather than a consequence of reduced egg output as suggested by Filmer (1974). He indicated that birds with low feed intake on low protein (amino acids) diets immediately recover their normal feed intake when switched to control diets. Production however took one month to return to the level of the control diets.

#### PREDICTION EQUATIONS TO DETERMINE METABOLISABLE ENERGY INTAKE.

In recent years a number of regression equations have been produced, for the prediction of food intake in the laying fowl. Byerly (1941) working with a flock of 102 laying hens, based prediction of food intake upon metabolic body size, egg mass and daily live mass gain.

$$F = 0,523 W^{0,653} + 1,126 W + 1,135 E.$$

where  $F$  = daily food intake (g)

$W^{0,653}$  = metabolic body size (g)

$\Delta W$  = daily live mass change (g)

$E$  = daily egg mass (g)

Brody (1945) produced a similar equation but incorporated an intercept value:

$$F = 0,273 W^{0,73} + 1,09 \Delta W + 0,688 E + 7,77$$

Combs (1962) proposed that the daily ME intake of a laying hen could be predicted by the equation

$$ME = T(1,52 W^{0,653} + 3,26 \Delta W + 3,29 E)$$

where  $ME$  = daily ME intake of the hen in kcal

$W$  = average body mass (g)

$\Delta W$  = daily mass gain (g)

$E$  = egg mass produced (g)

$T$  = approximate temperature correction factor for each season\*.

This was derived from Byerly's (1941) equation predicting daily feed intake by multiplying by the estimated ME level of Byerly's diet (2 900 kcal ME/kg).

As Hill (1956) had demonstrated a cyclical seasonal variation in the accuracy of Byerly's equation, the hen under favourable conditions only consuming 94 percent of that predicted by Combs, Thomas (1966) modified Combs' equation, multiplying all regression coefficients by 0,94. His equation was:

$$ME = 1,45 W^{0,653} + 3,13 \Delta W + 3,15 E$$

---

\* Autumn = 0,98  
 Winter = 1,05  
 Spring = 1,00  
 Summer = 0,94



Janssen (1970) cited by Grimbergen (1974) has provided us with yet another equation based on data obtained using laying hens in cages:

$$ME = 0,676 W^{0,75} + 2,866 E + 19$$

Leeson et al. (1973) kept two laying strains at a constant temperature of 18,3 °C and by means of multiple regression analyses produced the following equation to predict the food intake of the laying fowl:

$$F = 0,136 W^{0,75} + 1,605 \Delta W + 0,929 E + 21,68$$

where  $F$  = daily food intake (g)

$\Delta W$  = daily live mass change (g)

$W^{0,75}$  = metabolic body size (g)

$E$  = daily egg mass (g)

The diet they used contained 2 990 kcal/kg and the ME intake can therefore be calculated by multiplying all regression coefficients by 2,99.

$$ME = 0,41 W^{0,75} + 4,8 \Delta W + 2,78 E + 64,8$$

Emmans (1974) cited by Emmans and Charles (1977) give prediction equations for ME intake, which includes a term for the effect of temperature. These equations were validated by comparing predicted and measured intake for 49 8-week periods for 20 commercial laying flocks. The prediction equations were:

White strains -

$$ME = W[115 + 2,2(25-T)] + 2 E + 5 \Delta W$$

Brown strains -

$$ME = W[90 + 2,0(25-T)] + 2 E + 5 \Delta W$$

Tinted strains -

$$ME = W[102,5 + 2,1(25-T)] + 2 E + 5 \Delta W$$

where ME = ME intake kcal per bird per day

W = mean period body mass (kg)

T = mean environmental temperature, °C

E = egg output, g per bird per day

$\Delta W$  = body mass gain g/bird per day.

For each 8 week period, measurements were made of W,  $\Delta W$ , T, E and feed intake. Feed intake was predicted from the equations by assuming that all feeds contained 2,7 kcal ME/g.

Actual feed intake averaged 122,9 g per bird per day with a coefficient of variation (cv) of 9,13 percent. The ratio of actual to predicted feed averaged 1,030 with a cv of 7,05 percent.

McDonald (1978) accumulated data from published reports from 16 locations, for which ME intake, body mass, mass gain and daily egg mass were available. Breeds ranged from light bodied layers to meat breeders, in cages or floor pens, controlled environment or open pens. By multiple regression analyses the following equation to predict ME intake was produced from the data:

$$ME = 0,532 W^{0,75} + 14,5 \Delta W + 0,20 E + 147$$

This equation produced a correlation coefficient of 0,9 between observed and predicted ME intake.

The A R C (1975) assembled results from random sample laying trials and from feeding experiments published in the period 1965 - 1969 to provide estimates of ad libitum feed intake and hence, by calculation, energy intake averaged over periods of 8 to 12 months. The collection of data was limited to trials conducted in the U K.

Results were included only if the diet was composed of commonly used feedstuffs and contained 140 to 165 g crude protein/kg and 2,4 to 3,0 Mcal (10,0 to 12,6 MJ) ME/kg. The range of mean body mass in the data was from 1,5 kg to 2,75 kg and the range of egg



output was from 33 g to 53 g of egg material per bird per day.

The data were analysed and the equation obtained was:

$$ME = 65, W^{0,75} + 2,75(E+\Delta W) + 125,3$$

where ME = ME intake (kcal/bird day)

W = mean body mass (kg)

E = egg output (g/bird day)

$\Delta W$  = change in body mass (g/bird day)

or  $M(KJ) = 275,3 W^{0,75} + 11,51(E+\Delta W) + 524,3$

Byerly (1978) suggested the following equation to predict the feed intake of layers at different environmental temperatures.

$$F = (0,259 - 0,002 T)W^{0,75} + 1,983 \Delta W + 0,7859 E$$

where F = feed intake (g/bird d)

W = livemass/bird (g)

$\Delta W$  = change in live mass (g/bird d)

E = egg mass (g/bird d)

T = temperature (°C)

Gous et al. (1978) presented an equation for predicting food intake which was derived from data collected from five breeds varying considerably in body mass and egg output.

The equation obtained was:

$$F = 0,0255 W + 2,45 \Delta W + 0,97 E + 10,674$$

where F = daily food intake (of a 12,09 MJ ME/kg diet)

W = body mass (g)

$\Delta W$  = daily change in body mass (g)

E = daily egg mass output (g)

These authors concluded that in conjunction with a correction factor for dietary energy concentration proposed by Morris (1968) the

above equation accurately predicts food intake when applied to previously published data, and is valid over a range of body mass values from 1 200 g to 4 500 g.

Bearing in mind that their diet contained 2 890 kcal ME/kg (12,09 MJ/kg) the caloric consumption can be determined by modifying their equation as follows:

$$ME = 0,074 W + 7,11 \Delta W + 2,8 E + 30,85$$

or  $ME \text{ intake KJ/bird d} = 0,3 W + 29,74 \Delta W + 11,74 E + 129$

Hurwitz and Bornstein (1977) suggested that the energy requirements of layers could be calculated from the equation:

$$ME(\text{kcal/day}) = 145 W^{0,67} + 2 \Delta W + 1,8 E$$

where  $W$  = body mass (kg)

$\Delta W$  = body mass change (g/day)

$E$  = egg mass (g/day)

The maintenance requirement was recalculated from values determined by Waring and Brown (1967); the requirement of 2 kcal/g body mass was derived from chick data (Hurwitz, unpublished); the requirement for egg formation was calculated from the energy content of the egg, multiplied by a utilisation value of 0,85. The authors state that energy requirements calculated from this equation compared well with those observed in some of their previous experiments.

Combs (1968) used the following equation to determine the energy requirements of layers at different ambient temperatures at bird level.

$$ME = (1,78 - 0,012 T) 1,45 W^{0,653} + 3,13 \Delta W + 3,15 E$$

where  $E$  = egg mass (g/day)

$T$  = temperature in °F

$W$  = body mass (g)

$\Delta W$  = body mass change (g/day)



## CHAPTER 3

### RESPONSE OF LAYING HENS TO DIETARY ISOLEUCINE INTAKE

#### INTRODUCTION

Isoleucine is an aliphatic amino acid, similar in structure to leucine and valine, and because it cannot be synthesised by chickens, is classified as an essential amino acid (Scott et al., 1976). It is present in relatively low concentrations in most feedingstuffs, but is particularly low in bloodmeal due to the fact that the haemoglobin molecule does not contain isoleucine. In attempting to meet the daily requirements of laying hens for isoleucine as suggested by Scott et al., (1976) and Morris (1980) (between 850 and 880 mg isoleucine/bird d) this amino acid could be expected to be first-limiting when using typical values for raw ingredients available in South Africa.

The above values for the optimum daily intake of isoleucine are by no means typical of the majority of published values. A wide range of requirements have been published, and a summary of these results is presented in Table 3.1 together with notes regarding the maximum daily egg output achieved in the experiments, the type of diet fed and whether the requirement was based on actual experimental evidence or on a calculation from a theoretical model. It will be noted that the number of published reports on this topic are relatively few.

Two main reasons for the wide diversity of requirements as suggested by these authors are first, the fact that the isoleucine content of raw materials, as published by various research workers, differs markedly, and secondly, that there has been shown to be an inter-relationship between isoleucine, leucine and valine.

TABLE 3.1: Summary of experimental evidence about the isoleucine requirement of the laying hen and the recommendations of the Agricultural Research Council (A R C 1975) and National Research Council (N R C 1971). In addition to the calculated requirements of Johnson and Fisher (1958) and Moran, Summers and Pepper (1967) shown here, calculated requirements based on models suggested by Hurwitz and Bornstein (1973), Wilgus (1976) and Smith (1978) have also been given.

Reference	No and range of dietary contents used. No/g/kg.	Requirement g/kg	Intake at requirement. (mg/day)	Egg production. g/day or %	Notes
Miller et al (1954)	5/3,3 to 5,3	5,30	-	66 %	Young pullet. Available isoleucine. Two experiments.
Johnson & Fisher (1958)	-	5,0	-	-	Calculated, egg-ratio method.
Combs (1962)	2/5,3 to 6,42	6,42	614	42,9 g	Corn-soya diet.
Combs (1962)	2/5,17 to 6,51	6,51	626	43,75g	Corn-soya-fish diet.
Moran, Summers & Pepper (1967)	-	-	535	46,5 g	Calculated requirement.
Bray (1969)	9/2,5 to 6,2	4,0	472	45 g	Used young pullets. Basal diet provided 64,75% of requirement.
Hurwitz & Bornstein (1973)	-	-	689	50 g	(Calculated on model B. Birds with 2 kg body mass. ( Gaining 1,5 g/d.
Hurwitz & Bornstein (1973)	-	-	636	45 g	(Calculated on model B. Birds with 2 kg body mass. ( Gaining 1,5 g/d. (Hurwitz & Bornstein (1973) requirements based on total isoleucine.
A R C (1975)	-	5,5	550	50 g	The 550 mg/day is available isoleucine. The concentration of 5,5 g/kg assumes 110 g feed intake, 90% availability.
Scott, Nesheim & Young (1976)	-	-	850	-	Total isoleucine requirement. (Not available).
Wilgus (1976)	-	-	709	50 g	(Calculated on modified Model B. Birds with 2 kg body mass. ( Gaining 1,5 g/d.
Wilgus (1976)	-	-	656	45 g	(Calculated on modified Model B. Birds with 2 kg body mass. ( Gaining 1,5 g/d. (Wilgus (1976) requirements based on total isoleucine.
N R C (1977)	-	5,0	550	-	Based on feed consumption of 110 g/bird/day.
Smith (1978)	-	-	586	50 g	(Calculated on model 2. Birds with 2 kg body mass. ( Gaining 1,5 g/d.
Smith (1978)	-	-	544	45 g	(Calculated on model 2. Birds with 2 kg body mass. ( Gaining 1,5 g/d. (Smith (1978) requirements based on total isoleucine.
Morris (1980)	5/4,4 to 7,7	-	886	54,23g	Corn, Fish meal diets. Figure of 886 mg is optimum intake when marginal cost of isoleucine is 96 p/kg and value of egg output is 50 p/kg.



The wide range of isoleucine values in feed ingredients is shown in Table 3.2. One possible explanation for these diverse values is the fact that isoleucine and valine are released more slowly by acid hydrolysis prior to amino acid analysis (Davies and Thomas, 1973) and the lower values in the Table could represent results obtained after 24 h hydrolysis as opposed to those samples hydrolysed for 72 h.

The interrelationship between isoleucine and leucine was first noted by Harper (1955) who reported an "antagonism" in which a growth depression in rats caused by adding excess leucine to a basal diet could be reversed in part by isoleucine supplementation. Nesheim (1974) showed that growth depression in chickens due to high levels of leucine in the diet could be alleviated by increasing the valine and isoleucine contents of the diet. Harper (1970) states that an excessive supplement of any of these three amino acids seems to increase the requirement for the other two.

The low isoleucine requirement suggested by Bray (1969) could thus be due to the relatively low level of leucine used in his basal diet compared with the high level of leucine used, and hence the high requirement for isoleucine suggested by Miller et al., (1954). In these previous experiments the ratio between isoleucine and leucine has not been held constant throughout the range of diets fed, and if the ratio between these amino acids is of importance then the results of such trials would be confounded by this effect. The dilution technique of Fisher and Morris (1970) is ideally suited for determining responses to amino acids that might be affected by antagonistic amino acids, as the balance between these amino acids is kept constant throughout the range of diets fed. For this reason this dilution technique was used here to determine the response of laying hens to isoleucine intake, keeping the ratio of isoleucine, leucine and valine constant.

Table 3.2 Published Isoleucine values of different ingredients (g/kg).

Ingredient	N.R.C. (1977)	Scott <u>et al.</u> (1976)	Maryland (1978)	Dennison and Gous (1980)
Maize (ground)	3,70	4,00	3,60	2,60
Wheat bran	5,90	6,00	3,70	3,80
Lucerne meal	8,40	7,50	6,50	6,00
Soya Oilcake Meal	23,90	25,00	23,80	--
Sunflower Oilcake meal	27,80	21,00	16,40	14,80
Peanut Oilcake meal	21,00	22,00	--	13,40
Cotton seed Oilcake meal	13,30	16,00	12,00	--
Full fat Soyabean meal	20,00	20,00	19,10	--
Fish meal (Anchovy)	30,10	36,00	26,80	25,70
Bloodmeal	9,50	10,00	6,20	6,10

## MATERIALS AND METHODS

### *Dietary treatments.*

Using the recommended levels of amino acids for layers at varying energy levels in feeds (Scott, Nesheim and Young, 1976) "summit" and "dilution" diets at two energy levels were formulated. The minimum content of all essential amino acids except isoleucine in the summit diets were set at 140 percent of the suggested requirements. The isoleucine levels however, were fixed at 110 percent of these requirements. In the dilution diets minimum levels of all the essential amino acids except isoleucine were fixed at 90 percent while isoleucine was fixed at 42 percent of requirement. In an effort to reduce as far as possible unwanted surpluses of amino acids in the summit diets, the crude protein content of each diet was kept at a minimum. The composition of these four diets is shown in Table 3.3. The specified amino acid contents as set out by the



TABLE 3.3: Compositions (g/kg) of the summit and dilution diets.

Ingredients	Diet 1 (Summit diet)	Diet 5 (Dilution diet)	Diet 11 (Summit diet)	Diet 15 (Dilution diet)
Maize	427,00	363,00	454,00	485,00
Wheat bran	50,00	78,50	-----	-----
Lucerne meal	-----	165,00	-----	127,00
Full fat Soyabean meal (380)*	-----	-----	170,00	11,00
Peanut meal (400)*	234,00	35,00	62,00	48,00
Sunflower meal (380)*	130,00	-----	130,00	-----
Fish meal (660)*	50,00	-----	61,00	-----
Bone meal	19,00	-----	16,30	-----
Monocalcium Phosphate	-----	22,00	-----	23,50
Limestone Powder	83,50	90,80	83,8	85,80
Blood meal (800)*	-----	40,00	-----	50,00
Starch (maize)	-----	200,00	-----	163,00
Poultry Feathermeal (840)*	-----	-----	18,00	-----
Salt	2,00	3,50	1,50	3,50
Methionine (98%)	-----	-----	0,20	-----
Lysine (80%)	1,30	-----	-----	-----
Choline chloride	1,00	1,00	1,00	1,00
Vitamin Premix**	1,20	1,20	1,20	1,20
Mineral Premix***	1,00	1,00	1,00	1,00

Calculated analysis

Metabolisable energy (MJ/kg)	10,30	10,30	11,50	11,50
Crude protein (g N x 6,25/kg)	225,82	116,51	237,50	126,39
Isoleucine (g/kg)	7,8	3,08	9,0	3,3
Calcium (g/kg)	37,5	40,0	37,4	38,3
Phosphorus (g/kg)	6,9	6,8	6,6	6,9

\* Assumed crude protein (g N x 6,25/kg)

\*\* Vitamin Premix provides per kg of diet: 10 035 I.U. Vit A, 2 000 I.U. Vit. D3, 12,94 mg Vit. E, 2 mg menadionsodium-bisulfite, 1,0 mg thiamin, 5,16 mg riboflavin, 14,8 mg calcium pantothenate, 43,6 nicotinic acid, 10 micrograms Vit. B12, 0,4 mg folic acid, 300 mg choline.

\*\*\* Mineral Premix provides per kg of diet: 4 mg copper, 40 mg zinc, 2 mg iodine, 80 mg manganese.

TABLE 3.4:

Calculated amino acid contents of the summit and dilution diets relative to the suggested requirements of the laying hen.

	Requirement according to Scott, Nes- heim and Young (1976) in (g/kg) for diet with ME con- tent of 10,3 MJ/kg.	Amino acid con- tents expressed as multiples of requirements for diet with ME con- tent of 10,3 MJ/kg.		Requirement according to Scott, Nes- heim and Young (1976) in (g/kg) for diet with ME con- tent of 11,50 MJ/kg.	Amino acid con- tents expressed as multiples of requirements for diet with ME con- tent of 11,50 MJ/kg.	
		( Diet 1 (summit diet)	Diet 5 (dilution diet)	( Diet 11 (summit diet)	Diet 15 (dilution diet)	)
Arginine	7,04	2,74	0,88	7,87	2,17	0,86
Lysine	5,93	1,82	1,02	6,63	1,77	1,02
Methionine	3,03	1,38	0,60	3,38	1,39	0,60
Cystine	2,24	1,88	0,97	2,50	1,79	0,94
Tryptophan	1,45	1,86	0,97	1,62	1,78	0,89
Histidine	2,81	1,88	1,23	3,14	1,68	1,28
Leucine	10,64	1,61	1,09	11,88	1,61	1,13
Isoleucine*	7,04	1,10	0,44	7,86	1,14	0,42
Phenylalanine	6,50	1,57	0,95	7,26	1,44	0,97
Tyrosine	2,81	2,30	1,38	3,14	1,93	1,39
Threonine	5,15	1,46	0,84	5,75	1,50	0,82
Valine	6,03	1,72	1,07	6,74	1,69	1,07

A.R.C. (1975) recommendation used in preference to Scott, Nesheim and Young (1976) which was considered too high.



TABLE 3.5: Summary of dilution technique and calculated analysis of the experimental diets.

Diet	Blending ratio				Calculated dietary isoleucine (g/kg)	Calculated dietary protein (gNx6,25/kg)	Calculated dietary energy MJ/kg
	Diet 1 (summit diet)	Diet 5 (dilution diet)	Diet 11 (summit diet)	Diet 15 (dilution diet)			
1 a	1 000	-	-	-	7,8	225,82	10,3
1 b	750	250	-	-	6,62	198,49	10,3
1 c	500	500	-	-	5,44	171,17	10,3
1 d	250	750	-	-	4,26	143,84	10,3
1 e	-	1 000	-	-	3,08	116,51	10,3
2 a	500	-	500	-	8,4	231,66	10,9
2 b	375	125	375	125	7,1	204,11	10,9
2 c	250	250	250	250	5,8	176,56	10,9
2 d	125	375	125	375	4,49	149,00	10,9
2 e	-	500	-	500	3,19	121,45	10,9
3 a	-	-	1 000	-	9,0	237,50	11,5
3 b	-	-	750	250	7,58	209,72	11,5
3 c	-	-	500	500	6,15	181,95	11,5
3 d	-	-	250	750	4,7	154,17	11,5
3 e	-	-	-	1 000	3,3	126,39	11,5

procedure for the summit and dilution diets are shown in Table 3.4. The calculated contents achieved in the formulated diets relative to the suggested requirements are also indicated.

By blending the summit and dilution diets in appropriate proportions as indicated in Table 3.5, fifteen experimental diets were produced, consisting of three energy levels viz. 10,3, 10,9 and 11,5 MJ ME/kg and five isoleucine concentrations at each energy level representing 110, 92,5, 75, 57,5 and 40 percent of the isoleucine requirement.

#### *Preliminary trial.*

To confirm that isoleucine was the first limiting amino acid in the experimental diets, a preliminary trial was conducted prior to the main experiment. This trial was used also to confirm that methionine, the next-limiting amino acid, would not give a response when added to these diets.

The trial was conducted in a convection house using 1 440 laying hens 46 weeks of age. Twelve treatments, indicated in Table 3,6 were used each treatment being tested on three replications of 40 birds each. Prior to the introduction of these experimental diets all birds received a normal commercial layer diet for at least six weeks. Mean rate of lay in the flock at the start of the trial was 74 percent.

For the first three weeks diets 1, 5, 11 and 15 were each allocated to nine pens for adaptation purposes. During the three week observation period each of the abovementioned diets were supplemented with isoleucine or methionine giving three variations of each diet, the first serving as a control. (Table 3.6.) Egg numbers and total egg mass were recorded on a daily basis while feed intake was measured weekly.



Table 3.6: Diets used in preliminary trial

<u>Diet code</u>	<u>Calculated isoleucine content (g/kg)</u>	<u>Additions made to the diet (g/kg)</u>
1	7,80	---
5	3,08	---
11	9,00	---
15	3.30	---
1	9,40	1,6 Isoleucine
5	4,68	1,6 Isoleucine
11	10,60	1,6 Isoleucine
15	4,90	1,6 Isoleucine
1	7,80	0,6 DL-methionine
5	3,08	0,6 DL-methionine
11	9,00	0,6 DL-methionine
15	3,30	0,6 DL-methionine

*Main Experiment.*

A total of 3 600 Ross Tint pullets was used in this trial. They were reared in a controlled environment house and fed ad lib. on normal commercial rearing diets. A constant 13 hour photoperiod was used in the rearing period. After 18 weeks of age lighting was increased by 30 minutes per week to a maximum of 17 hours per day whereafter the daylength was held constant.

At 18 weeks of age the birds were moved into a convection laying house. Fifteen treatments were randomly allocated to 60 pens, giving 4 replicates of 60 birds for each treatment. Three pullets were housed per 45,7 cm x 30,5 cm cage.

Prior to the introduction of the 15 experimental diets at 21 weeks of age all the caged pullets received a layer diet containing 155 g crude protein (gN x 6,25/kg) and 37 g calcium/kg.

The trial commenced on 4th August 1978 when the birds were 21 w of age and continued for five 28 d periods.

Egg numbers, total egg mass and mortality were recorded daily. The body mass of twelve birds per replication was determined at the commencement of each 28 d period. Body mass of the same birds was measured on each occasion. From this data the mean body mass and mass gain/d was calculated. Feed intake was measured weekly.

The data was analysed per 28 d period and in addition the three periods encompassing peak production were combined for a separate analysis.

## RESULTS AND DISCUSSION.

### *Preliminary trial.*

As expected egg output declined drastically in birds switched from the commercial diet to the two dilution diets (diets 5 and 15). However, the birds changed from the commercial diet to the summit diets (diets 1 and 11) maintained an even output.

The egg output during the adaptation and observation periods is shown in Table 3.7 and Fig 3.1. From these results it is evident that no response was obtained from the summit diets supplemented with isoleucine or methionine.

The calculated intake of isoleucine on control diets 5 and 15 during the three week experimental period was 315 and 312 mg/bird d. At these low intake levels a response was expected.

The addition of isoleucine to diets 5 and 15 evoked a highly significant response, whereas the control and methionine supplemented diets showed no response.

It can therefore be concluded that isoleucine was the first-limiting amino acid in the experimental diets used.



TABLE 3.7: Egg output response of laying hens (g/hen d) fed diets 1, 5, 11 and 15 supplemented with isoleucine or methionine

Diet code	Three week adaptation period			Diet code	Three week experimental (observation) period.		
	Week 1	Week 2	Week 3		Week 4	Week 5	Week 6
1	44,6	41,3	43,5	1 Control	40,5	41,1	44,1
1	46,70	43,1	44,2	1 plus isoleucine	46,05	43,1	45,9
1	43,82	40,68	41,97	1 plus methionine	42,50	41,4	43,08
5	40,7	24,9	21,20	5 Control	17,40	19,5	19,9
5	40,64	23,33	20,77	5 plus isoleucine	19,78	25,88	30,61
5	40,71	22,95	20,35	5 plus methionine	16,86	17,64	19,88
11	44,2	40,6	43,60	11 Control	44,7	44,6	43,9
11	44,7	43,8	44,70	11 plus isoleucine	45,2	47,0	46,02
11	46,52	41,73	46,23	11 plus methionine	44,33	44,35	46,15
15	40,3	23,5	19,6	15 Control	17,7	19,4	21,1
15	39,84	25,14	21,38	15 plus isoleucine	17,88	24,09	32,76
15	40,71	24,51	20,38	15 plus methionine	15,99	17,38	19,49

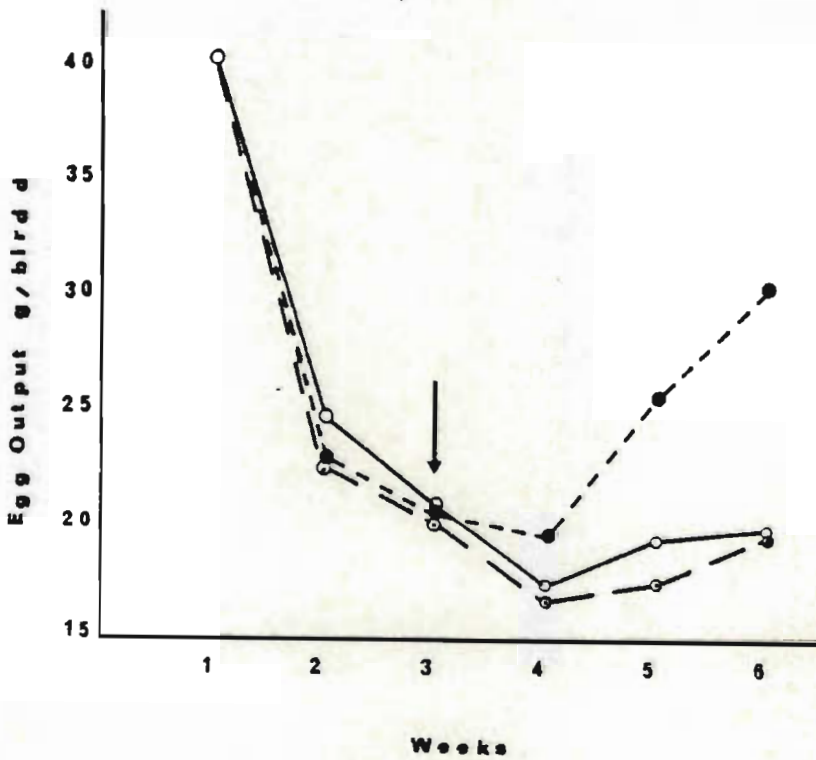
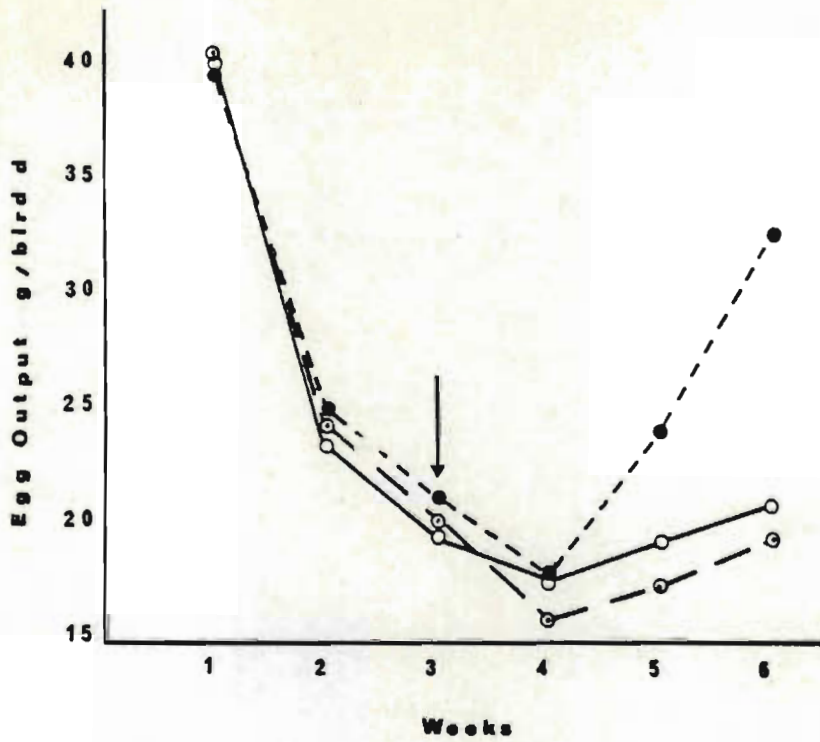


Figure 3.1 Response to the supplementation of two dilution diets (o—o) with isoleucine (●—●) and methionine (o—•). Supplementation commenced where indicated by arrow.



## RESULTS BY PERIOD (MAIN EXPERIMENT)

The following production parameters for each of the five periods of the trial are presented in Tables 3.8 to 3.12: Rate of lay (egg number/100 bird d), egg mass (g/egg), egg output (g/bird d), food intake (g/bird d), food conversion efficiency (g egg produced/g feed consumed) and body mass gain (g/bird d).

In each table the main effects of the isoleucine/energy ratio (I) and of energy (E) are shown together with the interaction terms (I x E). Also the standard errors of each mean (SEM) and the least significant differences (LSD) at  $P < 0,05$  and  $P < 0,01$  are indicated.

These same results are illustrated in Figures 3.2 to 3.7 where the effects of the five levels of dietary isoleucine concentrations have been plotted at each energy level for the five periods of the trial.

The effects of dietary isoleucine only will be considered in the present discussion as the effects of energy on the production parameters measured will be discussed in Chapter 6.

### *Egg production.*

From Tables 3.8 to 3.12 and figure 3.2, it is clear that egg production from the birds fed on diets adequate in isoleucine (summit, first and second dilution diets) was satisfactory throughout the experiment.

Rate of lay of birds on the third and fourth dilution diet was however significantly lower than that of birds fed the three diets containing higher isoleucine concentrations.

TABLE 3.8: Response to Isoleucine and Energy intake during period 1. (21 - 25 weeks of age)

3.8.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	23,30	23,87	25,90	22,10	12,82	21,60
2	23,12	27,25	23,62	24,30	15,90	22,84
3	27,95	24,97	27,55	21,82	21,72	24,80
Mean	24,79	25,37	25,69	22,74	16,82	23,08
	SEM		LSD (0,05)		LSD (0,01)	
I	0,77		2,20		3,00	
E	0,60		1,70		2,30	
I x E	1,37		3,90		5,20	

3.8.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	50,15	50,49	48,16	48,89	46,42	48,82
2	49,98	50,48	50,57	49,46	46,62	49,42
3	50,82	51,33	49,77	48,85	47,29	49,61
Mean	50,32	50,76	49,50	49,07	46,78	49,29
	SEM		LSD (0,05)		LSD (0,01)	
I	0,438		1,25		1,67	
E	0,339		0,967		1,29	
I x E	0,757		2,16		2,89	

3.8.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	11,69	12,07	12,48	10,82	5,96	10,60
2	11,55	13,74	11,95	12,00	7,42	11,33
3	14,21	12,80	13,72	10,66	10,27	12,33
Mean	12,48	12,87	12,72	11,16	7,88	11,42
	SEM		LSD ((0,05)		LSD (0,01)	
I	0,396		1,13		1,51	
E	0,308		0,88		1,17	
I x E	0,687		1,96		2,62	



TABLE 3.8 (Continued)

3.8.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	110,06	114,09	113,35	113,79	110,10	112,28
2	105,94	110,22	111,37	109,26	103,27	108,01
3	104,52	105,97	107,22	106,39	99,15	104,65
Mean	106,84	110,10	110,65	109,82	104,18	108,31
	SEM		LSD (0,05)		LSD (0,01)	
I	0,560		1,60		2,14	
E	0,434		1,24		1,66	
I x E	0,970		2,77		3,71	

3.8.5: Feed conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	0,1060	0,1055	0,1102	0,0952	0,0545	0,0943
2	0,1090	0,1250	0,1072	0,1100	0,0725	0,1047
3	0,1357	0,1210	0,1282	0,1000	0,1040	0,1178
Mean	0,1169	0,1172	0,1152	0,1017	0,0770	0,1056
	SEM		LSD (0,05)		LSD (0,01)	
I	0,0039		0,011		0,015	
E	0,0028		0,008		0,011	
I x E	0,0067		0,019		0,025	

3.8.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	12,29	10,87	11,22	8,87	6,67	9,97
2	12,50	10,93	10,79	8,26	7,29	9,95
3	11,15	13,38	11,21	9,80	5,14	10,14
Mean	11,98	11,73	11,07	8,98	6,35	10,02
	SEM		LSD (0,05)		LSD (0,01)	
I	0,385		1,10		1,47	
E	0,298		0,85		1,14	
I x E	0,665		1,90		2,55	

TABLE 3.9: Response to Isoleucine and Energy intake during period 2. (25 - 29 weeks of age)

3.1.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	88,32	87,88	87,97	80,73	58,50	80,68
2	88,35	88,95	88,58	82,25	60,60	81,75
3	89,27	90,47	90,37	86,02	60,05	83,24
Mean	88,65	89,10	88,97	83,00	59,72	81,89
	SEM		LSD (0,05)		LSD (0,01)	
I	0,46		1,30		1,70	
E	0,35		1,00		1,30	
I x E	0,77		2,20		3,00	

3.9.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	54,62	54,18	53,93	52,09	48,38	52,64
2	54,80	55,16	54,57	52,42	48,54	53,10
3	55,41	55,29	54,90	52,56	47,80	53,19
Mean	54,95	54,88	54,46	52,36	48,24	52,98
	SEM		LSD (0,05)		LSD (0,01)	
I	0,151		0,431		0,576	
E	0,117		0,334		0,447	
I x E	0,262		0,747		0,998	

3.9.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	48,24	47,59	47,45	42,07	28,30	42,73
2	48,43	49,06	48,33	43,11	29,41	43,67
3	49,47	50,02	49,61	45,21	28,69	44,60
Mean	48,71	48,89	48,46	43,46	28,80	43,67
	SEM		LSD (0,05)		LSD (0,01)	
I	0,224		0,64		0,86	
E	0,175		0,499		0,667	
I x E	0,392		1,12		1,49	



TABLE 3.9 (Continued)

3.9.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	116,61	119,02	119,66	117,13	95,98	113,68
2	114,41	115,30	114,39	111,70	93,71	109,90
3	110,10	110,93	112,13	109,58	85,86	105,72
Mean	113,71	115,08	115,39	112,80	91,85	109,77
	SEM		LSD (0,05)		LSD (0,01)	
I	1,825		5,21		6,97	
E	1,415		4,04		5,40	
I x E	3,163		9,03		12,07	

3.9.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	0,4137	0,4000	0,3965	0,3592	0,2952	0,3729
2	0,4237	0,4255	0,4225	0,3860	0,3140	0,394
3	0,4490	0,4510	0,4425	0,4127	0,3342	0,4179
Mean	0,4288	0,4255	0,4205	0,386	0,3145	0,3950
	SEM		LSD (0,05)		LSD (0,01)	
I	0,010		0,029		0,039	
E	0,008		0,023		0,030	
I x E	0,018		0,051		0,068	

3.9.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	- 0,16	- 0,44	- 0,87	- 2,07	- 5,50	- 1,81
2	0,08	0,33	0,22	- 0,29	- 4,97	- 0,93
3	1,04	0,75	0,70	0,09	- 4,33	- 0,35
Mean	0,32	0,21	0,02	- 0,76	- 4,93	- 1,03
	SEM		LSD (0,05)		LSD (0,01)	
I	0,312		0,89		1,19	
E	0,242		0,69		0,92	
I x E	0,539		1,54		2,06	

TABLE 3.10: Response to Isoleucine and Energy intake during period 3. (29 - 33 weeks of age)

3.10.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	89,12	87,62	87,90	80,00	41,42	77,21
2	89,45	88,87	89,62	80,82	42,37	78,23
3	88,72	89,42	89,55	84,00	37,00	77,74
Mean	89,10	88,64	89,02	81,61	40,27	77,73
	SEM		LSD (0,05)		LSD (0,01)	
I	0,67		1,90		2,60	
E	0,53		1,50		2,00	
I x E	1,2		3,30		4,50	

3.10.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	57,13	56,90	55,47	54,75	48,39	54,53
2	57,55	58,14	56,92	54,94	46,21	54,75
3	58,72	58,07	57,29	54,55	47,43	55,21
Mean	57,80	57,70	56,56	54,75	47,34	54,83
	SEM		LSD (0,05)		LSD (0,01)	
I	0,364		1,04		1,39	
E	0,282		0,806		1,08	
I x E	0,630		1,80		2,41	

3.10.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	50,91	49,84	48,76	43,81	20,05	42,67
2	51,48	51,67	51,02	44,41	19,53	43,62
3	52,10	51,93	51,30	45,80	17,54	43,73
Mean	51,50	51,15	50,36	44,67	19,04	43,34
	SEM		LSD (0,05)		LSD (0,01)	
I	0,392		1,120		1,490	
E	0,303		0,866		1,160	
I x E	0,676		1,950		2,590	



TABLE 3.10 (Continued)

3.10.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	118,76	117,51	119,92	119,06	91,30	113,31
2	116,34	115,58	118,10	113,18	85,30	109,70
3	109,42	110,43	113,91	112,47	73,81	104,01
Mean	114,84	114,50	117,31	114,90	83,47	109,01
	SEM		LSD (0,05)		LSD (0,01)	
I	0,858		2,45		3,28	
E	0,665		1,90		2,54	
I x E	1,485		4,24		5,68	

3.10.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	0,4288	0,4242	0,4065	0,3682	0,2195	0,3694
2	0,4425	0,4470	0,4320	0,3927	0,2292	0,3887
3	0,4760	0,4705	0,4507	0,4072	0,2375	0,4084
Mean	0,4491	0,4472	0,4297	0,3894	0,2287	0,3888
	SEM		LSD (0,05)		LSD (0,01)	
I	0,0025		0,007		0,010	
E	0,0021		0,006		0,008	
I x E	0,0046		0,013		0,017	

3.10.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	2,75	2,32	1,46	0,96	0,67	1,63
2	2,08	2,10	1,72	0,21	- 0,82	1,06
3	1,26	2,32	1,34	1,38	- 1,52	0,96
Mean	2,01	2,25	1,51	0,85	- 0,56	1,22
	SEM		LSD (0,05)		LSD (0,01)	
I	0,270		0,77		1,03	
E	0,210		0,60		0,80	
I x E	0,469		1,34		1,79	

TABLE 3.11: Response to Isoleucine and Energy intake during period 4. (33 - 37 weeks of age)

3.11.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	86,87	86,07	85,55	77,87	40,22	75,32
2	86,65	86,72	87,47	79,17	39,05	75,81
3	87,37	87,45	87,82	80,62	33,92	75,44
Mean	86,97	86,75	86,95	79,22	37,73	75,52
	SEM		LSD (0,05)		LSD (0,01)	
I	0,67		1,90		2,60	
E	0,53		1,50		2,00	
I x E	1,20		3,30		4,40	

3.11.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	58,44	58,16	57,55	55,81	50,02	56,00
2	58,69	58,93	58,36	56,36	49,87	56,44
3	59,64	59,14	58,60	56,44	49,84	56,73
Mean	58,92	58,75	58,17	56,20	49,91	56,39
	SEM		LSD (0,05)		LSD (0,01)	
I	0,130		0,371		0,496	
E	0,101		0,287		0,384	
I x E	0,225		0,642		0,859	

3.11.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	50,77	50,07	49,22	43,48	20,12	42,73
2	50,85	51,12	51,05	44,63	19,46	43,42
3	52,12	51,70	51,47	45,48	16,91	43,53
Mean	51,25	50,96	50,58	44,53	18,83	43,23
	SEM		LSD (0,05)		LSD (0,01)	
I	0,382		1,090		1,460	
E	0,297		0,848		1,130	
I x E	0,662		1,890		2,530	



TABLE 3.11 (Continued)

3.11.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	120,57	123,10	121,06	123,87	92,63	116,24
2	110,77	123,17	116,72	116,10	89,76	111,31
3	110,41	112,18	114,25	115,19	80,97	106,60
Mean	113,92	119,49	117,34	118,39	87,79	111,38
	SEM		LSD (0,05)		LSD (0,01)	
I	0,557		1,59		2,13	
E	0,431		1,23		1,65	
I x E	0,967		2,76		3,69	

3.11.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	0,4212	0,4067	0,4067	0,3513	0,2172	0,3606
2	0,4592	0,4152	0,4372	0,3842	0,2167	0,3825
3	0,4717	0,4610	0,4507	0,3947	0,2087	0,3974
Mean	0,4507	0,4277	0,4316	0,3767	0,2142	0,3802
	SEM		LSD (0,05)		LSD (0,01)	
I	0,0035		0,010		0,013	
E	0,0025		0,007		0,010	
I x E	0,0060		0,017		0,022	

3.11.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	0,27	2,29	0,84	0,36	- 1,12	0,53
2	1,98	2,20	3,09	8,21	- 0,60	2,98
3	2,09	1,94	1,95	- 0,36	0,62	1,25
Mean	1,45	2,14	1,96	2,74	- 0,36	1,58
	SEM		LSD (0,05)		LSD (0,01)	
I	1,163		3,32		4,44	
E	0,900		2,57		3,43	
I x E	2,01		5,74		7,68	

TABLE 3.12: Response to Isoleucine and Energy intake during period 5. (37 - 41 weeks of age)

3.12.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	83,65	83,37	82,43	76,27	39,53	73,05
2	83,82	83,57	84,25	75,62	42,32	73,92
3	84,15	84,22	83,33	77,27	39,65	73,72
Mean	83,87	83,72	83,33	76,39	40,50	73,56
	SEM		LSD (0,05)		LSD (0,01)	
I	0,74		2,1		2,80	
E	0,56		1,6		2,20	
I x E	1,29		3,67		4,90	

3.12.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	59,53	59,08	58,54	57,00	51,83	57,20
2	59,94	59,89	59,03	57,36	51,64	57,57
3	60,95	60,20	59,53	57,76	52,00	58,09
Mean	60,14	59,72	59,03	57,38	51,82	57,62
	SEM		LSD (0,05)		LSD (0,01)	
I	0,127		0,363		0,486	
E	0,098		0,281		0,376	
I x E	0,220		0,629		0,841	

3.12.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	49,79	49,27	48,25	43,49	20,48	42,26
2	50,25	50,07	49,72	43,37	21,88	43,06
3	51,31	50,70	49,60	44,61	20,64	43,37
Mean	50,45	50,01	49,19	43,82	21,00	42,90
	SEM		LSD (0,05)		LSD (0,01)	
I	0,440		1,256		1,68	
E	0,340		0,971		1,30	
I x E	0,760		2,17		2,91	



TABLE 3.12 (Continued)

3.12.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	116,33	112,15	118,86	118,99	91,80	111,63
2	116,88	112,20	118,03	111,56	90,77	109,89
3	104,41	107,44	106,13	109,94	84,18	102,42
Mean	112,54	110,60	114,34	113,50	88,92	107,98
	SEM		LSD (0,05)		LSD (0,01)	
I	1,797		5,13		6,86	
E	1,391		3,97		5,31	
I x E	3,11		8,88		11,87	

3.12.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	0,4287	0,4417	0,4060	0,3657	0,2230	0,3730
2	0,4305	0,4475	0,4232	0,3885	0,2407	0,3861
3	0,4927	0,4720	0,4677	0,4057	0,2457	0,4168
Mean	0,4507	0,4537	0,4323	0,3867	0,2365	0,3920
	SEM		LSD (0,05)		LSD (0,01)	
I	0,0060		0,017		0,022	
E	0,0046		0,013		0,017	
I x E	0,0102		0,029		0,039	

3.12.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	1,46	- 1,22	- 0,10	- 1,02	- 0,02	- 0,18
2	- 0,70	- 0,31	- 1,62	- 1,79	- 0,92	- 1,07
3	0,81	0,71	- 0,46	0,38	- 1,02	0,08
Mean	0,53	- 0,28	- 0,73	- 0,81	- 0,65	- 0,39
	SEM		LSD (0,05)		LSD (0,01)	
I	0,451		1,288		1,72	
E	0,349		0,997		1,33	
I x E	0,781		2,23		2,98	

It is interesting to note that in spite of the very low isoleucine concentration in the dilution diets, egg production nevertheless increased to approximately 60 percent in the second period. Thereafter egg production declined sharply to approximately 40 percent during the third period. Egg production from then onwards remained relatively constant until the termination of the experiment. Furthermore, it is obvious (Figure 3.2), that egg production response to isoleucine intake at the three dietary energy concentrations followed a very similar pattern.

No attempt was made to relate egg production response to actual isoleucine intake as this will form the subject of further discussion (see egg production during peak production periods).

#### *Egg mass.*

The egg mass (g/egg) response pattern to isoleucine intake was similar to that of rate of lay (Table 3.8 to 3.12 and Figure 3.3). The egg mass (g/egg) graph however, contrary to rate of lay, continued to increase as the laying period progressed.

No significant difference was found between the three diets with the highest isoleucine concentrations. There was however a definite trend towards reduced egg mass (g/egg) as the dietary isoleucine level decreased. The egg mass (g/egg) from layers on the third dilution diet exhibited a similar pattern to those of birds on the higher isoleucine diets but in this case egg mass was significantly lower in all periods.

The egg mass (g/egg) from birds on the lowest isoleucine diets followed a somewhat different pattern to the egg production curve (Figure 3.3) during the second and third production periods in that the sharp decline was not observed.



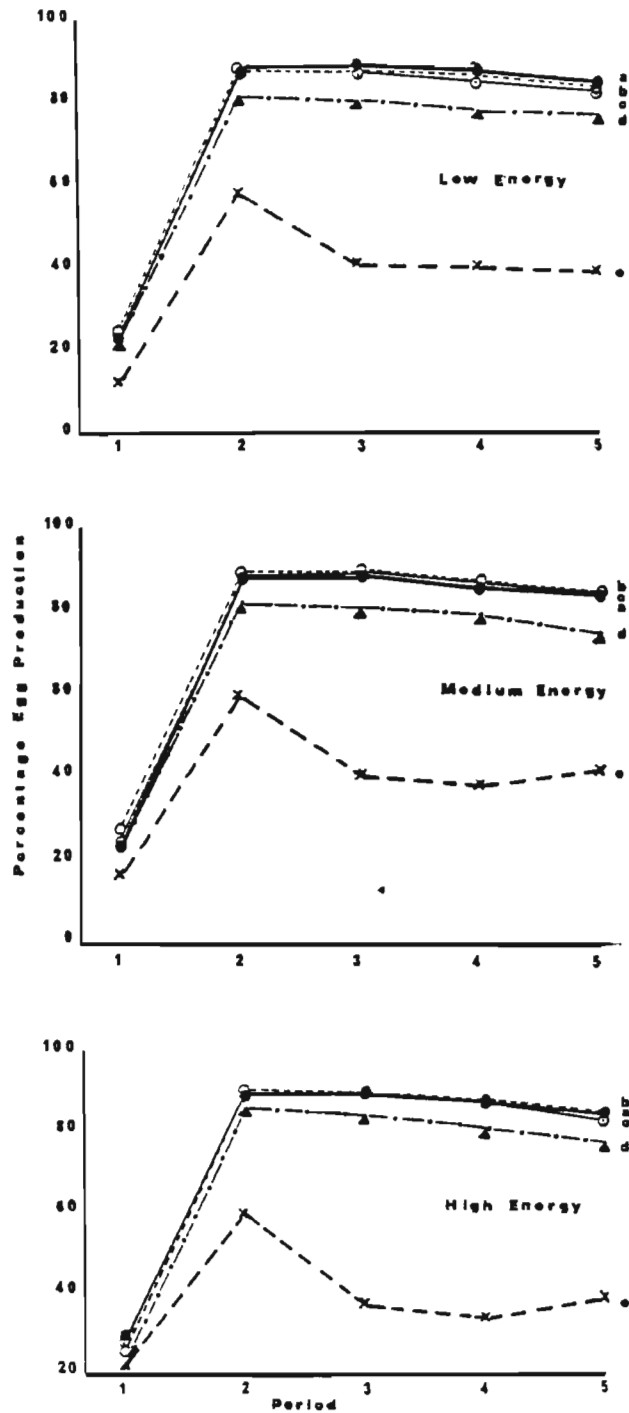


Figure 3.2 The effect of energy and isoleucine/energy ratio on percentage egg production during five 28 d periods. (Refer to Table 3.4 for a description of the diets fed).

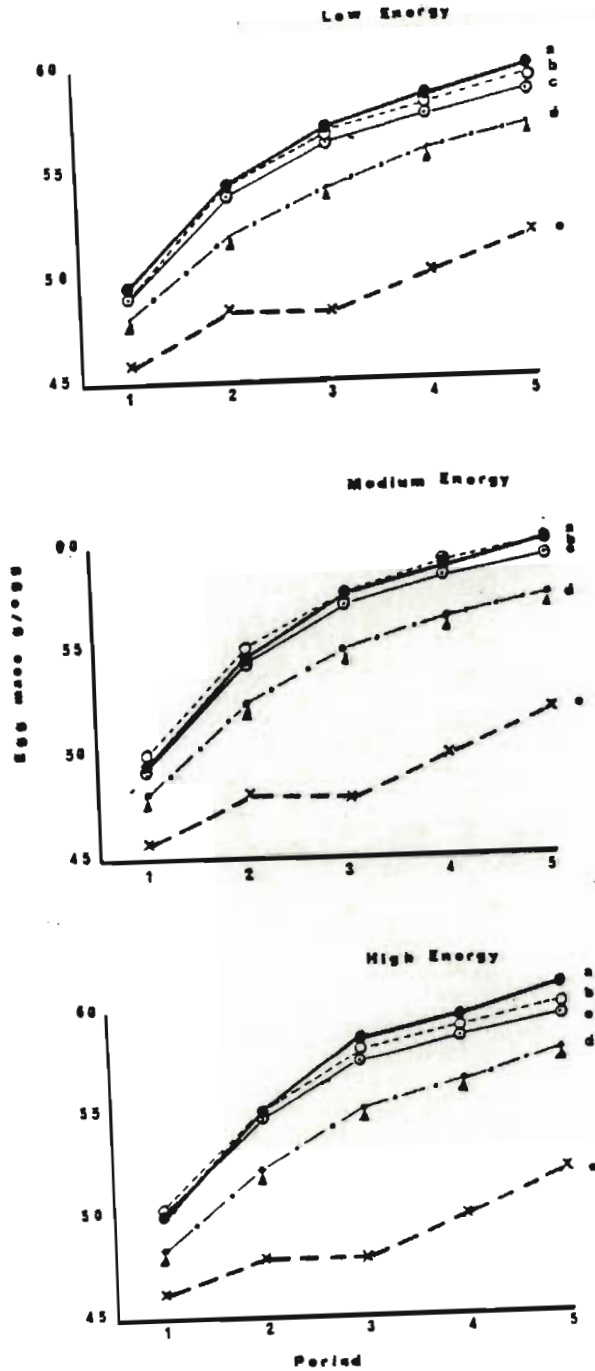


Figure 3.3 The effect of energy and isoleucine/energy ratio on egg mass (g/egg) during five 28 d periods. (Refer to Table 3.4 for a description of the diets fed.)



In this case a flattening out of the curve was evidenced followed by a gradual increase in egg mass until the experiment was ended.

The effect of isoleucine intake on egg mass (g/egg) during periods of peak production (periods 3, 4 and 5) was subjected to an analysis of variance and will be discussed at a later stage. (See egg mass during peak production periods.)

#### *Egg output.*

From Tables 3.8 to 3.12 and Figure 3.4 it will be noted that egg output from birds on the different isoleucine diets followed the same pattern as that of egg production.

Egg output from the summit, first and second dilution diets escalated sharply to approximately 48,5 g/bird d in the second period. Egg output from birds on the third and fourth dilution diets also increased sharply but was significantly lower than that from birds on the three higher dietary isoleucine concentrations (43,5 and 28,8 g/bird d respectively).

During periods two and three the egg output continued to increase marginally whilst a levelling out was observed from birds on the third dilution diet. Birds on diets with the lowest isoleucine concentrations, as opposed to the other diets, showed a marked egg output decline during the second and third periods. Thereafter a slight increase was evident until the termination of the experiment.

The egg output rise in the first two periods found by birds receiving the lowest dietary isoleucine concentrations can only be attributed to the hormonal stimulus for egg production but at the expense of body protein reserves.

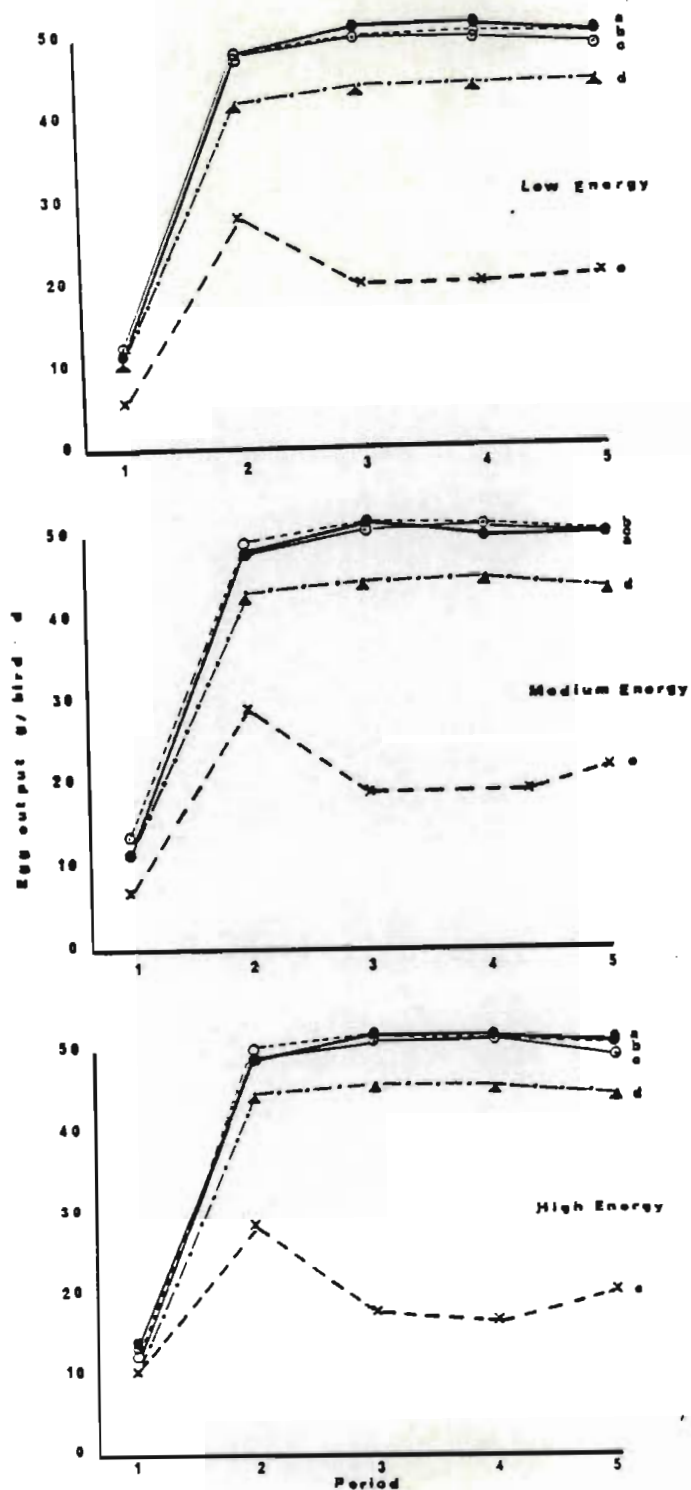


Fig 3.4 The effect of energy and isoleucine/energy ratio on egg output (g/bird d) during five 28 d periods. (Refer to Table 3.4 for a description of the diets fed.)



No further attention will be given to egg output response to isoleucine intake since this will be discussed later in this section (see egg output during peak periods).

#### *Food intake.*

Tables 3.8 to 3.12 and Figure 3.5 indicate a tendency for birds on the second and third dilution diets to have a higher voluntary food intake than birds on the summit and first dilution diets.

An unexpected increase of food intake by birds was observed during the fourth period on the first dilution medium energy diets (2b) which is difficult to explain. It is also difficult to determine the reason why the third dilution medium energy diet (2d) deviated from the expected pattern.

Voluntary food intake of birds on the lowest isoleucine diets showed a marked drop from the first to the third period. At this stage the food intake was 91,3, 85,3 and 73,81 g/bird d on the 10,3, 10,9 and 11,5 MJ ME/kg diets respectively which was significantly lower than that on the other diets. From the third period onward food intake increased gradually but more prominently on the higher energy diets.

All these findings are in agreement with earlier reports indicating that laying pullets receiving diets marginally inadequate in protein or methionine, eat more food than birds receiving adequate diets (Harms et al., 1962; Fisher and Morris, 1967; Harms et al., 1967). Further, in agreement with the findings of Pilbrow and Morris (1974) there are indications of a long term food intake adaptation by pullets receiving the low protein diets (diets 1e, 2e and 3e) resulting in higher intakes..

These workers have indicated that food intake by birds on protein deficient diets eventually exceeds that of birds receiving adequate

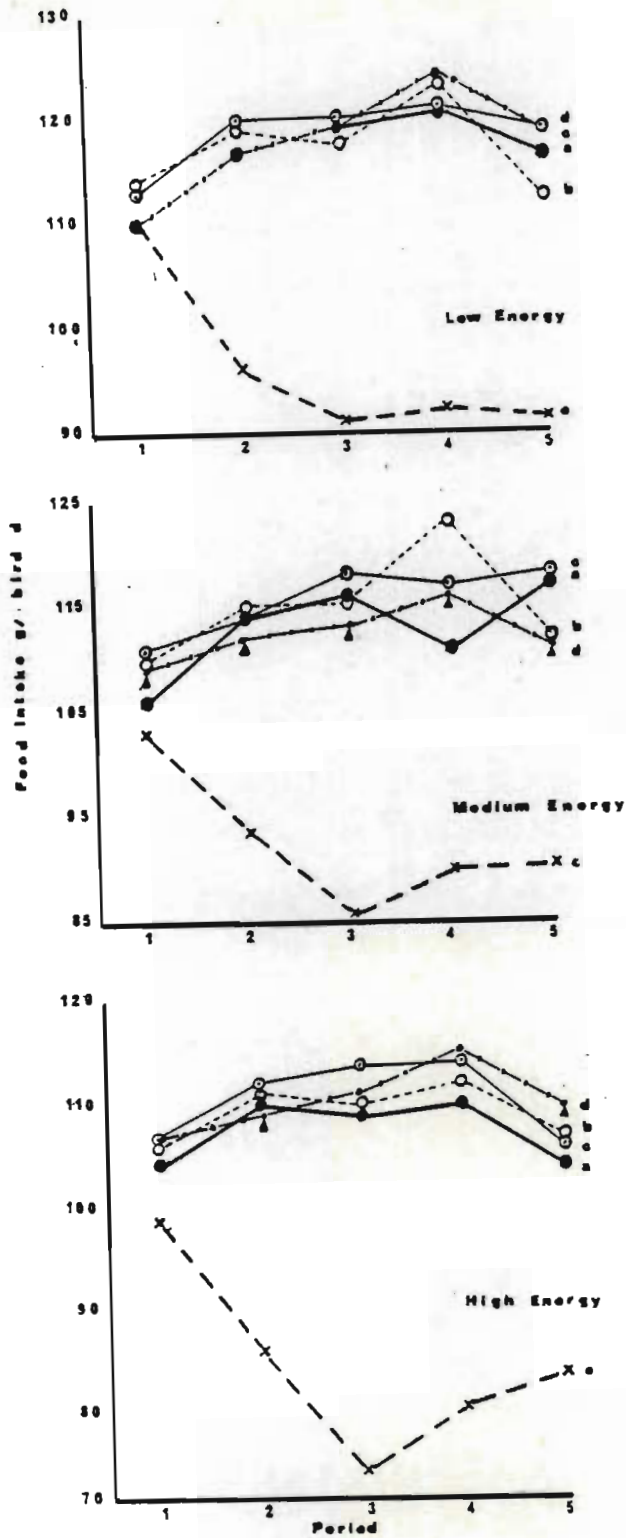


Fig 3.5 The effect of energy and isoleucine/energy ratio on food intake (g/bird d) during five 28 d periods. (Refer to Table 3.4 for a description of the diets fed.)



protein diets. Whether birds receiving the abovementioned isoleucine deficient diets would have adapted sufficiently so as to eventually exceed food intake of birds on adequate diets, is open to question.

*Food conversion efficiency.*

It will be observed from Tables 3.8 to 3.12 and Figure 3.6 that there is little difference in the food conversion efficiency of birds receiving the three diets with the highest isoleucine concentrations. The third and fourth dilution diets however exhibited significantly poorer food conversion efficiencies in all the experimental periods. The reason for this must be found from the lowered egg output observed from birds on these diets in all periods.

As for egg output, the food conversion efficiency of birds on the dilution diets improved during the first two periods followed by a sharp deterioration from period two to period three. Food conversion then gradually improved.

*Body mass gain.*

During the first period birds on all treatments showed a marked daily body mass increment (Tables 3.8 to 3.12 and Figure 3.7). Birds receiving the third and fourth dilution diets however showed a significantly lower daily increment than those fed the three higher dietary isoleucine levels (8,98 and 6,35 g/bird d respectively compared to approximately 11,5 g/bird d on the three higher dietary isoleucine concentrations). It would therefore appear that isoleucine has an important effect on daily body mass gain.

The daily body mass increments in the following periods were considerably lower which would seem to indicate that this is dependent on the body mass achieved at point of lay. Furthermore,

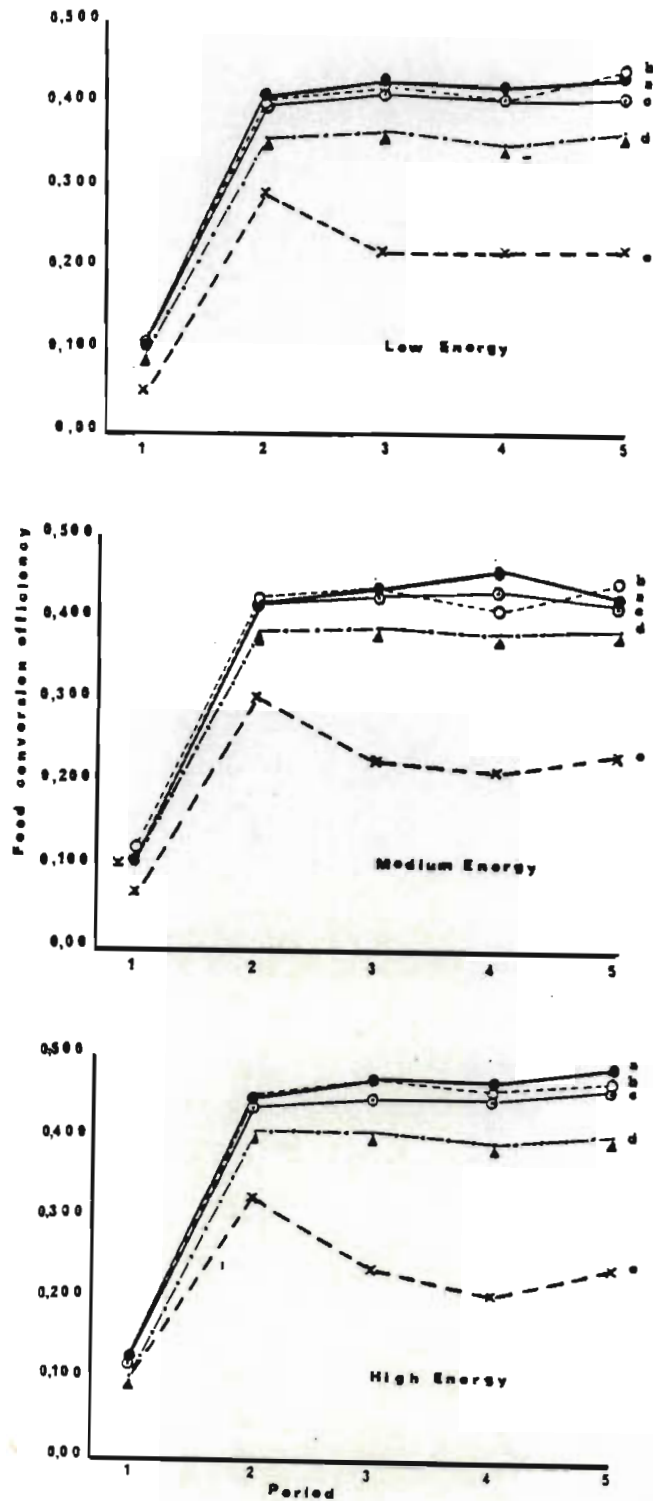


Fig 3.6 The effect of energy and isoleucine/energy ratio on food conversion efficiency (g egg/g food) during five 28 d periods. (Refer to Table 3.4 for a description of the diets fed.)

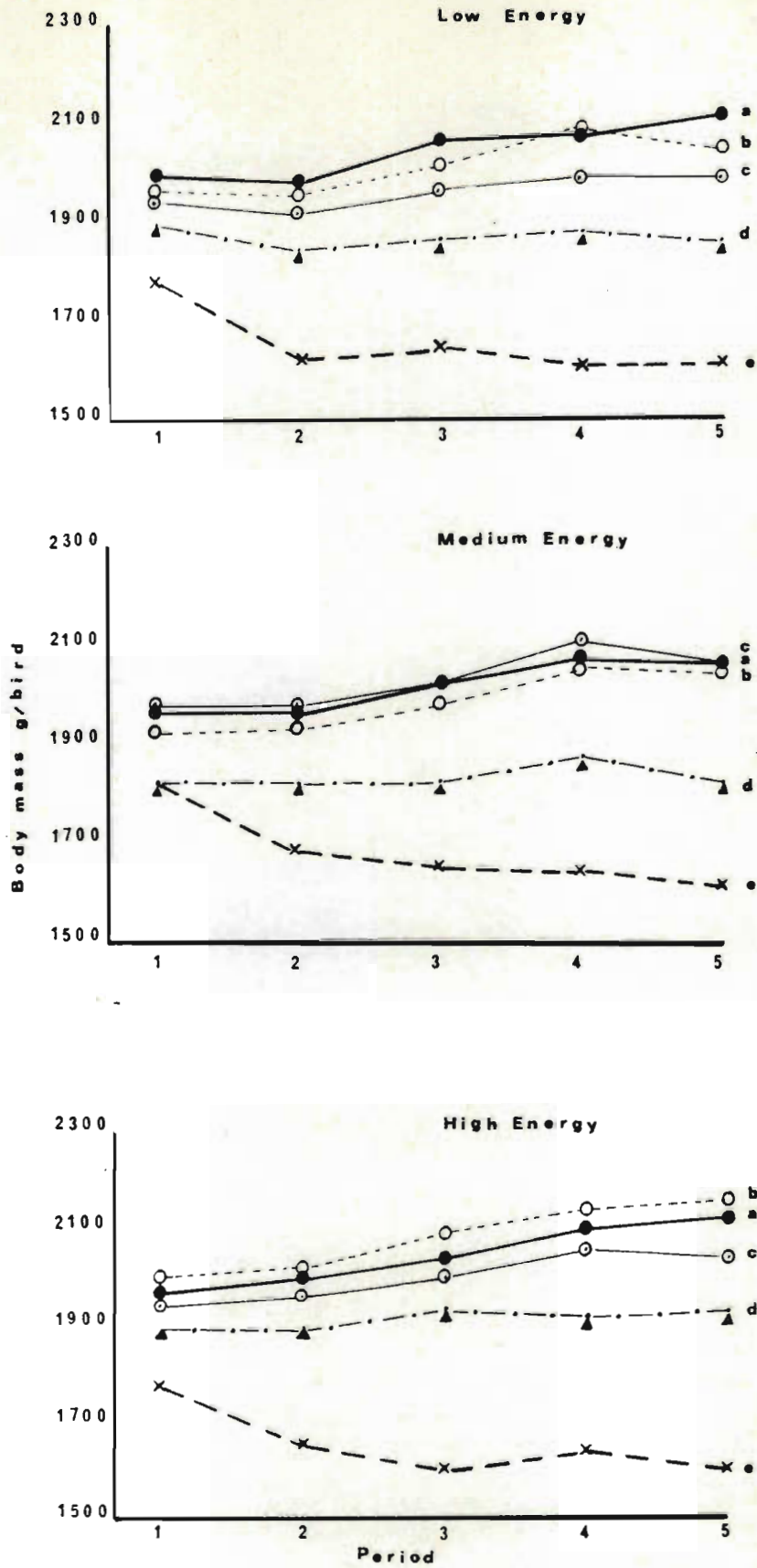


Figure 3.7 The effect of energy and or isoleucine/energy ratio on body mass (g/bird) during five 28 d periods. (Refer to Table 3.4 for a description of the diets fed.)



all treatments showed a low egg output in the first period which would suggest that most of the growth that takes place is prior to the time each pullet lays her first egg.

Birds on the dilution diets showed a negative daily gain during the second period. This was the only treatment showing a significant difference, and it continued into the third period.

RESULTS DURING PERIOD OF MAXIMUM PRODUCTION (periods 3, 4 and 5 29 - 41 weeks of age).

At the time of peak egg production, individual egg outputs are normally distributed, but later in lay, pausing, moulting and morbidity result in considerable skewness and an increase in non-laying birds. In fact, Wethli and Morris (1978) concluded that the best available method of estimating the amino acid requirement of an older flock would seem to be to calculate its requirement at peak output, and to assume that the requirement, expressed as a daily intake, does not change for the remainder of the pullet laying year. Similarly, different responses may be observed if earlier periods are used, because young pullets on low protein diets may still sacrifice body protein at this stage.

From Figure 3.4 it will be observed that maximum egg output was obtained between 29 and 41 weeks of age (periods 3, 4 and 5). Since response to dietary amino acid intake is normally distributed during peak production periods and since the response in the three periods was very similar, it was decided to combine the data in order to accurately assess the effect of isoleucine intake on the production parameters rate of lay (eggs/100 bird d), egg mass (g/egg), egg output (g/bird d), food intake (g/bird d), food conversion efficiency (g egg/g food) and mass gain (g/bird d).

The combined results of periods 3, 4 and 5 are shown in Table 3.13. The main effects of isoleucine/energy ratio (I) and of energy (E) are shown together with the interaction terms (I x E).

TABLE 3.13: Response to Isoleucine and Energy intake during combined periods 3, 4 and 5.  
(29 - 41 weeks of age)

3.13.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	86,55	85,69	85,29	78,05	40,39	75,20
2	86,64	86,39	87,12	78,54	41,25	75,99
3	86,75	87,03	86,90	80,63	36,86	75,63
Mean	86,65	86,37	86,44	79,07	39,50	75,61
	SEM		LSD (0,05)		LSD (0,01)	
I	0,50		1,40		1,90	
E	0,40		1,10		1,50	
I x E	0,90		2,50		3,30	

3.13.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	58,37	58,05	57,19	55,85	50,08	55,91
2	58,72	58,99	58,10	56,22	49,24	56,26
3	59,77	59,14	58,47	56,25	49,75	56,68
Mean	58,95	58,72	57,92	56,11	49,69	56,28
	SEM		LSD (0,05)		LSD (0,01)	
I	0,245		0,68		0,90	
E	0,191		0,53		0,69	
I x E	0,426		1,18		1,55	

3.13.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	50,49	49,73	48,74	43,60	20,22	42,55
2	50,86	50,95	50,60	44,14	20,29	43,37
3	51,84	51,44	50,79	45,30	18,36	43,55
Mean	51,07	50,71	50,04	44,34	19,62	43,16
	SEM		LSD (0,05)		LSD (0,01)	
I	0,242		0,67		0,88	
E	0,188		0,52		0,68	
I x E	0,418		1,16		1,52	



TABLE 3.13 (Continued)

3.13.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	118,55	117,59	119,94	120,64	91,91	113,73
2	114,67	116,98	117,62	113,61	88,61	110,30
3	108,08	110,02	111,43	112,53	79,65	104,34
Mean	113,77	114,86	116,33	115,60	86,72	109,46
	SEM		LSD (0,05)		LSD (0,01)	
I	0,794		2,20		2,89	
E	0,613		1,70		2,24	
I x E	1,37		3,81		5,01	

3.13.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	0,426	0,424	0,406	0,362	0,220	0,368
2	0,444	0,437	0,431	0,389	0,229	0,386
3	0,480	0,468	0,456	0,403	0,231	0,408
Mean	0,450	0,443	0,431	0,384	0,227	0,387
	SEM		LSD (0,05)		LSD (0,01)	
I	0,0025		0,007		0,010	
E	0,0022		0,006		0,008	
I x E	0,0047		0,013		0,017	

3.13.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	3rd Dilution (d)	Dilution Diet (e)	Mean
1	1,49	1,13	0,74	0,10	- 0,15	0,66
2	1,12	1,33	1,07	2,21	- 0,78	0,99
3	1,39	1,65	0,94	0,46	- 0,64	0,76
Mean	1,33	1,37	0,91	0,92	- 0,52	0,80
	SEM		LSD (0,05)		LSD (0,01)	
I	0,465		1,29		1,70	
E	0,361		1,00		1,31	
I x E	0,804		2,23		2,94	



Also the standard errors of each mean (SEM) and the least significant differences (LSD) at  $P < 0,05$  and  $P < 0,01$  are indicated.

These same results are also illustrated in Figures 3.8 to 3.12. where isoleucine intake has been plotted against some of the production parameters mentioned above.

The effects of dietary isoleucine only will be considered in the present discussion as the effects of energy on the production parameters measured will be discussed in Chapter 6.

#### *Egg production.*

Table 3.13.1 shows that the mean egg production rates from birds on diets of descending isoleucine content were 86,65, 86,37, 86,44, 79,07 and 39,5 percent. The mean isoleucine intake of birds in the same dietary sequence were 954, 814, 673, 518 and 276 mg/bird d respectively.

There was no significant difference in rate of lay between the first three dietary isoleucine concentrations. However, the mean production rates of 79,07 and 39,5 percent on the respective isoleucine intakes of 518 and 276 mg/bird d was very much poorer than those on the three diets of higher isoleucine concentration, ( $P < 0,01$ ).

The linear regression equation fitted to data where intake of isoleucine was regarded as adequate (in excess of 650 mg/bird d) is shown in Figure 3.8 together with observations indicating the response of isoleucine intake on percentage egg production. This equation suggests that maximum egg production is achieved at an intake of 650 mg isoleucine/bird d, as the positive slope ( $b = 0,00137$ ) is not significant.

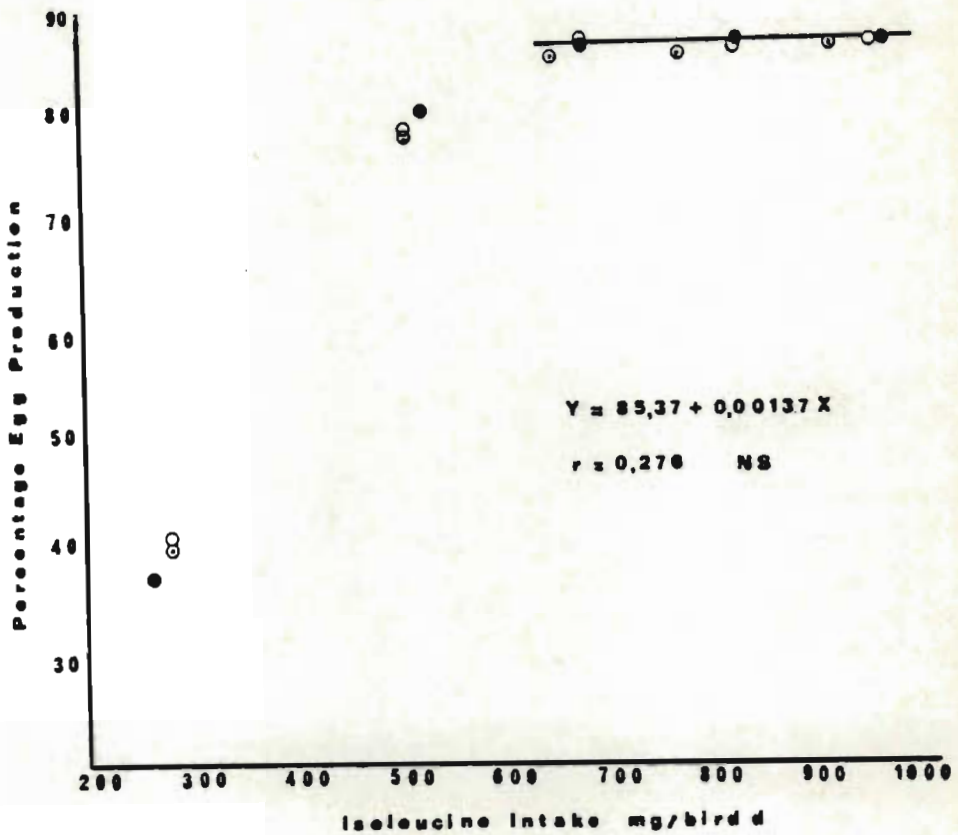


Figure 3.8 The relationship between percentage egg production and isoleucine intake. Linear regression equation calculated from data where intake of isoleucine was 650 mg or more.



### *Egg mass.*

The egg mass (g/egg) data from the combined periods was subjected to a factorial analysis. From Table 3.13.2 it is evident that the mean egg mass numerically declined as the isoleucine content of the diets decreased. The mean egg mass (g/egg) was 58,95, 58,72, 57,92, 56,11 and 49,69 on respective isoleucine intakes of 954, 814, 673, 518 and 276 mg/bird d. The lowered mean egg mass from progressively declining isoleucine intakes was significantly different in all treatments except those from the summit and first dilution diets.

Bray (1969) also reported a lowered egg mass with decreased isoleucine intakes. At intakes of 606 and 284 mg isoleucine/bird d he found egg masses of 55,1 and 52,6 grams respectively. This egg mass decrease is substantially less than that found in this study.

Using the combined data of periods three, four and five a regression equation relating isoleucine intake (X) to egg mass (g/egg) (Y) was computed, which is:

$$Y = 39,116 + 0,0460X - 0,0000265X^2$$

with a correlation of 0,922.

This curvilinear regression is presented in Figure 3.9. It would appear from this graph that maximum egg mass is reached on an isoleucine intake of approximately 870 mg/bird d. From this it would appear that egg mass (g/egg) is more sensitive to isoleucine intake than rate of production.

### *Egg output.*

The Reading model (Fisher et al., 1973) was used to analyse this data.



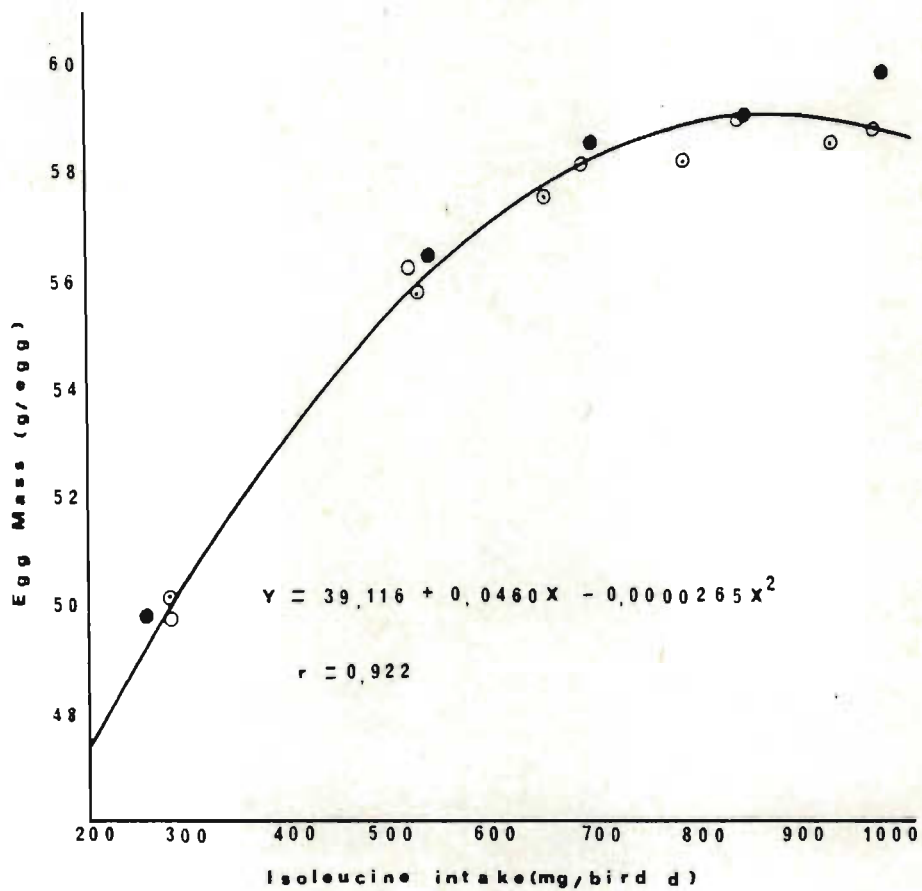


Figure 3.9 Relationship between egg mass (g/egg) and isoleucine intake.

Essentially, this model assumes that for the individual hen, daily amino acid requirements bear a simple linear relationship to egg output and body mass. Using isoleucine (isoleucine, mg/d) as an example we can write:

$$I = \underline{a}E + \underline{b}W + \underline{c}\Delta W$$

where  $I$  = daily isoleucine intake (mg/bird d)

$E$  = daily egg output (g/bird d)

$W$  = mean body mass (kg/bird)

$\Delta W$  = daily change in body mass (g/bird d)

The population response curve is obtained by averaging the individual responses taking into account variations in maintenance requirements (s.d.W) and variations in the maximum potential level of egg production (s.d.EMax).

This response curve is defined by seven parameters, namely - the average egg production,  $E_{max}$ ; variation in potential egg production (defined by s.d.E); average body mass,  $W$ ; variation in body mass (defined by s.d.W); the correlation between  $E$  and  $W$  and two constant representing the amount of amino acid required per unit of egg production ( $\underline{a}$  mg/day) and per unit body mass ( $\underline{b}$  mg/day).

To obtain estimates of optimum isoleucine intake from fitted response curves Fisher et al., (1976) used the following equation:

$$Y = \underline{a}\bar{E}_{max} + \underline{b}\bar{W} + X(\sqrt{\underline{a}^2 s.d.^2 E + \underline{b}^2 s.d.^2 W - 2\underline{a}\underline{b} s.d.E s.d.W})$$

where  $X$  = the deviation from the mean of a normal distribution which is exceeded with probability  $\underline{a}k$  in one tail.

$$k = \frac{(\text{cost per 1 mg isoleucine input})}{(\text{marginal value of 1 g egg output})}$$

The value of  $X$  in standard units is obtained from tables and multiplied by the standard deviation of an individual's amino acid requirement.

If  $rEW$  is zero this simplifies to

$$(\sqrt{\underline{a}^2 s.d.^2 E + \underline{b}^2 s.d.^2 W})$$

From this equation the amount of amino acid which is worth providing above the average requirement can be calculated, and depends on the coefficients  $\underline{a}$  and  $\underline{b}$ , on flock variability and the cost of the input and the value of the output. The more variable the flock and the smaller the cost relative to the value of the product, the higher is the amino acid intake which maximises profit. It is of course assumed that the individual bird's requirements are normally distributed about the mean, which requires the distributions of  $E$  and  $W$  also to be normal.

In this trial the body mass changes of all birds were very small during the combined periods and so  $\Delta W$  was ignored, and thus the coefficient for mass gain  $\underline{c}$  was eliminated from the calculations. The parameters of the resulting response curve (Figure 3.10) are:

$$\underline{a} = 9,076 \text{ mg isoleucine/g egg}$$

$$\underline{b} = 48,747 \text{ mg isoleucine/kg Wd}$$

$$\bar{E}_{\max} = 50,783 \text{ g egg/hen d}$$

$$W = 2,0 \text{ kg}$$

$$s.d.E = 10,0 \text{ g/hen d}$$

$$s.d.W = 0,2 \text{ kg}$$

$$rEW = 0,0$$

$$\text{residual sum of squares} = 4,6137 \text{ (11 d.f.)}$$

$$\text{residual s.d.} = 0,65 \text{ g egg.}$$

From Figure 3.10 it will be observed that essentially equal response increments occurred for equal increments of isoleucine input when isoleucine is inadequate. Diminishing response increments however are evident when isoleucine adequacy is approached.



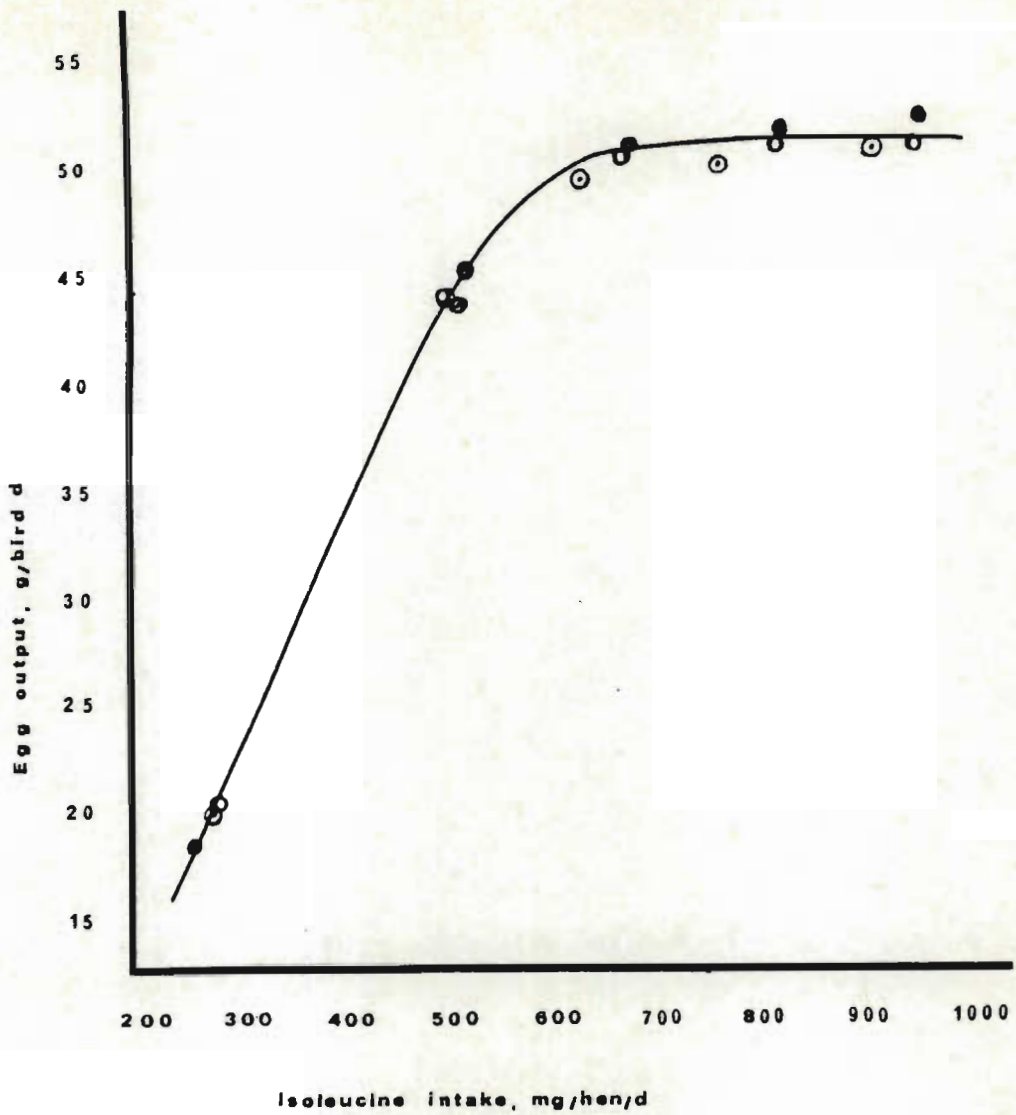


Figure 3.10 The relationship between egg output and isoleucine intake. The curve was fitted by the method of Curnow (1973) using the parameters described in the text.

From these results it can be concluded that the isoleucine requirement of an individual pullet in the early stages of lay is adequately represented (neglecting change in body mass) by the equation:

$$\text{Isoleucine req.} = 9,076 \text{ Emax} + 48,747 \text{ W.}$$

This equation should not be used directly by substituting average values of Emax and W to predict the "requirement" of a flock, since it makes no allowance for the variations in yield and body mass which exist within the flock and the consequent curvilinear responses to be expected when diets of differing isoleucine concentration are fed to different groups of birds. Once curvilinear responses are assumed a requirement can only usefully be defined in economic terms.

#### *Optimum Isoleucine intake.*

To obtain estimates of optimum isoleucine intake from fitted response curves, as indicated, it is necessary to know the marginal cost of isoleucine and the value of eggs.

Eggs were assigned a price of 60 cents/kg in this study. The cost of supplying isoleucine was estimated by formulating a series of least-cost diets for layers, using current (1980) South African ingredient prices, with isoleucine contents increasing with 0,5 g/kg increments from 6,5 g/kg to 9,5 g/kg. A linear relationship was obtained between price/kg and isoleucine content, the cost of the diet being increased by 1 cent/kg for each 1 g isoleucine/kg added to the specification. Thus under these conditions the cost of supplying extra isoleucine in the form of protein was R10,00/kg isoleucine. In 1980 the price of free L-isoleucine was about R160,00/kg.

Using the price of R10,00/kg, the optimum intakes of isoleucine were calculated from the abovementioned equation.

With the present cost ratio of  $k = 0,017$ , the optimum isoleucine intake was found to be 651 mg/bird d.

Because the cost ratio  $k$  will no doubt change in future, calculated optimum isoleucine allowances for various price structures are presented in Table 3.14.

TALBE 3.14: Optimum isoleucine intakes (mg/hen d) for flocks of young laying pullets (2kg body mass and egg output of 50 g/bird d) as affected by the marginal cost of isoleucine and the value of egg output.

Marginal value of egg output (cents/kg)	Marginal cost of Isoleucine (R/kg)		
	10-00	12-50	15-00
	Requirements, mg/day		
60	651	638	626
70	661	648	637
80	668	656	644
90	674	662	652
100	679	668	658
110	684	673	663

Evans et al., (1949) found a variation of 6,8 to 7.1 g isoleucine per 16 g nitrogen in the egg. Assuming that eggs contain 11,25 percent protein (Fisher, 1976) and using a mean value of 7,0 g isoleucine per 16 g nitrogen the calculated amount of isoleucine per gram of egg is 7,9 mg.

The coefficients for egg output ( $\underline{a}$ ) and body mass maintenance ( $\underline{b}$ ) in the equation presented by the author earlier is 9,076 mg/g and 48,75 mg/kg respectively. This indicates a net efficiency of isoleucine utilisation for egg production of 87 percent.

Direct estimates of maintenance requirements in adult roosters range from 72 to 40 mg isoleucine per kg W (Leveille and Fisher, 1960; Kandatsu and Ishibashi, 1966). The maintenance requirement of 48,75 mg isoleucine per kg body mass found in this study is therefore in close agreement with the recommendation of Kandatsu and Ishibashi (1966) of 40 mg/kg body mass per day.



The optimum requirement of 651 mg isoleucine/bird d found in this study is slightly higher than the 605 mg total isoleucine (550 mg available isoleucine) recommended by the A.R.C. (1975), higher than that of 550 mg total isoleucine advocated by the N.R.C., (1977), and higher than the 535 mg/bird d calculated by Moran et al., (1967) for layers producing 46,5 g egg mass per day.

Bray (1969) found that maximum egg output could be obtained when the diet supplied 472 mg of isoleucine per day. He further speculated that this low requirement compared to that found by Miller (1954) and Johnson and Fisher (1958), may have been due to the relatively low level of leucine in the basal diet he used.

As shown in Chapter 1 there are many reasons why different amino acid requirement levels are reported variously by researchers. It would however appear that the lower requirement reported by Moran et al., (1967) and Bray, (1969) can be partly explained by higher rates of production found in this study.

Bray's findings of 472 mg/bird d to produce a daily egg mass of 45 g must not however be ascribed solely to low egg output but also possibly to the statistical analyses or technique employed.

Fisher and Morris, (1970) indicated that the method of fitting two straight lines as used by Bray will always underestimate the requirement for maximum output if the true response line is a continuous curve.

Bray's technique does not show a zone of diminishing returns; responses ceasing abruptly at 45 g egg output per day. According to Morris et al., (1978) this "bent stick" response is typical of an experiment in which free amino acid supplementation of a low protein basal diet is used and probably indicates that the amino acid under test ceases to be the limiting factor in the diet at the point of inflection.

Should the 50,78 g egg output from a 2 kg layer with a daily mass gain of 1,25 g, as found in this study, be used in the "B model" equations of Hurwitz and Bornstein, (1973) and Wilgus (1976), the calculated isoleucine requirements would be 695 mg and 716 mg/bird d respectively.

These requirements are somewhat higher than that found in this study. Nevertheless it would appear that the equations provide a reasonable isoleucine requirement estimate for a layer.

When the same production data used above are fitted to the "model 2" equation of Smith (1978), the isoleucine requirement is 590 mg/bird d. Thus, this model provides an underestimate of isoleucine requirement.

Scott, et al., (1976) recommend 850 mg isoleucine/bird d. This is in close agreement with the optimum level of 886 mg isoleucine input found by Morris (1980). Due to the anomalous response to supplementation in some diets the latter author however was a little doubtful as to whether the responses obtained from increased isoleucine inputs were entirely due to this amino acid.

The difference in recommended isoleucine requirements of 650 mg isoleucine per hen day in this study and the 850 mg per hen day advocated by Scott, et al. (1976) could conceivably be attributed to different isoleucine values assigned to feedstuffs. This in turn could be attributed to hydrolysis time, use of different hydrolytic agents or equipment used.

Very often nutritionists use raw material matrix values from one source and yet use requirement standards from another source. This study clearly illustrates the pitfall of following this procedure. It is of interest to note that the identical diet providing 650 mg isoleucine per hen per day on the author's raw material matrix will provide a theoretical intake of 920 mg/hen/day when using the Scott, Nesheim and Young (1976) ingredient



composition.

One of the most interesting findings in the present study is the fact that the utilisation of isoleucine for egg output is unaffected by the dietary energy concentration. Amino acid requirements expressed as a percentage will differ because of variations in food intake due to such factors as energy concentration. However, when the response is expressed in terms of actual isoleucine intake, the utilisation of this amino acid is the same on the three series of dietary energy concentrations.

#### *Food intake.*

From Table 3.13.4 it is evident that the mean food intake of birds on the lowest dilution diet was significantly lower than that of birds on the diets with higher dietary isoleucine concentrations. Intakes of birds receiving the other diets were fairly similar.

Three separate graphs were fitted relating food intake to isoleucine intake due to the fact that energy concentration had a significant effect on food intake independent of the isoleucine effect. The highest intake corresponded to the sub-optimal isoleucine intake of 514 mg/bird d. (Figure 3.11.)

#### *Food conversion efficiency.*

The food conversion efficiencies during the periods of peak production were 0,4502, 0,4429, 0,4312, 0,3843 and 0,2265 (Table 3.13.5) on respective isoleucine intakes of 954, 814, 673, 518 and 276 mg/bird d.

From the above it is clear that as the isoleucine intake decreased the food conversion efficiency deteriorated progressively. This difference was significant between each treatment.



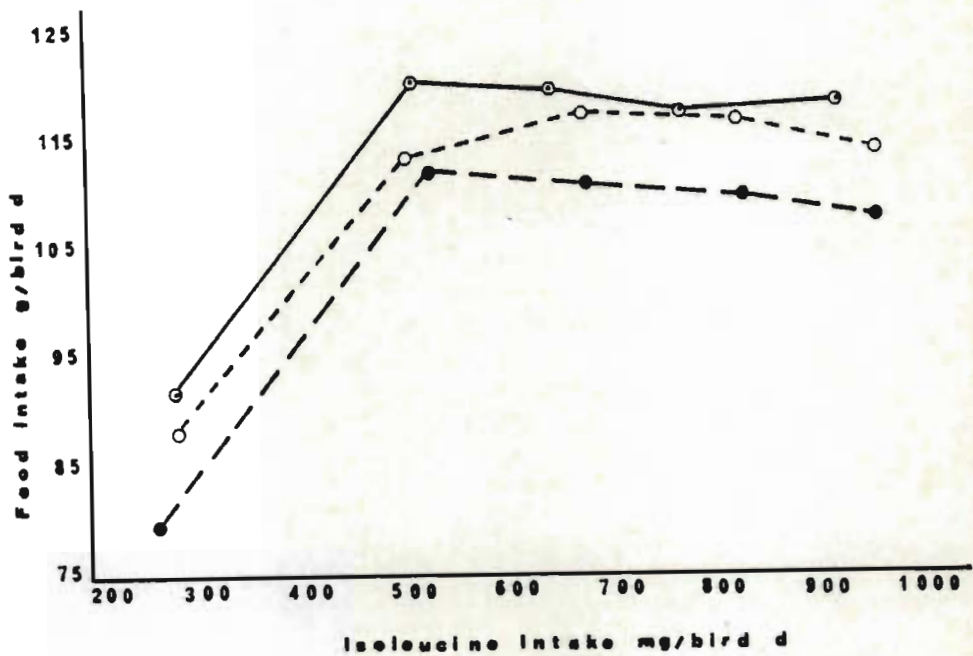


Figure 3.11 The relationship between food intake (g/bird d) and isoleucine intake (mg/bird d) when fed high energy diets (●—●), medium energy diets (○-----○) and low energy diets (○—○).

From this it can be deduced that isoleucine intake during peak production periods has a profound effect on food conversion efficiency.

Three regression equations were computed relating food conversion efficiency (Y) to isoleucine intake (X) due to the fact that energy concentration had a profound effect on food conversion efficiency independent of the isoleucine effect. The three equations are as follows:

Low energy (10,3 MJ ME/kg).

$$Y = -0,056 + 0,00119X - 0,00000072X^2$$

$$r = 0,973.$$

Medium energy (10,9 MJ ME/kg)

$$Y = -0,057 + 0,00124X - 0,00000076X^2$$

$$r = 0,972.$$

High energy (11,5 MJ ME/kg)

$$Y = -0,019 + 0,00113X - 0,00000065X^2$$

$$r = 0,978.$$

From these equations it was calculated that optimum food conversion efficiency is obtained from an isoleucine intake in excess of 815 mg/bird d. This is substantially higher than the requirement for optimum egg output.

#### *Body mass gain.*

Birds on the dilution diets had significantly lower daily gains than those on diets containing higher isoleucine levels (Table 3.13.6). Birds receiving the summit and first dilution diets gained more than those on the second and third dilution diets but these differences were not statistically significant.

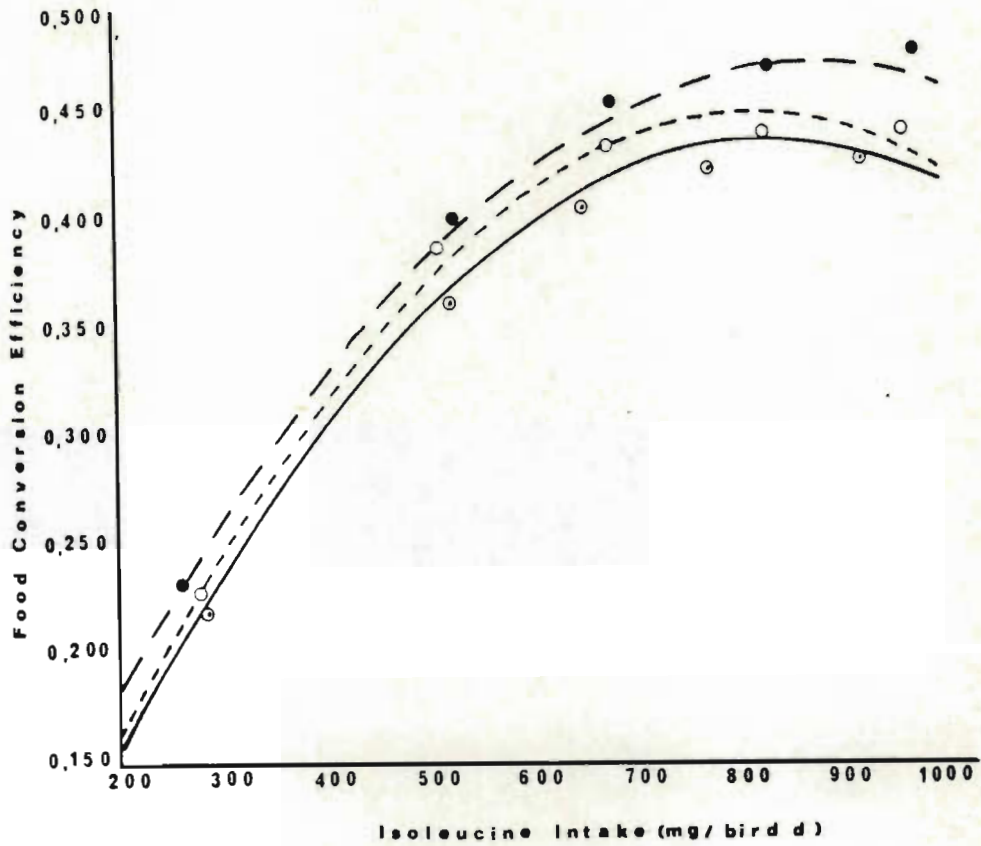


Figure 3.12 The relationship between food conversion efficiency and isoleucine intake when hens were fed the high energy diets (●—●), medium energy diets (○----○) and low energy diets (○—○).



## SUMMARY.

1. A total of 3 600 laying hens were used in this study designed to determine the response of laying hens to increasing concentrations of isoleucine.
2. Using a dilution technique, five isoleucine levels at three different energy concentrations were each fed to four replicates of 60 birds.
3. The isoleucine requirement of an individual pullet is represented (ignoring change in body mass) by the equation
$$\text{Isoleucine req.} = 9,076 \text{ Emax} + 48,747$$
4. Relating marginal cost of eggs and isoleucine it was determined that optimal egg output was achieved with an isoleucine intake of 651 mg/bird d.
5. A table is presented from which optimal response to isoleucine intake can be determined (2 kg layers producing 50 g egg mass/bird d) should the value of eggs or marginal cost of isoleucine change.
6. Maximum egg mass was achieved with an isoleucine intake of 870 mg/bird d.
7. Three separate graphs were fitted relating food intake to isoleucine intake due to the fact that energy concentration had a significant effect on food intake independent of isoleucine effect. The highest food intake corresponded to the sub-optimal isoleucine intake of 514 mg/bird d. It was concluded that with marginally sub-optimal levels of isoleucine in the diet food intake increased in an attempt to sustain production.
8. Three regression equations were computed relating food conversion efficiency to isoleucine intake due to the fact that energy had

a profound effect on this parameter independent of the isoleucine effect. From these equations it was calculated that optimal food conversion efficiency is obtained with an isoleucine intake in excess of 815 mg/bird d. This is substantially higher than the requirement for optimal egg output.

9. Body mass gain of birds on inadequate isoleucine diets were significantly poorer than that of birds receiving diets with adequate isoleucine levels.
10. The utilisation of isoleucine was not affected by dietary energy concentration.



## CHAPTER 4

### RESPONSE OF LAYING HENS TO METHIONINE INTAKE

#### INTRODUCTION

Methionine is one of the most limiting amino acids in practical diets fed to laying hens and following the first study of Ingram et al. (1951) several laboratories have investigated the quantitative requirement of hens for this amino acid as well as for total sulphur-containing amino acids. There are variations in recommended requirements and Table 4.1 lists some of the wide range of values suggested as to the amount of methionine required to support the most efficient production.

The wide range of requirements is indicative of the different methods used to determine these requirements, the different raw material matrix values used and the different methods on interpretation of the data.

Fisher and Morris (1970) used a diet dilution technique to determine the response of laying hens to methionine intake. This was the first time that such a technique had been reported in the literature, and has resulted in a new approach to laying hen nutrition. The experiment reported in this Chapter is an extension of their work, the aim being to confirm that the response in egg output is primarily a function of intake of the most-limiting nutrient (in this case methionine) and is not affected by changes in food intake brought about by different energy concentrations.



TABLE 4.1: Summary of experimental evidence about the methionine requirement of the laying hen and the recommendations of the Agricultural Research Council (ARC, 1975) and National Research Council (NRC, 1971). In addition to the calculated requirements of Johnson and Fisher (1958) and Moran, Summers and Pepper (1967) shown here, calculated requirements based on models suggested by Hurwitz and Bornstein (1973), Wilgus (1976) and Smith (1978) have also been given.

Reference	No and range of dietary contents used. No/g/kg.	Requirement g/kg	Intake at requirement. (mg/day)	Egg production. g/day or %	Notes
Ingram et al (1951)	4/2,2 to 7,0	3,80	-	63 %	Used young pullets. Microbiological assay methionine.
Leong & McGinnis (1952)	4/1,8	2,80	-	76 %	Used young pullets. Microbiological assay methionine.
Ingram & Little (1958)	-	2,50	-	-	Age not stipulated. Abstract only.
Johnson & Fisher (1958)	-	2,40	-	-	Calculated - egg - ratio method. Used purified diets.
Spandorf cited Combs (1962)	5/1,7 to 2,8	2,50	257	41,5 g	Does not stipulate total or available methionine.
Harms & Waldroup (1963)	2/2,6 to 3,50	3,50	333	38,5 g	Used young pullets. On calculation appears to be total methionine.
Combs (1964)	7/1,8 to 3,6	3,04	337	46,5 g	Young selected pullets. Total methionine.
Bray (1965)	8/1,6 to 3,3	2,20	224	40,6 g	Used young pullets. Appears to be available methionine.
Harms et al (1967)	5/2,1 to 3,5	3,10	308	40 - 43 g	Two breeds. Young pullets. Available methionine(Maddy,1963)
Moran, Summers & Pepper (1967)	-	-	312	46,5 g	Calculated requirement.
Moran (1969)	5/2,0 to 3,2	2,90	363	46,3 g	Age of birds not stipulated. On calculation appears to be total methionine.
Harms & Damron (1969)	5/1,9 to 3,5	2,70	257	42,6 g	Used young pullets. Available methionine(Maddy 1963) matrix
Harms et al (1969)	5/1,9 to 3,5	3,10	290 - 295	42 - 43 g	Used young pullets. Available methionine(Maddy 1963) matrix
Fisher & Morris (1970)	5/1,9 to 3,5	2,70	275	40 g	Used young pullets. Available methionine.
Roberson (1970)	2/2,4 to 2,9	2,4	269	46,5 g	Corn-cottonseed meal diets.

/Continued .....

TABLE 4.1 (Continued)

Reference	No and range of dietary contents used. No/g/kg.	Requirement g/kg	Intake at requirement. (mg/day)	Egg production. g/day or %	Notes
Damron & Harms (1973)	5/1,9 to 3,5	3,10	288	39,3 g	Corn-soya diets. Appears to be total methionine.
Reid & Weber (1973)	3/2,4 to 3,3	2,90	260	41,4 g	Corn-soya diets. Young pullets. Total methionine.
Hurwitz & Bornstein (1973)	-	-	433	50 g	(Calculated on Model B. Birds with 2 kg body mass. Gaining 1,5 g/day).
Hurwitz & Bornstein (1973)	-	-	405	45 g	(Calculated on Model B. Birds with 2kg body mass. Gaining 1,5 g/day. Hurwitz & Bornstein (1973) requirements based on total methionine).
Jensen et al (1974)	-	-	290 - 300	-	After number of experiments authors concluded 290 - 300 mg.
Sell & Johnson (1974)	8/2,1 to 3,1	2,7 & 2,80	289 - 314	44,5 - 44,8g	Used young birds. On calculation appears to be total methionine. Corn-soya diets. Two energy levels.
A R C (1975)	-	3,50	350	50 g	Available methionine. Assuming 110 g feed intake/day 90% availability of A A.
Wilgus (1976)	-	-	337	50 g	(Calculated on modified Model B. Birds with 2 kg body mass gaining 1,5 g/b/d).
Wilgus (1976)	-	-	309	45 g	(Calculated on modified Model B. Birds with 2 kg body mass gaining 1,5 g/b/d. Wilgus (1976) requirements based on total methionine).
Scott, Nesheim & Young (1976)	-	3,04-3,27	340	52,7 - 53,4g	Total methionine. Used two energy diets over 11 week period.
N R C (1977)	-	2,70	300	-	Requirement based on total methionine.
Smith (1978)	-	-	378	50 g	Calculated on Model 2. Birds with 2 kg body mass. Gaining 1,5 g/day.
Smith (1978)	-	-	356	45 g	Calculated on Model 2. Birds with 2 kg body mass. Gaining 1,5 g/day.
Daub et al (1978)	4/2,3 to 3,8	3,3	410	50,73g	On calculation appears to be total methionine.
Schutte & van Weerden (1978)	2,3 to 3,7	3,1 - 3,7	390 - 440	51,1 - 51,5g	Used pullets from point of lay to 78 weeks. Diets based on total methionine. Indications requirement for maximum egg output was less than for maximum F C R.



## MATERIALS AND METHODS.

### *Dietary treatments.*

Using the recommended levels of amino acids for layers at varying energy levels in feeds (Scott et al. 1976) "summit" and "dilution" diets at two energy levels were formulated. The minimum content of all essential amino acids except methionine in the summit diets were set at 175 percent of the suggested requirements. The methionine levels however were fixed at 135 percent of these requirements. In the dilution diets minimum levels of all the essential amino acids except methionine were fixed at 90 percent while methionine was fixed at 60 percent of requirements. In an effort to reduce as far as possible unwanted surpluses of amino acids in the summit diets, the crude protein content of each diet was kept at a minimum. The composition of these four diets is shown in Table 4.2. The specified amino acid contents as set out by the procedure for the summit and dilution diets are shown in Table 4.3. The calculated contents achieved in the formulated diets relative to the suggested requirements are also indicated.

By blending the summit and dilution diets in appropriate proportions as indicated in Table 4.4 twelve experimental diets were produced, consisting of three energy levels viz. 10,49, 11,09 and 11,7 MJ ME/kg and four methionine concentrations at each energy level representing 135, 110, 85 and 60 percent of the methionine requirement.

### *Preliminary trial.*

To confirm that methionine was the first limiting amino acid in the experimental diets, a preliminary trial was conducted prior to the main experiment.

The trial was conducted in a convection house using 504 laying hens



TABLE 4.2: Compositions (g/kg) of the summit and dilution diets.

Ingredients	Diet 1 (Summit diet)	Diet 4 (Dilution diet)	Diet 9 (Summit diet)	Diet 12 (Dilution diet)
Maize (ground)	379,00	436,50	420,00	503,00
Wheat bran	100,00	60,00	-----	-----
Lucerne meal	20,00	40,00	-----	40,00
Full fat Soyabean meal (380)*	123,00	-----	123,00	150,00
Peanut meal (400)*	100,00	108,00	227,00	20,00
Sunflower meal (380)*	135,00	-----	-----	-----
Fish meal (660)*	42,00	-----	50,00	-----
Monocalcium Phosphate	5,00	12,50	5,50	12,00
Starch (maize)	-----	150,00	-----	118,00
Poultry feathermeal (840)*	-----	36,00	-----	-----
Ground Sunflower hulls	-----	57,10	-----	53,70
Maize gluten meal (600)*	-----	-----	63,00	-----
Blood meal (800)*	-----	-----	15,00	3,50
L - Lysine HCl	-----	1,70	-----	0,60
Limestone Powder	90,80	92,00	91,80	93,00
Salt	2,00	3,00	1,50	3,00
Choline chloride	1,00	1,00	1,00	1,00
Vitamin Premix **	1,20	1,20	1,20	1,20
Mineral Premix ***	1,00	1,00	1,00	1,00

Calculated analysis

Metabolisable energy (MJ/kg)	10,49	10,49	11,70	11,70
Crude protein (g N x 6,25/kg)	216,60	130,90	256,50	120,80
Methionine (g/kg)	4,1	1,8	4,5	2,0
Calcium (g/kg)	36,5	36,9	36,7	37,0
Phosphorus (g/kg)	6,3	5,2	5,6	4,7

\* Assumed crude protein (g N x 6,25/kg)

\*\* Vitamin Premix provides per kg of diet: 10 035 I.U. Vit. A, 2 000 I.U. Vit. D3, 12,94 mg Vit. E, 2 mg menadionsodium-bisulfite, 1,0 mg thiamin, 5,16 mg riboflavin, 14,8 mg calcium pantothenate, 43,6 nicotinic acid, 10 micrograms Vit. B12, 0,4 mg folic acid, 300 mg choline.

\*\*\* Mineral Premix provides per kg of diet: 4 mg copper, 40 mg zinc, 2 mg iodine, 80 mg manganese.

TABLE 4.3: Calculated amino acid contents of the summit and dilution diets relative to the suggested requirements of the laying hen.

	Requirement according to Scott, Nes- heim and Young (1976) in (g/kg) for diet with ME con- tent of 10,49 MJ/kg.	Amino acid con- tents expressed as multiples of requirements for diet with ME con- tent of 10,49 MJ/kg.		Requirement according to Scott, Nes- heim and Young (1976) in (g/kg) for diet with ME con- tent of 11,70 MJ/kg.	Amino acid con- tents expressed as multiples of requirements for diet with ME Con- tent of 11,70 MJ/kg.	
		( Diet 1 (summit diet)	Diet 4 (dilution diet)	( Diet 9 (summit diet)	Diet 12 (dilution diet)	)
Arginine	7,10	2,38	1,24	8,00	2,36	0,93
Lysine	6,00	1,75	0,90	6,70	1,79	0,94
Methionine	3,00	1,35	0,60	3,36	1,35	0,60
Cystine	2,30	1,83	1,22	2,60	1,69	0,96
Tryptophan	1,50	1,87	0,87	1,70	1,65	0,88
Histidine	2,90	1,76	0,79	3,20	2,03	0,88
Leucine	11,00	1,54	1,05	12,10	1,98	0,92
Isoleucine*	5,08	1,61	0,81	5,7	1,63	0,79
Phenylalanine	6,60	1,50	0,83	7,40	1,76	0,76
Tyrosine	2,90	2,03	1,34	3,20	2,94	1,38
Threonine	5,20	1,48	0,81	5,80	1,57	0,76
Valine	6,10	1,70	1,00	6,90	1,88	0,83

\* Optimum isoleucine found in Chapter 4 used in preference to Scott, Nesheim and Young (1976) which was considered too high. (Also related to adjusted energy intake suggested by Morris, (1968).

TABLE 4.4: Summary of dilution technique and calculated analysis of the experimental diets.

Diet	Blending ratio				Calculated dietary methionine (g/kg)	Calculated dietary protein (gNx6,25/kg)	Calculated dietary energy MJ/kg
	Diet 1 (summit diet)	Diet 9 (summit diet)	Diet 4 (dilution diet)	Diet 12 (dilution diet)			
1 a	1 000	-	-	-	4,1	216,6	10,49
1 b	666,7	-	333,3	-	3,3	188,0	10,49
1 c	333,3	-	666,7	-	2,3	159,5	10,49
1 d	-	-	1 000	-	1,8	130,9	10,49
2 a	500	500	-	-	4,3	236,6	11,09
2 b	333,35	333,35	166,65	166,65	3,5	199,6	11,09
2 c	166,65	166,65	333,35	333,35	2,7	162,8	11,09
2 d	-	-	500	500	1,9	125,9	11,09
3 a	-	1 000	-	-	4,5	256,5	11,70
3 b	-	666,7	-	333,3	3,7	211,3	11,70
3 c	-	333,3	-	666,7	2,9	166,0	11,70
3 d	-	-	-	1 000	2,0	120,8	11,70



TABLE 4.10: Response to Methionine and Energy intake for period 3. (32 - 35 weeks of age)

4.10.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	90,36	88,18	88,79	57,57	81,22
2	93,56	91,74	88,26	72,47	86,51
3	89,33	92,29	90,77	68,34	85,18
Mean	91,08	90,73	89,27	66,13	84,31
	SEM		LSD (0,05)		LSD (0,01)
M	0,96		2,80		3,81
E	0,83		2,43		3,30
M x E	1,65		4,85		6,60

4.10.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	54,96	54,99	54,72	48,74	53,35
2	54,96	54,89	54,95	51,14	53,98
3	55,21	55,49	55,17	51,69	54,39
Mean	55,05	55,12	54,94	50,53	53,91
	SEM		LSD (0,05)		LSD (0,01)
M	0,26		0,77		1,05
E	0,23		0,67		0,91
M x E	0,46		1,34		1,82

4.10.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	49,66	48,48	48,58	28,06	43,70
2	51,42	50,35	48,53	37,11	46,85
3	49,33	51,20	50,07	35,39	46,50
Mean	50,14	50,02	49,06	33,52	45,68
	SEM		LSD (0,05)		LSD (0,01)
M	0,61		1,80		2,45
E	0,53		1,56		2,11
M x E	1,06		3,12		4,25

TABLE 4.10 (Continued)

4.10.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	121,65	121,77	123,27	101,72	117,10
2	119,52	117,24	122,41	110,37	117,38
3	109,51	105,69	114,56	103,76	108,38
Mean	116,89	114,90	120,08	105,28	114,29
	SEM		LSD (0,05)		LSD (0,01)
M	1,38		4,05		5,50
E	1,20		3,51		4,77
M x E	2,40		7,02		9,50

4.10.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,410	0,400	0,397	0,277	0,371
2	0,433	0,427	0,400	0,337	0,399
3	0,450	0,490	0,437	0,347	0,431
Mean	0,431	0,439	0,411	0,320	0,400
	SEM		LSD (0,05)		LSD (0,01)
M	0,0058		0,017		0,024
E	0,0051		0,015		0,020
M x E	0,010		0,03		0,041

4.10.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,41	0,83	2,49	- 0,02	0,93
2	0,72	1,94	2,27	0,90	1,46
3	1,31	1,58	2,75	1,66	1,82
Mean	0,81	1,45	2,50	0,84	1,40
	SEM		LSD (0,05)		LSD (0,01)
M	0,50		1,47		2,00
E	0,44		1,29		1,75
M x E	0,87		2,55		3,47

TABLE 4.11: Response to Methionine and Energy intake during period 4. (36 - 39 weeks of age)

4.11.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	88,41	85,40	85,60	65,64	81,26
2	90,78	89,54	87,06	77,47	86,21
3	87,70	90,32	89,09	70,42	84,38
Mean	88,96	88,42	87,25	71,18	83,95
	SEM		LSD (0,05)		LSD (0,01)
M	1,33		3,90		5,30
E	1,15		3,37		4,59
M x E	2,30		6,76		9,19

4.11.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	56,15	56,03	56,30	51,0	54,87
2	56,43	56,65	57,11	53,85	56,01
3	56,73	56,27	56,51	53,78	55,82
Mean	56,44	56,32	56,64	52,87	55,57
	SEM		LSD (0,05)		LSD (0,01)
M	0,28		0,83		1,13
E	0,25		0,72		0,98
M x E	0,49		1,44		1,96

4.11.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	49,63	47,84	48,19	33,57	44,81
2	51,22	50,71	49,72	41,73	48,35
3	49,78	50,81	50,35	37,89	47,21
Mean	50,21	49,79	49,42	37,73	46,79
	SEM		LSD (0,05)		LSD (0,01)
M	0,81		2,38		3,24
E	0,70		2,06		2,80
M x E	1,41		4,13		5,61



TABLE 4.11 (Continued)

4.11.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	122,21	119,88	126,51	117,69	121,57
2	121,66	120,19	120,78	122,42	121,26
3	115,23	119,62	120,47	113,19	117,12
Mean	119,70	119,90	122,58	117,77	119,99
	SEM		LSD (0,05)		LSD (0,01)
M	1,45		4,25		5,77
E	1,25		3,68		4,99
M x E	2,51		7,36		10,01

4.11.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,407	0,400	0,383	0,283	0,368
2	0,420	0,423	0,410	0,343	0,399
3	0,433	0,427	0,420	0,337	0,404
Mean	0,420	0,417	0,404	0,321	0,391
	SEM		LSD (0,05)		LSD (0,01)
M	0,0058		0,017		0,023
E	0,0051		0,015		0,020
M x E	0,0099		0,029		0,039

4.11.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	2,25	2,23	0,24	0,27	1,25
2	1,30	0,61	1,05	1,88	1,21
3	0,83	2,29	0,87	0,89	1,22
Mean	1,46	1,71	0,72	1,02	1,23
	SEM		LSD (0,05)		LSD (0,01)
M	0,43		1,25		1,70
E	0,37		1,08		1,47
M x E	0,74		2,16		2,94

TABLE 4.12: Response to Methionine and Energy intake during period 5. (40 - 43 weeks of age)

4.12.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	85,30	82,86	80,80	64,01	78,24
2	88,48	86,01	85,46	71,60	82,89
3	86,84	88,66	86,70	69,91	83,03
Mean	86,87	85,84	84,32	68,51	81,39
	SEM		LSD (0,05)		LSD (0,01)
M	1,18		3,46		4,70
E	1,02		2,99		4,07
M x E	2,04		5,99		8,15

4.12.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	57,99	58,08	55,83	53,97	56,47
2	57,74	58,08	57,88	54,41	57,03
3	58,05	57,58	58,25	55,25	57,28
Mean	57,93	57,92	57,32	54,54	56,93
	SEM		LSD (0,05)		LSD (0,01)
M	0,26		0,75		1,02
E	0,22		0,65		0,89
M x E	0,44		1,30		1,77

4.12.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	49,46	48,12	45,20	34,64	44,35
2	51,09	49,94	49,46	38,98	47,37
3	50,42	51,05	50,50	38,64	47,65
Mean	50,32	49,71	48,39	37,42	46,46
	SEM		LSD (0,05)		LSD (0,01)
M	0,76		2,24		3,05
E	0,66		1,94		2,64
M x E	1,32		3,88		5,27

TABLE 4.12 (Continued)

4.12.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	120,53	114,62	119,07	118,42	118,16
2	119,62	118,60	120,76	115,81	118,70
3	112,77	116,28	121,03	112,43	115,63
Mean	117,64	116,50	120,29	115,55	117,50
	SEM		LSD (0,05)		LSD (0,01)
M	1,18		3,46		4,71
E	1,03		3,01		4,09
M x E	2,04		5,99		8,15

4.12.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,413	0,423	0,377	0,293	0,377
2	0,430	0,423	0,410	0,337	0,400
3	0,450	0,440	0,417	0,347	0,413
Mean	0,431	0,429	0,401	0,326	0,397
	SEM		LSD (0,05)		LSD (0,01)
M	0,0048		0,014		0,019
E	0,0041		0,012		0,016
M x E	0,0082		0,024		0,032

4.12.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	1,52	1,40	- 1,77	2,50	0,91
2	2,11	2,19	0,74	- 1,45	0,90
3	2,05	1,19	2,97	- 0,36	1,46
Mean	1,89	1,59	0,65	0,23	1,09
	SEM		LSD (0,05)		LSD (0,01)
M	0,47		1,38		1,87
E	0,41		1,19		1,62
M x E	0,81		2,37		3,24



TABLE 4.13: Response to Methionine and Energy intake during period 6. (44 - 47 weeks of age)

4.13.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	81,10	79,59	76,03	61,79	74,63
2	84,20	82,74	81,78	64,20	79,23
3	84,45	84,84	85,19	60,68	78,79
Mean	83,25	82,39	81,00	62,23	77,22
	SEM		LSD (0,05)		LSD (0,01)
M	0,93		2,74		3,72
E	0,81		2,37		3,22
M x E	1,62		4,75		6,46

4.13.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	58,88	58,81	58,18	54,94	57,70
2	58,63	58,97	58,83	55,80	58,06
3	59,23	58,51	59,18	56,37	58,32
Mean	58,92	58,76	58,73	55,70	58,03
	SEM		LSD (0,05)		LSD (0,01)
M	0,24		0,69		0,94
E	0,20		0,60		0,81
M x E	0,41		1,20		1,63

4.13.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	47,74	46,81	44,28	34,01	43,21
2	49,36	48,79	48,11	35,82	45,52
3	50,04	49,65	50,41	34,20	46,08
Mean	49,05	48,42	47,60	34,68	44,94
	SEM		LSD (0,05)		LSD (0,01)
M	0,60		1,75		2,38
E	0,52		1,52		2,06
M x E	1,03		3,03		4,12

TABLE 4.13 (Continued)

4.13.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	129,65	128,49	132,23	129,32	129,92
2	130,72	127,72	128,32	130,19	129,24
3	124,62	124,01	132,89	125,58	126,78
Mean	128,33	126,74	131,14	128,37	128,65
	SEM		LSD (0,05)		LSD (0,01)
M	0,96		2,82		3,83
E	0,83		2,44		3,33
M x E	1,67		4,89		6,65

4.13.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,367	0,367	0,337	0,263	0,333
2	0,377	0,383	0,377	0,273	0,353
3	0,403	0,400	0,383	0,273	0,365
Mean	0,382	0,383	0,366	0,270	0,350
	SEM		LSD (0,05)		LSD (0,01)
M	0,0051		0,015		0,021
E	0,0044		0,013		0,018
M x E	0,0088		0,026		0,036

4.13.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,00	2,13	3,71	- 0,16	1,42
2	0,48	1,15	0,27	1,62	0,88
3	0,92	1,38	- 0,69	0,24	0,46
Mean	0,47	1,55	1,09	0,57	0,92
	SEM		LSD (0,05)		LSD (0,01)
M	0,57		1,68		2,28
E	0,49		1,45		1,97
M x E	1,00		2,92		3,97

TABLE 4.14: Response to Methionine and Energy intake during period 7. (48 - 51 weeks of age)

4.14.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	78,68	76,96	76,10	53,85	71,40
2	81,96	79,69	77,92	64,30	75,97
3	80,05	80,57	80,32	63,39	76,08
Mean	80,23	79,07	78,11	60,52	74,48
	SEM		LSD (0,05)		LSD (0,01)
M	1,04		3,07		4,17
E	0,90		2,65		3,61
M x E	1,81		5,31		7,21

4.14.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	60,06	60,01	60,12	56,22	59,10
2	59,87	60,31	60,44	58,14	59,69
3	60,79	59,72	59,54	58,80	59,71
Mean	60,24	60,01	60,03	57,72	59,50
	SEM		LSD (0,05)		LSD (0,01)
M	0,24		0,70		0,95
E	0,20		0,60		0,82
M x E	0,41		1,20		1,64

4.14.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	47,26	46,18	45,73	30,28	42,36
2	49,07	48,06	47,09	37,41	45,41
3	48,65	48,12	47,83	37,31	45,48
Mean	48,32	47,45	46,88	35,00	44,42
	SEM		LSD (0,05)		LSD (0,01)
M	0,69		2,02		2,75
E	0,60		1,75		2,33
M x E	1,19		3,50		4,75



TABLE 4.14 (Continued)

4.14.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	124,82	122,83	130,59	128,16	126,60
2	124,85	122,55	124,40	128,25	125,01
3	120,02	119,96	124,16	122,93	121,77
Mean	123,23	121,78	126,38	126,45	124,46
	SEM		LSD (0,05)		LSD (0,01)
M	1,03		3,03		4,11
E	0,89		2,62		3,56
M x E	1,79		5,24		7,13

4.14.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,380	0,373	0,350	0,237	0,335
2	0,390	0,393	0,377	0,290	0,363
3	0,403	0,397	0,387	0,307	0,373
Mean	0,391	0,388	0,371	0,278	0,357
	SEM		LSD (0,05)		LSD (0,01)
M	0,0048		0,014		0,019
E	0,0041		0,012		0,016
M x E	0,0082		0,024		0,033

4.14.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	1,06	0,08	0,31	0,09	0,38
2	0,34	1,00	0,11	- 0,05	0,35
3	- 0,19	- 0,07	- 0,55	0,97	0,04
Mean	0,40	0,34	- 0,04	0,34	0,26
	SEM		LSD (0,05)		LSD (0,01)
M	0,43		1,25		1,70
E	0,37		1,08		1,47
M x E	0,74		2,17		2,95

TABLE 4.15: Response to Methionine and Energy intake during period 8. (52 - 55 weeks of age)

4.15.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	74,24	74,41	74,17	57,28	70,03
2	77,10	74,83	73,67	63,57	72,29
3	76,67	76,80	73,11	60,88	71,86
Mean	76,00	75,35	73,65	60,58	71,39
	SEM		LSD (0,05)		LSD (0,01)
M	0,81		2,37		3,22
E	0,70		2,06		2,79
M x E	1,40		4,11		5,58

4.15.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	60,47	60,99	60,58	57,93	59,99
2	60,53	60,72	60,89	59,26	60,35
3	60,43	59,92	60,26	59,13	59,94
Mean	60,48	60,54	60,58	58,77	60,09
	SEM		LSD (0,05)		LSD (0,01)
M	0,17		0,49		0,66
E	0,14		0,42		0,57
M x E	0,29		0,84		1,14

4.15.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	44,90	45,37	44,93	33,13	42,10
2	46,66	45,42	44,86	37,68	43,65
3	46,32	46,02	44,05	36,00	43,10
Mean	45,96	45,60	44,61	35,62	42,95
	SEM		LSD (0,05)		LSD (0,01)
M	0,48		1,41		1,92
E	0,42		1,22		1,66
M x E	0,83		2,44		3,32

TABLE 4.15 (Continued)

4.15.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	124,4	124,37	129,05	125,74	125,89
2	123,10	120,00	126,83	128,59	124,63
3	117,82	119,45	123,21	124,67	121,29
Mean	121,77	121,28	126,36	126,33	123,93
	SEM		LSD (0,05)		LSD (0,01)
M	1,33		3,9		5,30
F	1,15		3,38		4,59
M x E	2,31		6,76		9,18

4.15.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,360	0,367	0,350	0,267	0,336
2	0,380	0,380	0,357	0,293	0,353
3	0,393	0,387	0,360	0,290	0,358
Mean	0,378	0,378	0,356	0,283	0,349
	SEM		LSD (0,05)		LSD (0,01)
M	0,0034		0,010		0,014
E	0,0031		0,009		0,012
M x E	0,0061		0,018		0,024

4.15.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,39	0,04	1,24	2,07	0,74
2	1,33	0,78	0,91	1,41	0,65
3	0,87	1,02	1,44	0,22	0,78
Mean	0,61	0,62	0,59	1,09	0,72
	SEM		LSD (0,05)		LSD (0,01)
M	0,41		1,20		1,63
E	0,40		1,17		1,59
M x E	0,71		2,08		2,83



TABLE 4.16: Response to Methionine and Energy intake for period 9. (56 - 59 weeks of age)

4.16.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	70,41	70,42	71,01	58,07	67,48
2	74,29	72,18	71,27	61,09	69,71
3	73,91	72,69	69,06	57,04	68,17
Mean	72,87	71,76	70,44	58,73	68,45
	SEM		LSD (0,05)		LSD (0,01)
M	0,87		2,55		3,47
E	0,75		2,21		3,00
M x E	1,51		4,42		6,00

4.16.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	60,23	61,02	60,73	58,47	60,11
2	60,90	61,10	61,18	59,27	60,61
3	61,31	60,42	60,74	60,03	60,63
Mean	60,81	60,85	60,88	59,26	60,45
	SEM		LSD (0,05)		LSD (0,01)
M	0,19		0,57		0,77
E	0,17		0,49		0,67
M x E	0,33		0,98		1,33

4.16.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	42,41	42,97	43,12	33,95	40,61
2	45,23	44,10	43,60	36,22	42,29
3	45,32	43,92	41,93	34,26	41,36
Mean	44,32	43,67	42,88	34,81	41,42
	SEM		LSD (0,05)		LSD (0,01)
M	0,52		1,53		2,08
E	0,45		1,32		1,80
M x E	0,90		2,65		3,60

TABLE 4.16 (Continued)

4.16.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	125,22	123,55	128,68	130,09	126,89
2	124,35	123,16	126,40	132,70	126,65
3	116,00	121,55	119,93	131,14	122,15
Mean	121,86	122,75	125,00	131,31	125,23
	SEM		LSD (0,05)		LSD (0,01)
M	1,80		5,27		7,16
E	1,56		4,56		6,20
M x E	3,11		9,12		12,40

4.16.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,340	0,350	0,333	0,260	0,321
2	0,363	0,357	0,347	0,273	0,335
3	0,390	0,360	0,353	0,260	0,341
Mean	0,364	0,356	0,344	0,264	0,332
	SEM		LSD (0,05)		LSD (0,01)
M	0,0038		0,011		0,015
E	0,0034		0,010		0,013
M x E	0,0065		0,019		0,026

4.16.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	- 0,27	- 0,38	- 0,35	- 0,42	- 0,35
2	0,08	- 0,14	1,16	0,54	0,41
3	0,23	0,50	- 0,17	- 0,55	0,00
Mean	0,01	- 0,01	- 0,21	- 0,14	0,02
	SEM		LSD (0,05)		LSD (0,01)
M	0,33		0,97		1,32
E	0,29		0,85		1,16
M x E	0,57		1,67		2,27

energy (E) are shown, together with the interaction terms (M x E). Also the standard errors of each mean (SEM) and the least significant differences (LSD) at  $P < 0,05$  and  $P < 0,01$  are indicated.

These same results are illustrated in Figures 4.1 to 4.6 where the effects of the four levels of dietary methionine concentrations have been plotted at each energy level for the nine periods of the trial.

The effects of dietary methionine only will be considered in the present discussion as the effects of energy on the production parameters measured, will be discussed in Chapter 6.

#### *Egg production.*

Mean egg production declined as the methionine content of the diets decreased. The difference between the summit, first and second dilution diets was however not significant in any of the nine periods of the experiment. The production of layers on the lowest dilution diets was significantly poorer than those on the higher dietary methionine concentrations.

It will be observed from Figure 4.1 that the egg production from layers receiving the second dilution diets at both the low and medium energy levels was consistently lower than those on the two higher methionine concentrations. It is however of interest that egg production of layers on the second dilution diets at the highest energy concentration did not differ to any marked degree from that of layers on the two higher dietary methionine concentrations. The reason for the different responses between the second dilution diets at different energy levels is most probably due to higher methionine intake at the higher dietary energy levels. In this trial methionine content of diets was strictly related to energy content. As reported by Morris (1968) birds on higher energy diets tend to overconsume energy. In this trial where the amino acid was strictly related to energy it would



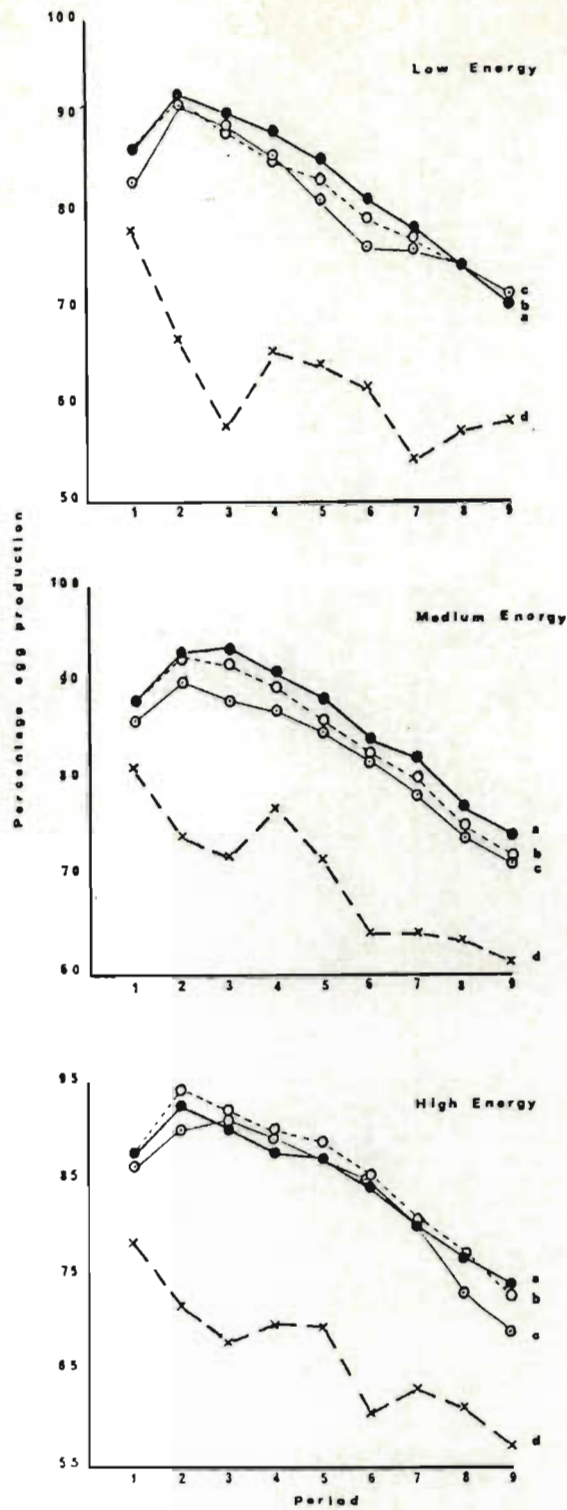


Figure 4.1 The effect of energy and methionine/energy ratio on percentage egg production during nine 28 d periods. (Refer to Table 4.4 for a description of the diets fed.)

automatically result in a higher methionine consumption on higher energy diets.

Egg production of layers on the dilution diets manifested a peculiar aberration. Peak production was attained during the first period and then sharply declined to the third period. Production increased from the third to the fourth period at all of the three dietary energy levels. Thereafter production declined with another slight increase during periods six to seven. Egg production in the isoleucine and lysine trials showed an even post-peak decline from the third period onwards and the aberrations in this trial are difficult to explain.

#### *Egg mass*

No statistical difference was found in the mean egg mass from layers on the summit, first and second dilution diets (Tables 4.8 to 4.16). Egg mass from birds on the dilution diets differed very significantly from that obtained on the three higher dietary methionine concentrations.

It is of interest to note that as the trial progressed egg mass on all the diets increased, although the egg mass range between the high methionine and dilution diets decreased. Furthermore it is evident that the narrowing of the range is more pronounced on the higher dietary energy levels than on the other energy levels. A possible explanation for this phenomenon is that feed intake on the dilution diets increased as the trial progressed. The methionine intake was consequently higher, particularly on the higher energy diets thus explaining the lower range of egg mass values on these diets.

Figure 4.2 illustrates a significantly lowered egg mass from birds on the low energy second dilution diet (1C) during periods five and six. The reason for this can again be ascribed to lower methionine intakes.

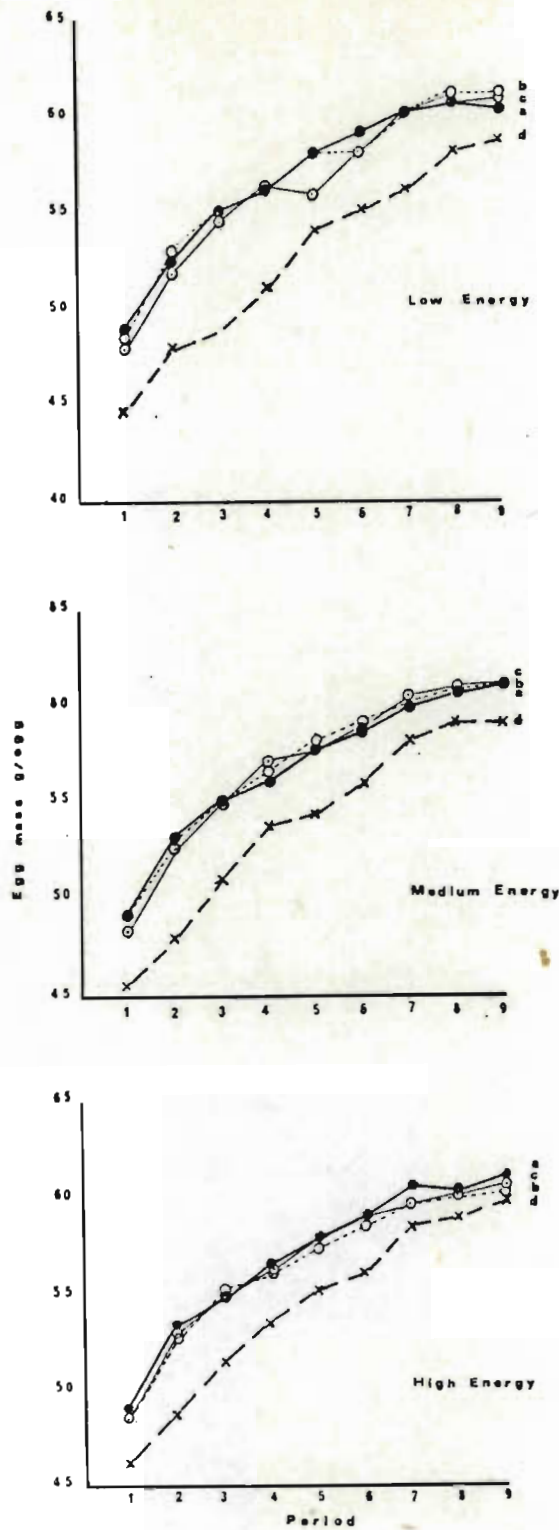


Figure 4.2

The effect of energy and methionine/energy ratio on egg mass (g/egg) during nine 28 d periods. (Refer to Table 4.4 for a description of the diets fed.)



#### *Egg output.*

As the methionine concentration of the diets decreased egg output declined. Egg output from birds on the dilution diets was significantly poorer than that of birds on higher dietary methionine concentrations.

Egg output from birds receiving the second dilution diets at the low and medium energy levels approached significance during most experimental periods and was in fact significantly poorer during periods five and six in the instance of the low energy dietary level. (Figure 4.3). The reason for the poorer egg output could be as a result of inadequate methionine intake. The different pattern in egg output exhibited by birds on the second dilution diets at the high energy level is again explained by higher methionine intakes.

#### *Food intake*

Food intake of birds on the dilution diets was significantly lower than that of birds on the three higher dietary methionine concentrations.

In agreement with Pilbrow and Morris (1974) food intake by birds on these diets increased markedly and was significantly higher than that of birds receiving the summit and first dilution diets towards the end of the trial.

Mean food intake on the second dilution diet did not differ significantly from the lowest dilution diet because the marginal methionine level of this diet resulted in compensatory food intake to sustain production.

It is of interest to note that the difference in food intake between the second and lowest dilution diets was substantially narrower on the lower energy diets than on the higher energy diets. This

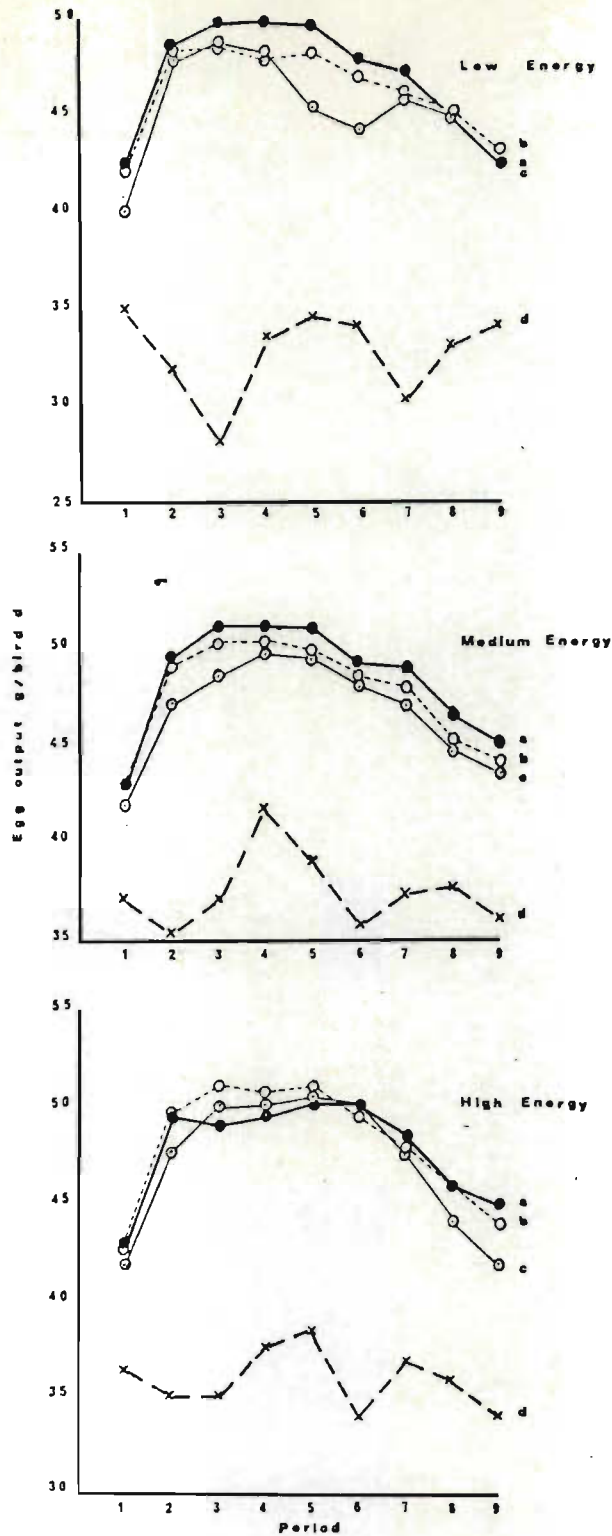


Figure 4.3 The effect of energy and methionine/energy ratio on egg output (g/bird d) during nine 28 d periods. (Refer to Table 4.4 for a description of the diets fed.)

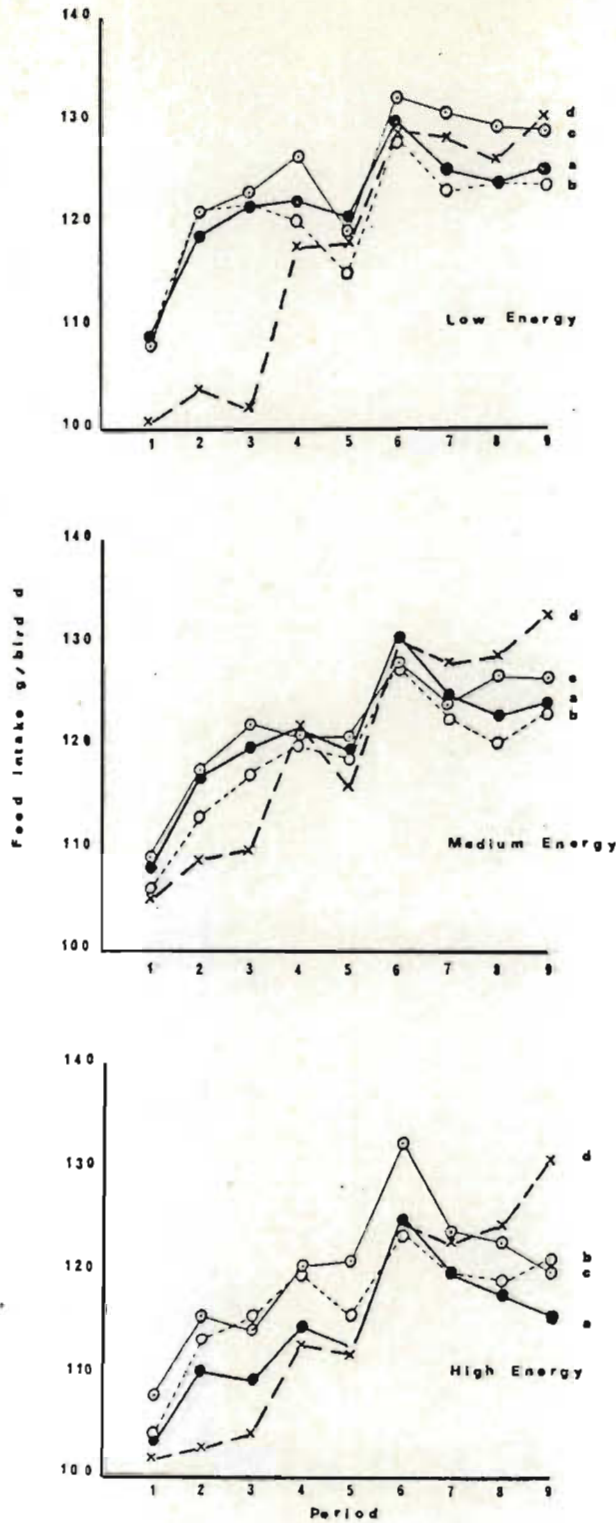


Figure 4.4 The effect of energy and methionine/energy ratio on food intake (g/bird d) during nine 28 d periods. (Refer to Table 4.4 for a description of the diets fed.)



is due to higher methionine intakes by birds fed the higher energy diets.

Filmer (1974) indicated that with severely deficient amino acid levels in the diet, increased food intake can no longer supply the daily nutrients necessary to sustain egg output. Egg output therefore falls and consequently the demand for all nutrients diminishes. Food intake therefore falls in sympathy with the lower demand.

Picard et al. (1979) however expressed the opinion that food intake on inadequate amino acid diets had a "direct action". He pointed out that birds with low feed intake switched from a low protein diet to an adequate diet immediately returned to normal, whereas production took some time to recover. This study however, indicates that although food intake progressively increased on the inadequate diets egg production nevertheless remained at a low level. It must therefore be concluded that the inadequate diets directly affect egg output.

#### *Food conversion efficiency.*

The mean food conversion efficiency of birds on the second and lowest dilution diets was significantly poorer than that of those on the two higher dietary methionine concentrations. The poorer efficiency was not unexpected since food intake on the second dilution diets was consistently higher in all periods in order to support production. On the other hand birds on the lowest dilution diets performed poorly whilst food intake increased during the latter periods of the trial. The food conversion efficiency during these periods thus reached its lowest level.

#### *Body mass gain*

The mean daily body mass increments of birds fed diets with higher methionine concentrations were greater in most of the nine

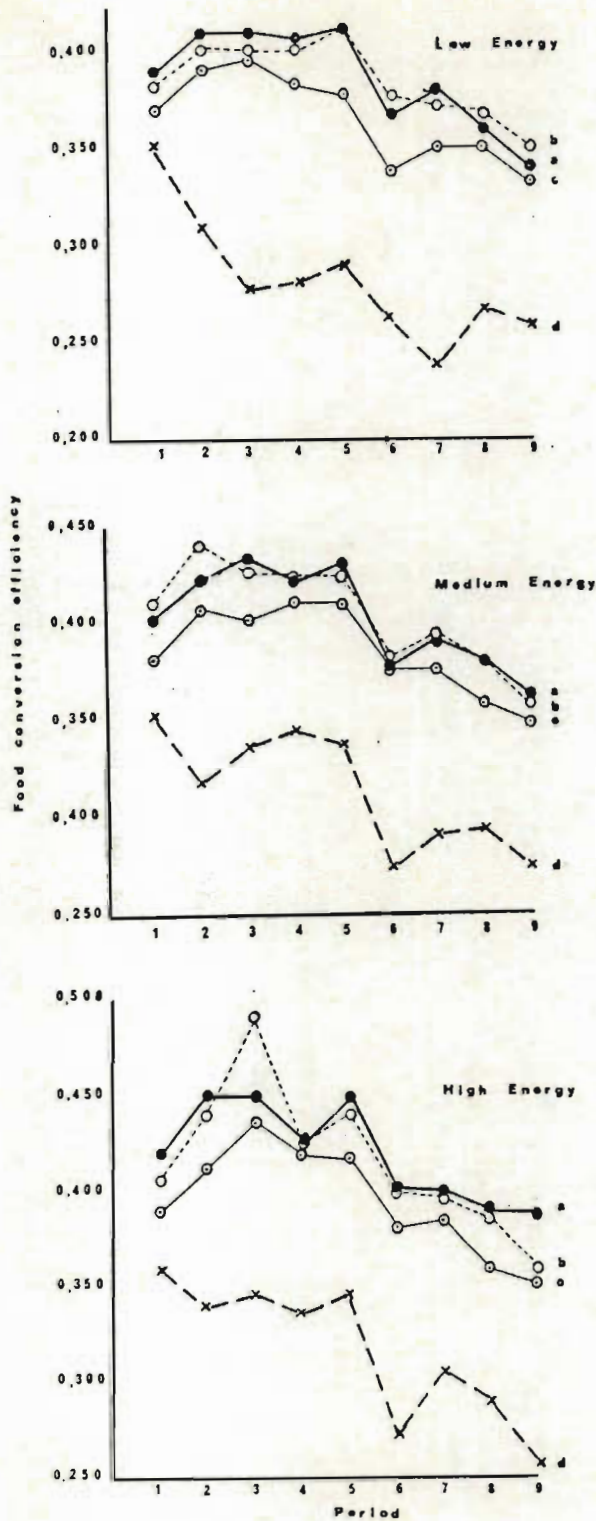


Figure 4.5 The effect of energy and methionine/energy ratio on food conversion efficiency (g egg/g food) during nine 28 d periods. (Refer to Table 4.4 for a description of the diets fed.)



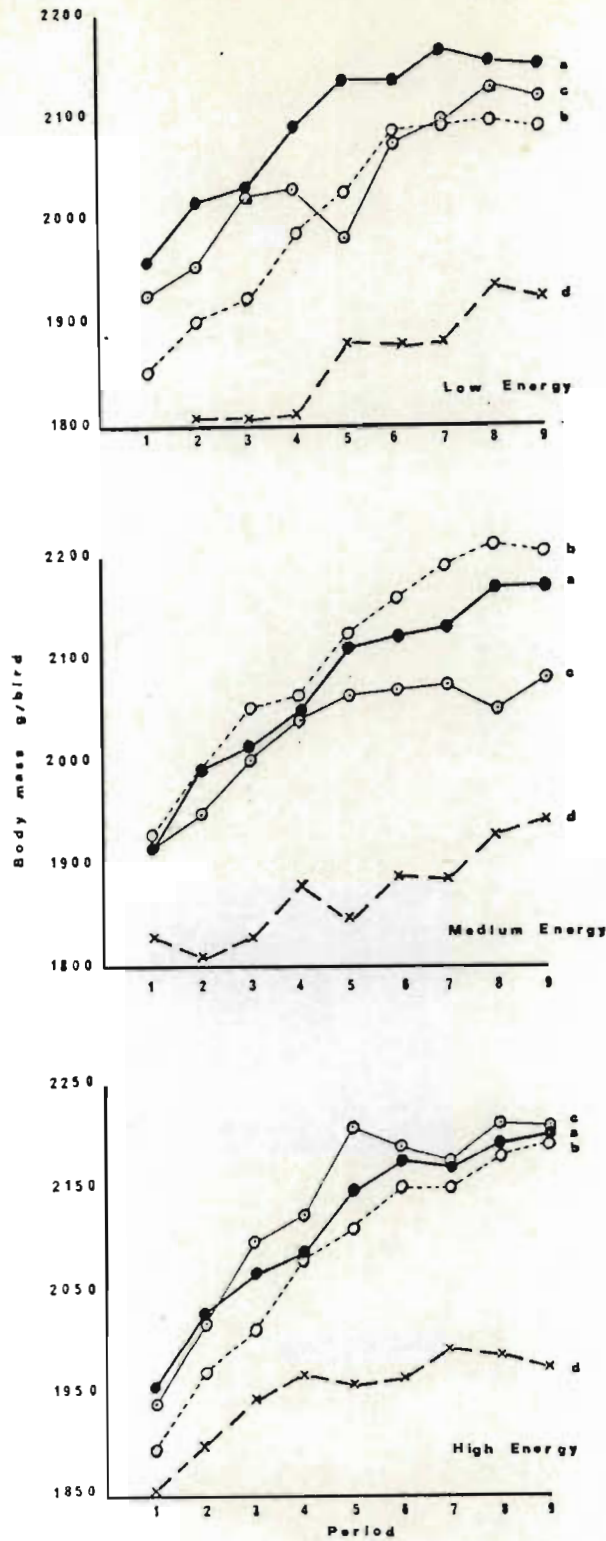


Figure 4.6 The effect of energy and methionine/energy ratio on body mass gain during nine 28 d periods. (Refer to Table 4.4 for a description of the diets fed.)



periods of the experiment. In only some periods were these differences significant.

The actual body mass (g/bird) of birds on the different dietary methionine and energy concentrations per period were plotted and the graphs are presented in Figure 4.6. It is clear that the lack of methionine in the lowest dilution diets had a distinct effect on final body mass, this being substantially lower at each energy level. It is also interesting to note that the body mass of birds on the higher methionine diets at the high energy level was very similar.

During the first two periods the daily mass gain on the lowest dilution diets was significantly lower than that of birds on the three higher dietary methionine levels. These mass gains improved from the third period onwards and although lower than those on other treatments, the differences were not significant for the remaining periods. The improvement can be attributed to higher food intake on these diets as the trial progressed.

RESULTS DURING PERIOD OF MAXIMUM PRODUCTION (periods 3, 4 and 5, 32 - 43 weeks of age).

From Figure 4.3 it will be observed that maximum egg output was obtained between 32 and 43 weeks of age (periods 3, 4 and 5). For reasons already explained in Chapter 3, it was decided to combine the data of these periods in order to accurately assess the effect of methionine intake on the production parameters rate of lay (eggs/100 bird d), egg mass (g/egg), egg output (g/bird d), food intake (g/bird d), food conversion efficiency (g egg/g food) and mass gain (g/bird d).

The combined results of periods 3, 4 and 5 are presented in Table 4.17. The main effects of methionine/energy ratio (M) and of energy (E) are shown together with the interaction terms (M x E). Also the standard errors of each mean (SEM) and the least significant differences (L.S.D.) at  $P < 0,05$  and  $P < 0,01$  are indicated.

TABLE 4.17: Response to Methionine and Energy intake during combined periods 3, 4 and 5.  
(32 - 43 weeks of age)

4.17.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	88,03	85,48	85,06	62,41	80,24
2	90,94	89,09	86,93	73,85	85,20
3	87,95	90,42	88,86	69,56	84,20
Mean	88,97	88,33	86,95	68,61	83,21
	SEM		LSD (0,05)		LSD (0,01)
M	0,722		2,03		2,70
E	0,626		1,76		2,34
M x E	1,251		3,52		4,67

4.17.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	56,37	56,37	55,62	51,24	54,90
2	56,38	56,54	56,65	53,13	55,67
3	56,66	56,45	56,64	53,57	55,83
Mean	56,47	56,45	56,30	52,65	55,47
	SEM		LSD (0,05)		LSD (0,01)
M	0,300		0,845		1,12
E	0,260		0,732		0,970
M x E	0,520		1,464		1,94

4.17.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	49,59	48,15	47,32	32,09	44,29
2	51,24	50,34	49,24	39,27	47,52
3	49,84	51,03	50,31	37,30	47,12
Mean	50,22	49,84	48,96	36,22	46,31
	SEM		LSD (0,05)		LSD (0,01)
M	0,434		1,22		1,62
E	0,377		1,06		1,40
M x E	0,750		2,11		2,80



TABLE 4.17 (Continued)

4.17.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	121,46	118,76	122,95	112,61	118,95
2	120,27	118,68	121,32	116,20	119,12
3	112,50	113,86	118,69	109,79	113,71
Mean	118,08	117,10	120,98	112,87	117,26
	SEM		LSD (0,05)		LSD (0,01)
M	1,03		2,91		3,86
E	0,896		2,52		3,34
M x E	1,792		5,04		6,68

4.17.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,410	0,408	0,386	0,284	0,372
2	0,428	0,424	0,407	0,339	0,399
3	0,444	0,452	0,424	0,343	0,416
Mean	0,427	0,428	0,406	0,322	0,396
	SEM		LSD (0,05)		LSD (0,01)
M	0,0034		0,0096		0,0127
E	0,0030		0,0083		0,0110
M x E	0,0059		0,0166		0,0220

4.17.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	1,39	1,49	0,32	0,91	1,03
2	1,38	1,58	1,36	0,45	1,19
3	1,40	1,69	2,20	0,73	1,50
Mean	1,39	1,59	1,29	0,70	1,24
	SEM		LSD (0,05)		LSD (0,01)
M	0,301		0,847		1,123
E	0,261		0,734		0,973
M x E	0,523		1,470		1,948



These same results are also illustrated in Figures 4,7 to 4.11 where methionine intake has been plotted against some of the production parameters mentioned above.

Only the effects of dietary methionine will be discussed in this Chapter as the effects of energy on the production parameters measured will be discussed in Chapter 6.

#### *Egg production.*

Mean egg production was 88, 97, 88, 33, 86, 95 and 68,61 percent during the combined peak periods (3, 4 and 5) on the diets containing progressively lower methionine levels. These production levels were obtained from respective mean methionine intakes of 506, 410, 315 and 215 mg per bird d. Only the lowest level of egg production was significantly inferior.

The relationship between methionine intake and percentage egg production is illustrated in Figure 4.7. A linear regression equation was fitted to data corresponding to an intake of 315 mg or more methionine/bird d in order to ascertain the effect of adequate intakes of methionine on egg production. The slope, although positive ( $b = +0,0092$ ), was not significant indicating very little improvement in egg production with higher intakes of methionine. This corresponds exactly with the effects of adequate isoleucine intakes on percentage egg production as indicated in Chapter 3.

#### *Egg mass*

Mean egg mass from laying hens on diminishing dietary methionine concentrations was 56,47, 56,45, 56,30 and 52,65 g/egg. These values were obtained with methionine intakes of 506, 410, 315 and 215 mg/bird d respectively. Only at the lowest methionine intakes was egg mass significantly lower than the other treatments.

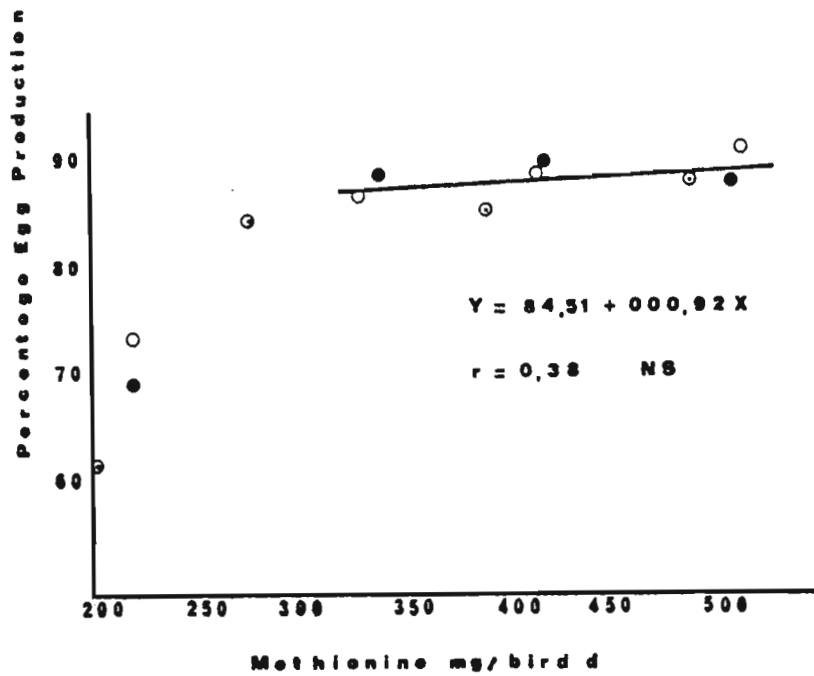


Figure 4.7 The relationship between methionine intake and percentage egg production. Regression equation fitted to data corresponding to an intake of more than 315 mg methionine/bird d.

The response of egg mass to methionine intake is illustrated in Figure 4.8. On adequate and marginally adequate diets the effect of methionine intake on egg mass was computed and the linear regression graph and equation are both shown in the figure. At methionine intakes in excess of 315 mg/bird d, egg mass no longer increases, but in fact declines slightly ( $b = -0,00075$ ). The slope, although negative, was not significant indicating very little deterioration in egg mass with higher intakes of methionine.

#### *Egg output.*

As in the isoleucine trial the Reading model (Fisher et al., 1973) was used to analyse the combined egg output data obtained in this experiment. During the three periods mentioned body mass changes of all birds were small and so  $\Delta W$  was ignored, and thus the coefficient for mass gain  $C$  was eliminated from the calculations.

After fitting the curve and examining the data it was noticed that the one low energy diet (1b) gave an unexpectedly low egg output. It was therefore considered necessary to fit response curves to the data of each of the three periods separately. Examining these data it was observed that the responses in all three periods were consistent over the three energy levels. The low energy diet (1b) however gave low egg outputs in all three periods. It was therefore decided to fit a curve where the data of the three periods were combined but where the data of this one diet was omitted. A summary of the parameters resulting from the model are given in Table 4.18.

The response curve of the combined data, but omitting diet (1b) is illustrated in Figure 4.9.

It can therefore be concluded that the methionine requirement of an individual pullet in the early stages of lay is adequately



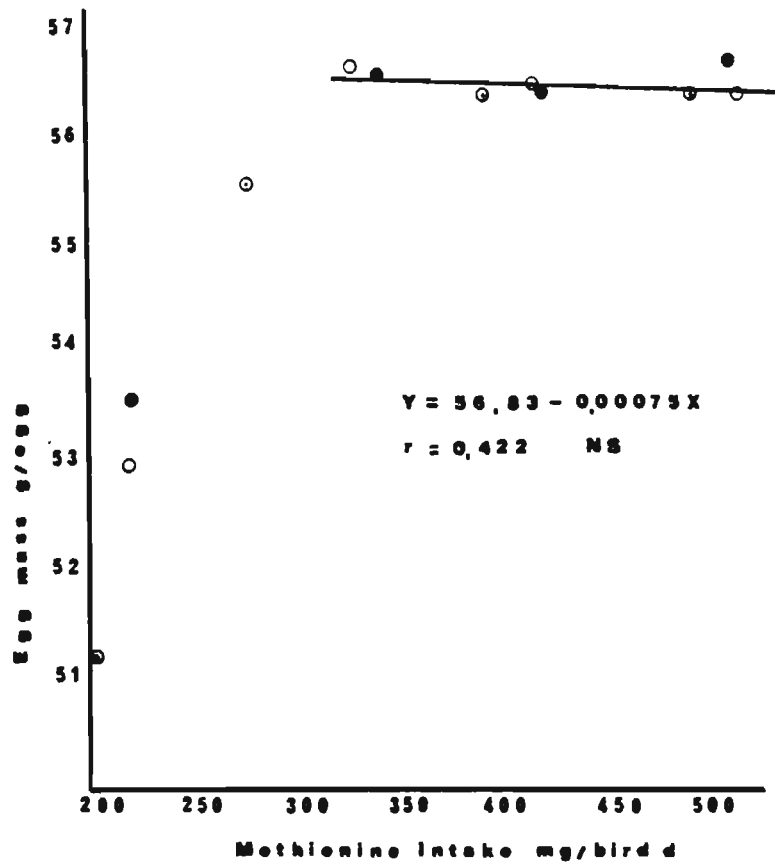


TABLE 4.18: Parameters of response curves and the estimated optimum methionine intakes assuming a marginal value of 60 cents/kg for eggs and a marginal cost of R3-00/kg for methionine.

Period	Mean live mass kg/bird	Maximum egg output g/hen d (E <sub>max</sub> )	mg amino acid per g egg (a)	mg amino acid per kg W daily (b)	Residual s.d. g/hen d	Optimum amino acid input mg/hen d.
3	1,95	49,91	3,09	49,29	1,021	318
4	2,00	50,01	3,08	53,00	1,117	327
5	2,05	50,18	4,24	29,06	1,123	359
3,4,5 (combined)	2,00	49,95	3,32	46,64	1,054	330
3,4,5 (combined and omitt- ing diet 1b	2,00	50,29	3,50	43,55	0,876	337

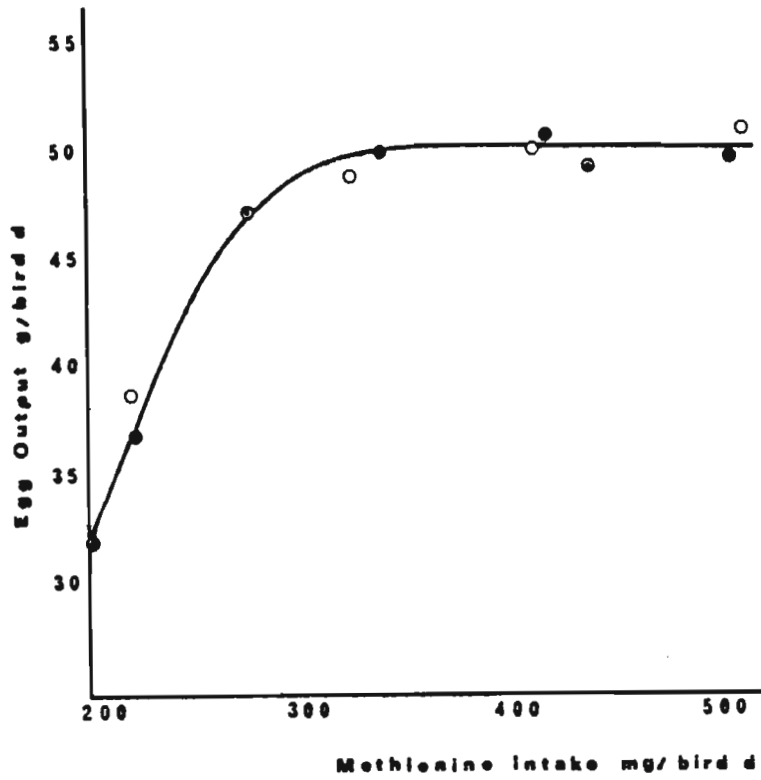


Figure 4.9 The relationship between egg output and methionine intake. The curve was fitted by the method of Curnow (1973) using the parameters described in the text.



represented (neglecting change in body mass) by the equation

$$\text{Methionine req.} = 3,5 \text{ Emax} + 43,55 \text{ W.}$$

This equation should not be used directly by substituting average values of Emax and W to predict the "requirement" of a flock, since it makes no allowance for the variations in yield and body mass which exist within the flock and the consequent curvilinear responses to be expected when diets of differing methionine concentration are fed to different groups of birds. Once curvilinear responses are assumed, a requirement can only usefully be defined in economic terms.

*Optimum Methionine intake.*

To obtain estimates of optimum methionine intake from fitted response curves, as previously indicated in Chapter 3, it is necessary to know the marginal cost of methionine and the value of eggs.

Eggs were assigned a price of 60 cents/kg in this study. The cost of supplying methionine was estimated by formulating a series of least-cost diets for layers at 0,5 g/kg increments, using current South African ingredient prices.

A linear relationship was obtained between price/kg and methionine content, the cost of the diet being increased by 0,3 cents/kg for each 1 g methionine/kg added to the specification. Thus, under these conditions the cost of supplying extra methionine in the form of protein was R3-00/kg methionine. In 1980 the price of DL methionine was R3-33/kg.

Using the price of R3-00/kg for methionine, the optimum intakes of methionine were calculated from the equation given in Chapter 3.

With the present cost ratio of  $k = 0,005$ , the optimum methionine intake was found to be 337 mg/bird d.

As in the case of isoleucine (Chapter 3) Table 4.19 was compiled from which the optimum levels of methionine can be determined should the price of eggs and the marginal cost of methionine change.

TABLE 4.19: Optimum methionine intakes (mg/hen d) for flocks of young laying pullets of 2 kg body mass and producing 50 g egg material as affected by the marginal cost of methionine and the value of egg output.

Marginal value of egg output (cents/kg)	Marginal cost of methionine (R/kg)		
	R3-00	R4-00	R5-00
	Methionine requirements, mg/hen d		
60	337	333	330
70	339	335	332
80	341	337	334
90	343	339	335
100	344	340	337
110	345	342	338

Carpenter et al. (1968) found 3,26 g "available" methionine per 16 g nitrogen in egg. Assuming that eggs contain 11,25 percent protein Fisher (1976) calculated a methionine content of 3,67 mg methionine/g egg. He further found that the methionine requirement of an individual pullet in the early stages of lay is adequately represented by the equation

$$M = 4,358E + 25W.$$

where  $M$  = mg available methionine/bird d

$E$  = g egg output/bird d

$W$  = body mass in kg.

Using the coefficient of 4,358 methionine per g egg output and

an available methionine content of 3,67 mg/g egg Fisher concluded that the nett efficiency of methionine utilisation for egg production is 84,2 percent.

In this study Table 4.18 shows that the coefficient for egg output of 4,24 during period 5 matches the findings of Fisher (1976) fairly well. However, the lower egg output coefficients found in periods 3 and 4 and the combined 3, 4 and 5 periods differ from his coefficient. It is interesting to note that in the earlier periods when the coefficient for egg output was low, the live mass coefficient was somewhat higher than that reported by Fisher.

Leveille and Fisher (1960) estimated the methionine requirement for maintenance of nitrogen balance in adult roosters to be 15 mg/kg day and for maintenance of "optimum" nitrogen balance to be 71 mg/kg day. Kandatsu and Ishibashi (1966) in turn estimated the latter figure as 25 mg/kg day.

The body mass coefficient determined by Fisher is therefore in total agreement with that found by Kandatsu and Ishibashi (1966).

Again the coefficient for body maintenance of 29,06 mg methionine per kg found in period 5 is in close agreement with that found by Fisher (1976).

The optimum requirement of 337 mg methionine/bird d found in this study agrees very well with the 340 mg/bird d recommended by Scott, Nesheim and Young (1976). It is however lower than the 385 mg total methionine (350 mg available methionine) recommended by the A.R.C. (1975) but slightly higher than the 300 mg total methionine advocated by the N.R.C. (1977).

Bray (1965) noted the lack of information on the methionine requirement of laying hens expressed on an intake basis and was able to compare his own estimate of 224 mg/bird d only with that



of Combs (1964) whose results indicated a requirement of 295 mg/bird d. According to Bray there were several bases for speculation as to why the requirement they determined was comparatively low. Comparing their results with those of Combs (1964) it appeared that the maximum egg yield of the "carefully selected" pullets used by the latter author was 46,5 g compared to their 40,58 g. Bray felt that this difference of 5,9 g in daily egg output could partly explain why Combs found an intake requirement of 295 mg of available methionine (chick assay) compared to their 224 mg.

Another possible contributory factor could have been the comparative amount of feather growth during the two assays involved. Bray (1965) indicated that the pullets (29 weeks old) used in their experiment had a mature coat of feathers judged by the complete absence of feathers that contained blood in the quill. However, another population of (24 week old) birds reared under similar conditions had immature feathers in all regions of the body, even though the flock was laying at a rate of 50 percent. This was especially obvious in the back feather tract.

Feathers contain a high concentration of cystine (Ward et al., 1955) cited by Bray (1965). Some basal diets may not contain enough cystine under conditions of intensive feather growth to prevent conversion of methionine to cystine for the synthesis of egg and feather proteins.

Fisher (1970) however plotted the calculated responses in egg yield from these two studies together with the data of his study and a further report by Combs (1962). Taken together these experiments indicated a requirement of approximately 275 mg available methionine per day. Considering the diversity of the conditions in these experiments there is remarkably good agreement between them and it is unlikely that they can be reconciled more closely.

Fisher (1970) attributed the low value found by Bray to the statistical method of analysis employed. He indicated that the method of fitting two straight lines used by Bray (1965) will always underestimate the requirement for maximum output if the true response line is a continuous curve.

Schutte and van Weerden (1978) found a requirement of approximately 775 to 880 mg sulfur amino acids/hen d, of which about 390 to 440 mg was methionine for an egg output of 80 - 83 egg/100 hen d. These figures are in close agreement with those of Moran (1969) who advocated 788 mg sulfur amino acids/hen d, of which 362 mg was methionine.

They indicated that the lower requirements estimated by Novacek and Carlson (1969), Jensen et al. (1974) and Sell and Johnson (1974) compared to their own results could be partly explained by the very high rates of production achieved in their experiments. In addition they point out that Fisher and Morris (1970) and Jensen et al. (1974) used mainly egg production criteria, rather than food conversion efficiency in their assessments.

Careful study of their work indicates that in one of their experiments no statistical improvement in respect of egg output was obtained on methionine intake levels above 347 mg/bird d. The egg output at this methionine intake was 50,17 g/hen d. A statistical improvement in food conversion ratio was however obtained at an intake level of 440 mg methionine/bird d.

In their second experiment a control diet containing 2,7 g/kg methionine was compared to the same diet but supplemented with 0,5 g methionine/kg. In this instance a statistical improvement in egg mass (g/egg), total egg output (g/bird) and feed efficiency was observed. The methionine intakes on the control and methionine supplemented diets were 343 mg and 390 mg/bird d while the egg outputs were 49 g and 51,9 g/bird d respectively. It cannot be

deduced from this trial that the optimum intake for egg output is 390 mg/bird d since the methionine increment was too wide. The figure of 337 mg/hen d found in the present study compares well with the 347 mg methionine/hen d in the first experiment reported by Schutte and van Weerden (1978).

The recommendation of 390 to 440 mg methionine/bird d by these workers is based on the production parameter of feed efficiency. For maximum egg output the requirement however is somewhat lower which emphasises the importance of stipulating parameters used when methionine intake recommendations are made.

The high methionine intake advocated by Daub et al. (1978) of 410 mg total methionine per bird d cannot be adequately evaluated since insufficient information with regard to statistical analysis was provided.

Should the marginal cost of methionine be 160 pence/kg and egg prices 40 pence/kg ( $k = 0,004$ ), Fisher (1976) finds that the optimum methionine requirement for 2 kg layers with an egg output of 50 g/bird d is 337 mg. Using the same  $k$ -value in this study the optimum methionine intake for layers producing 50,29 g egg/hen d appears to be 340 mg. (Table 4.19.) Fisher's finding however is based on available methionine while estimates from this work are based on total methionine.

It is generally accepted that approximately 90 percent of total methionine is available. Consequently it would appear that Fisher's recommendation for methionine is about 10 percent higher than that found in this study. Therefore the figure of 370 mg/hen d methionine should be compared to 337 mg/hen d reported by Fisher under similar circumstances.

The discrepancy in these methionine requirement figures is difficult to explain. Possible reasons for this are firstly a variation



of raw materials used in the summit and dilution diets. In the dilution technique all constituents of the diet are varied which means that the response might be partly attributable to a decreasing level of some component of the summit diet (other than methionine) or an increasing level of some component of the dilution mixture. Secondly, the response is determined under conditions of a deliberate amino acid imbalance. The extent of the effect of amino acid imbalance, if any, in this study is open to speculation.

The dilution technique of determining amino acid requirements assumes that the surpluses of amino acids in the diet have no effect on performance. This assumption is probably valid for the great majority of diets, but there is mounting evidence that amino acid imbalance can be an issue of practical importance. (Wethli, Morris and Shreshta, 1975.). Future research should be directed towards quantifying the effects of amino acid surpluses so that suitable conditions can be included in linear programme matrices to prevent the formulation of imbalanced diets.

Should the 50,29 g egg output from a 2 kg layer with a daily mass gain of 1,25 g, as found in this study, be used in the "B model" equations of Hurwitz and Bornstein (1973) and Wilgus (1976), the calculated methionine requirements would be 433 mg and 337 mg/bird d respectively.

The estimated methionine requirement of 337 mg/bird d when using the Wilgus (1976) equation is in total agreement with the results of this experiment. It would therefore appear that this equation provides a very good methionine requirement estimate for layers. The model used by Hurwitz and Bornstein probably overestimates the methionine requirements for layers.

Wilgus (1976) also concluded that the Hurwitz and Bornstein (1973) equation overestimated the methionine needs of layers. Using body mass ( $W'$ ) as suggested by the latter authors, Wilgus (1973) found

that the calculated sulfur amino acid estimates for both growing and adult turkeys were excessively high. He therefore modified the Hurwitz and Bornstein model by using metabolic body mass ( $W^{0.75}$ ) instead of body mass ( $W^1$ ). This modification has little effect on the calculated maintenance requirements of a small bird but a marked effect on those of large birds. Furthermore, the methionine content of maintenance protein, given as 4.1 percent or 80 percent of the total sulfur amino acids (TSAA) by Hurwitz and Bornstein was reduced to 1.3 percent or 25 percent of TSAA. This modification is based on the report of Graber et al. (1971) that the TSAA requirements of chickens can be met by increasing proportions of cystine, being about 55 percent at two weeks of age and about 70 percent at 8 weeks. They refer to evidence that this proportion approaches 90 percent in mature birds. This modification resulted in a decrease from 350 mg methionine per day by the 1.85 kg laying hen given by Hurwitz and Bornstein (1973) to 310 mg, a figure in close agreement with published research findings.

When the same production data found in this experiment are fitted to the "model 2" equation of Smith (1978) the methionine requirement is 378 mg/bird d. Thus, this model provides an estimate of methionine requirement which matches the A.R.C. (1975) recommendation fairly well.

Fitting the abovementioned production data to the equation cited by Combs (1964) the calculated methionine requirement is 359 mg/bird d.

Tolan and Morris (1969) determined the individual methionine requirements for a flock of 192 singly caged pullets in a change-over experiment using sets of balanced Latin squares. An assessment of the requirement of each individual bird was made by regressing her mean daily body mass change in each of four periods on her mean daily methionine intake. The requirement was then

defined as the regression estimate of methionine intake for zero body mass change. It was shown that the range of methionine intake requirements for a population of birds determined in this manner was normally distributed with a mean of 337 mg/bird d and a standard deviation of 44 mg/bird d. The range of requirements was from 220 to 470 mg per bird d.

The mean requirement of 337 mg/bird d is in exact agreement with the optimum methionine level found in this study.

In summary it can be said that acceptable reasons can be found for both excessively high and low methionine recommendations reported by various workers during the last three decades. It can also be concluded that the 337 mg daily intake suggested in this study is an accurate assessment of the true requirement for laying hens with production parameters similar to those used in this trial. A further observation, as in the isoleucine and lysine trials, is that the utilisation of methionine was not affected by dietary energy concentration.

#### *Food intake.*

Mean food intake by birds during the peak egg output periods was 118,08, 117,1, 120,98 and 112,87 g/bird d on diets with declining concentrations of methionine.

Food intake due to marginal methionine deficiency in the second dilution diets was significantly higher than that of birds fed the summit and first dilution diets. Conversely the food intake on the inadequate lowest dilution diet was significantly lower than all other treatments during periods of peak egg output.

Three separate graphs (Fig 4.10) were fitted relating food intake to methionine intake due to the fact that energy concentration had a significant effect on food intake independent of the



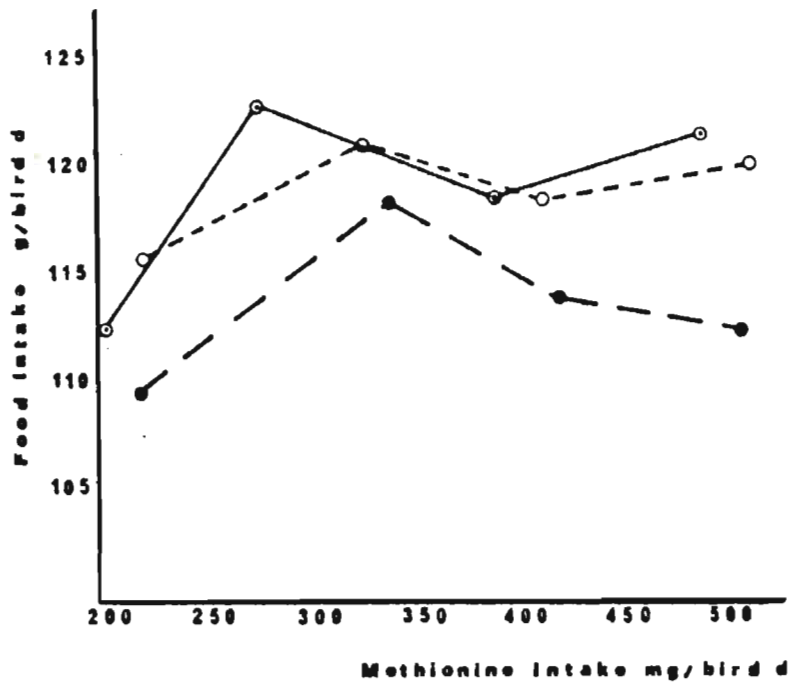


Figure 4.10 The relationship between food intake (g/bird d) and methionine intake (mg/bird d) when fed high energy diets (●—●), medium energy diets (○----○) and low energy diets (○—○).

methionine effect. The highest intake corresponded to the sub-optimal methionine intake of 277 mg/bird d.

*Food conversion efficiency.*

There was a highly significant difference in food conversion efficiency in favour of the birds receiving the summit and first dilution diets over those fed the second and lowest dilution diets.

Three regression equations were computed relating food conversion efficiency to methionine intake due to the fact that energy had a profound effect on this parameter independent of the methionine effect. The three equations are as follows:

Low energy (10,49 MJ ME/kg)

$$Y = -0,071 + 0,00239X - 0,00000289X^2$$

$$r = 0,935.$$

Medium energy (11,09 MJ ME/kg)

$$Y = 0,097 + 0,00145X - 0,00000157X^2$$

$$r = 0,951.$$

High energy (11,7 MJ ME/kg)

$$Y = 0,0496 + 0,00179X - 0,000002X^2$$

$$r = 0,836$$

where Y = food conversion efficiency

and X = methionine intake (mg/bird d).

From these regression equations and Figure 4.11 it would appear that optimum food conversion efficiency is obtained on a daily methionine intake of more than 413 mg per bird d which is substantially higher than the intake corresponding to the optimum

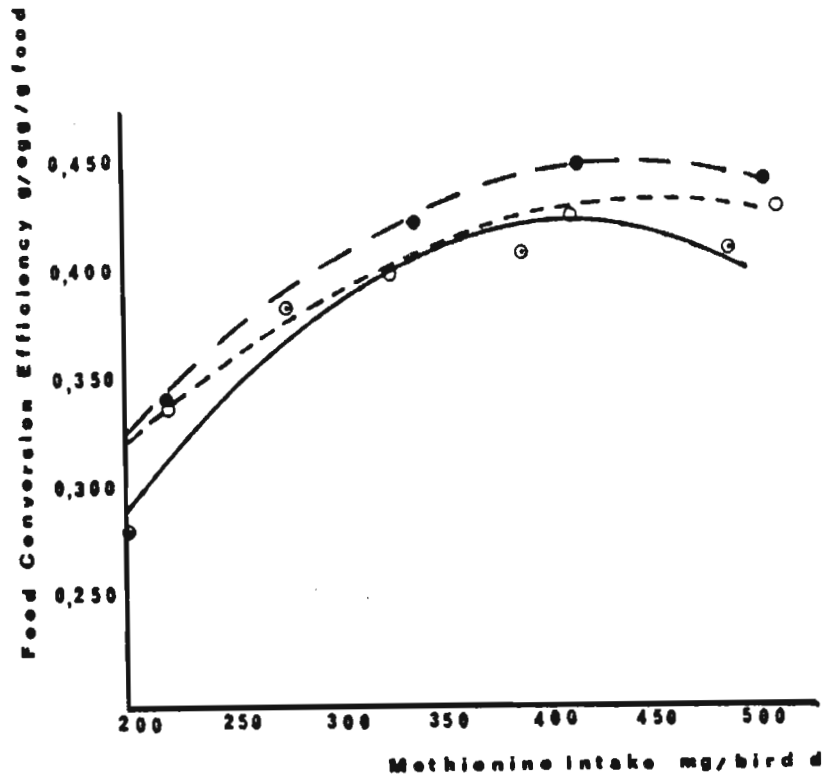


Figure 4.11 The relationship between food conversion efficiency and methionine intake when hens were fed the high energy diets (●—●), medium energy diets (○----○) and low energy diets (○—○)



egg output of 337 mg/bird d. This finding is in agreement with Janssen (1974) and Schutte et al. (1978) who concluded that the sulphur amino acid requirement for optimum food conversion efficiency is distinctly higher than that for maximum egg yield.

*Body mass gain.*

There was little difference in mean daily body mass gain during peak egg output periods between the three diets with the higher methionine levels. The inadequate dilution diets exhibited a lower daily body mass gain which differed significantly ( $P < 0,05$ ) only from that of birds on the first dilution diet.

SUMMARY.

1. A total of 2 376 laying hens were used in this study designed to determine the response of laying hens to increasing concentrations of methionine.
2. Using a dilution technique, four methionine levels at three different energy concentrations were each fed to three replicates of 66 birds.
3. The methionine requirement of an individual pullet is respresented (ignoring change in body mass) by the equation
$$\text{Methionine req.} = 3,5 \text{ Emax} + 43,55W.$$
4. Relating marginal cost of eggs and methionine it was determined that optimal egg output was achieved with a methionine intake of 337 mg/bird d.
5. A table is presented from which optimal response to methionine intake can be determined (2 kg layers producing 50 g egg mass/bird d) should the value of eggs or marginal cost of methionine change.

6. Egg mass with methionine intakes in excess of 315 mg/bird d no longer increased, but in fact slightly decreased. The slope ( $b = -0,00075$ ) although negative, was not significant indicating very little deterioration in egg mass with higher intakes of methionine.
7. Three separate graphs were fitted relating food intake to methionine intake due to the fact that energy concentration had a significant effect on food intake independent of methionine effect. The highest food intake corresponded to the sub-optimal methionine intake of 277 mg/bird d. It was concluded that with marginally sub-optimal levels of methionine, in the diet food intake increased in an attempt to sustain production.
8. Food intake of birds fed methionine deficient diets was significantly lower than that of birds fed adequate diets during the initial periods of the trial. Although food intake on the inadequate diets markedly increased towards the end of the trial, egg production nevertheless remained at a low level.
9. Three regression equations were computed relating food conversion efficiency to methionine intake due to the fact that energy had a profound effect on this parameter independent of the methionine effect. From these equations it was calculated that optimal food conversion efficiency is obtained with a methionine intake in excess of 413 mg/bird d. This is substantially higher than the requirement for both optimal egg output and egg mass.
10. Body mass gains of birds on inadequate isoleucine diets were significantly poorer than that of birds receiving diets with adequate methionine levels.
11. The utilisation of methionine was not affected by dietary energy concentration.

## CHAPTER 5

### RESPONSE OF LAYING HENS TO LYSINE INTAKE.

#### INTRODUCTION.

In South Africa lysine is the most limiting amino acid in diets for laying hens due mainly to the high levels of maize used in such diets. For this reason a curve describing the response of laying hens to increasing concentrations of dietary lysine is of great economic importance in this country.

A review of the literature concerning the requirements of laying hens for lysine reveals a very wide range of values (Table 5.1). These differences are due to the factors pointed out earlier (Chapter 1), and include differences in availability in ingredients used, methods of determination of requirements and statistical procedures used to interpret the data.

Pilbrow and Morris (1974) have described a response curve to lysine. However, their diets contained high levels of wheat, and it has been shown (Jensen et al. 1974; Morris, 1980) that lysine is apparently unavailable in wheat-based diets due possibly to an amino acid imbalance in this ingredient.

In this study maize was used as the main cereal source as this pertains to South African conditions. Also, three dietary energy levels were used in order to confirm that changes in food intake brought about by differences in energy concentrations will not affect the utilisation of lysine, the first-limiting amino acid in this experiment.



TABLE 5.1 : Summary of experimental evidence about the lysine requirement of the laying hen and the recommendations of the Agricultural Research Council (ARC, 1975) and National Research Council (NCR, 1971). In addition to the calculated requirements of Johnson and Fisher (1958) and Moran, Summers and Pepper (1967) shown here, calculated requirements based on models suggested by Hurwitz and Bornstein (1973), Wilgus (1976) and Smith (1978) have also been given.

Reference	No and range of dietary contents used. No/g/kg.	Requirement g/kg	Intake at requirement (mg/day)	Egg production. g/day or %.	Notes
Ingram et al (1951)	4/3,5 to 6,0	5,20	-	64 %	Requirement based on available lysine.
Ingram & Little (1958)	-	4,63 - 4,88	-	-	Abstract only. Presumably available lysine.
Johnson & Fisher (1958)	4/4,0 to 5,5	5,0	-	-	N - balance method. Purified diets. (Available lysine.)
Harms & Waldroup (1963)	2/5,2 to 6,2	5,2	495	38,5 g	Used young pullets. On calculation appears to be total lysine
Taylor, Payne & Lewis (1967)	-	-	794	48 g	Wheat, maize and Soyabean based diets. Total lysine.
Moran, Summers & Pepper (1967)	-	-	454	46,5 g	Calculated requirement.
Thomas cited by Combs (1968)	-	-	723	50 g	Calculated from equation. 2 kg body mass. Gaining 1,5 g/day. Available lysine.
Thomas cited by Combs (1968)	-	-	660	45 g	Calculated from equation. 2 kg body mass. Gaining 1,5 g/day. Available lysine.
Bray (1969)	9/3,1 to 6,7	4,93	522	45 g	Corn-soya based diets.
Carlson & Guenther (1969)	Various	-	645	36,9 g	Used various diets. Concluded requirement slightly less than 645 mg.
Roberson (1970)	2/5,2 to 6,3	5,3	594	46,5 g	Corn, milo, cottonseed diets. Total lysine.
Roberson (1970)	2/5,3 to 6,3	5,3	608	49,4 g	Corn, milo, cottonseed diets. Total lysine.
March & Biely (1972)	Various	7,0 - 7,5	800 - 850	-	Wheat based diets.
Hurwitz & Bornstein (1973)	-	-	641	50 g	(Calculated on Model B. Birds with 2 kg body mass. Gaining 1,5 g/day).
Hurwitz & Bornstein (1973)	-	-	586	45 g	(Calculated on Model B. Birds with 2 kg body mass. Gaining 1,5 g/day. Hurwitz & Bornstein (1973) requirements based on total lysine).
Jensen, Chang & Falen (1974)	3/5,72 to 7,86	7,02	765	42,03g	Total lysine. Young pullets. Wheat based diets.
Jensen, Chang & Falen (1974)	4/5,23 to 8,23	6,23	666	39,86g	Total lysine. Young pullets. Corn based diets.

/Continued .....

TABLE 5.1 (Continued)

Reference	No and range of dietary contents used. No/g/kg.	Requirement g/kg	Intake at requirement. (mg/day)	Egg production. g/day or %.	Notes
Sell & Johnson (1974)	8/4,9 to 6,5	5,4 - 6,0	594 - 624	38,15-39,07g	Wheat-soya diets. Two energy levels.
Pilbrow & Morris (1974)	-	-	655	50 g	Calculated from equation for individual bird. Body mass 2 kg. Gaining 1,5 g/d. Available lysine.
Pilbrow & Morris (1974)	-	-	608	45 g	Calculated from equation for individual bird. Body mass 2 kg. Gaining 1,5 g/d. Available lysine.
A R C (1975)	-	7,50	750	50 g	Available lysine. Assuming 110 g feed intake/day.
Scott, Nesheim & Young (1976)	-	6,43 - 7,21	720 - 750	52,7 - 53,4g	Total lysine. Used two energy diets over 11 week period.
Wilgus (1976)	-	-	735	50 g	(Calculated on modified Model B. Birds with 2 kg body mass. Gaining 1,5 g/day).
Wilgus (1976)	-	-	671	45 g	(Calculated on modified Model B. Birds with 2 kg body mass. Gaining 1,5 g/day. Wilgus (1976) requirements based on total lysine).
Latshaw (1976)	4/4,5 to 5,7	5,3	657	44,8 g	Young pullets. Total lysine. Corn based diets. Diet ME 2 880 kcal/kg.
Latshaw (1976)	4/4,9 to 6,1	5,3	636	42,11g	Young pullets. Total lysine. Diet ME 3 110 kcal/kg.
Chi & Speers (1976)	6/3,5 to 8,5	6,5	676	46 g	Total lysine. Young pullets.
N R C (1977)	-	6,0	660	-	Total lysine. Determined with corn-soyabean meal.
Gardiner & Dubetz (1977)	5/4,16 to 8,45	5,22	637	42,4 g	Using high protein wheat as a cereal.
Smith (1978)	-	-	702	50 g	(Calculated on Model 2. Birds with 2 kg body mass. Gaining 1,5 g/d).
Smith (1978)	-	-	641	45 g	(Calculated on Model 2. Birds with 2 kg body mass. Gaining 1,5 g/d. Smith (1978) requirements based on total lysine).
Daub et al (1978)	4/6,5 to 8,5	7,0	873	50,3 g	Corn based diet. On calculation appears to be total lysine.
Nathanael & Sell (1980)	8/5,7 to 7,8	6,6	700	44 g	Corn based diet. Young pullets. Total lysine.

## MATERIALS AND METHODS.

### *Dietary treatments.*

Using the recommended levels of amino acids for layers at varying energy levels in feeds (Scott et al. 1976) "summit" and "dilution" diets at two energy levels were formulated. The minimum content of all essential amino acids except lysine in the summit diets were set at 175 percent of the suggested requirements. The lysine levels however, were fixed at 135 percent of these requirements. In the dilution diets minimum levels of all the essential amino acids except lysine were fixed at 90 percent while lysine was fixed at 60 percent of requirements. In an effort to reduce as far as possible unwanted surpluses of amino acids in the summit diets, the crude protein content of each diet was kept at a minimum. The composition of these four diets is shown in Table 5.2. The specified amino acid contents as set out by the procedure for the summit and dilution diets are shown in Table 5.3. The calculated contents achieved in the formulated diets relative to the suggested requirements are also indicated.

By blending the summit and dilution diets in appropriate proportions as indicated in Table 5.4, twelve experimental diets were produced, consisting of three energy levels viz. 10,49, 11,09 and 11,7 MJ ME/kg and four lysine concentrations at each energy level representing 135, 110, 85 and 60 percent of the lysine requirement.

### *Preliminary trial*

To confirm that lysine was the first limiting amino acid in the experimental diets, a preliminary trial was conducted prior to the main experiment.

The trial was conducted in a convection house using 504 laying hens 38 weeks of age. Four treatments indicated in Table 5.5



TABLE 5.2: Compositions (g/kg) of the summit and dilution diets.

Ingredients	Diet 1 (Summit diet)	Diet 4 (Dilution diet)	Diet 9 (Summit diet)	Diet 12 (Dilution diet)
Maize (ground)	402,00	541,00	460,00	648,00
Wheat bran	177,50	216,00	-----	112,20
Peanut meal (400)*	34,00	14,00	100,00	-----
Sunflower meal (380)*	130,00	-----	130,00	70,00
Fish meal (660)*	50,00	-----	60,00	-----
Monocalcium Phosphate	-----	16,00	-----	17,00
Sunflower hulls (ground)	-----	52,80	-----	-----
Maize gluten meal (600)*	97,00	57,00	140,80	50,00
Limestone Powder	86,00	96,00	84,00	96,00
Bone meal	18,00	-----	20,00	-----
Salt	2,00	4,00	2,00	3,60
Methionine (98%)	0,30	-----	-----	-----
Choline chloride	1,00	1,00	1,00	1,00
Vitamin Premix **	1,20	1,20	1,20	1,20
Mineral Premix ***	1,00	1,00	1,00	1,00

Calculated analysis

Metabolisable energy (MJ/kg)	10,49	10,49	11,70	11,70
Crude protein (g N x 6,25/kg)	220,00	120,84	257,84	129,16
Lysine (g/kg)	8,1	3,6	9,1	4,2
Calcium (g/kg)	37,3	38,3	37,2	38,7
Phosphorus (g/kg)	7,0	6,8	6,6	6,8

\* Assumed crude protein (g N x 6,25?kg)

\*\* Vitamin Premix provides per kg of diet: 10 035 I.U. Vit. A, 2 000 I.U. Vit. D3, 12,94 mg Vit. E, 2 mg menadionsodiumbi-sulfite, 1,0 mg thiamin, 5,16 mg riboflavin, 14,8 mg calcium pantothenate, 43,6 nicotinic acid, 10 micrograms Vit. B12, 0,4 mg folic acid, 300 mg choline.

\*\*\* Mineral premix provides per kg of diet: 4 mg copper, 40 mg zinc, 2 mg iodine, 80 mg manganese.

TABLE 5.3: Calculated amino acid contents of the summit and dilution diets relative to the assumed requirements of the laying hen.

	Requirement according to Scott, Nesheim and Young (1976) in (g/kg) for diet with ME content of 10,49 MJ/kg	Amino acid contents expressed as multiples of requirements for diet with ME content of 10,49 MJ/kg.		Requirement according to Scott, Nesheim and Young (1976) in (g/kg) for diet with ME content of 11,70 MJ/kg.	Amino acid contents expressed as multiples of requirements for diet with ME content of 11,70 MJ/kg.	
		(Diet 1 (summit diet))	(Diet 4 (dilution diet))		(Diet 9 (summit diet))	(Diet 12 (dilution diet))
Arginine	7,10	1,94	0,89	8,00	2,05	0,91
Lysine	6,00	1,35	0,60	6,70	1,35	0,60
Methionine	3,00	1,80	0,90	3,40	1,76	0,91
Cystine	2,30	1,83	1,22	2,60	1,77	1,19
Tryptophan	1,50	1,53	0,80	1,70	1,47	0,76
Histidine	2,90	1,83	0,97	3,20	1,97	0,97
Leucine	11,00	1,98	1,25	12,10	2,25	1,21
Isoleucine*	5,08	1,56	0,79	5,7	1,67	0,79
Phenylalanine	6,60	1,59	0,88	7,40	1,76	0,86
Tyrosine	2,90	2,14	1,62	3,20	2,50	1,34
Threonine	5,20	1,56	0,83	5,80	1,66	0,83
Valine	6,10	1,85	1,02	6,90	1,94	0,97

\* Optimum isoleucine found in chapter 4 used in preference to Scott, Nesheim and Young (1976) which was considered too high. (Also related to adjusted energy intake suggested by Morris, 1968).

TABLE 5.4: Summary of dilution technique and calculated analyses of the experimental diets.

Diet	Blending ratio				Calculated dietary lysine (g/kg)	Calculated dietary protein (gNx6,25/kg)	Calculated dietary energy MJ/kg
	(Diet 1 (summit diet))	(Diet 9 (summit diet))	(Diet 4 (dilution diet))	(Diet 12 (dilution diet))			
1 a	1 000	-	-	-	8,1	220,00	10,49
1 b	666,7	-	333,3	-	6,6	186,95	10,49
1 c	333,3	-	666,7	-	5,1	153,89	10,49
1 d	-	-	1 000	-	3,6	120,84	10,49
2 a	500	500	-	-	8,6	238,92	11,09
2 b	333,35	333,35	166,65	166,65	7,0	200,95	11,09
2 c	166,65	166,65	333,35	333,35	5,4	162,97	11,09
2 d	-	-	500	500	3,8	125,00	11,09
3 a	-	1 000	-	-	9,1	257,84	11,70
3 b	-	666,7	-	333,3	7,4	214,95	11,70
3 c	-	333,3	-	666,7	5,7	172,05	11,70
3 d	-	-	-	1 000	4,0	129,16	11,70



were used, each treatment being tested on three replications of 42 birds each. Prior to the introduction of these experimental diets all birds received a normal commercial layer diet for at least six weeks. Mean rate of lay in the flock at the start of the trial was 84,5 percent.

For the first three weeks diets 4 and 12 were each allocated to six pens for adaptation purposes. During the three week observation period each of the above mentioned diets were supplemented with lysine giving two variations of each diet, the first serving as a control (Table 5.5).

TABLE 5.5: Diets used in preliminary experiment.

Diet code	Calculated lysine content (g/kg)	Additions made to diet
4	3,6	----
12	4,2	----
4	4,8	1,20
12	5,56	1,36

*Main experiment.*

Birds of the Amber Link strain were used in this trial. They were reared in a controlled environment house and fed ad lib. on normal commercial rearing diets. A constant eight hour photoperiod was used in the rearing period. After 20 weeks of age lighting was increased to 14 hours and then by 30 minutes per week to a maximum of 16 hours per day, whereafter the daylength was held constant.

At 21 weeks of age the birds were moved into a convection laying house containing reverse faced wire cages (45,7 cm x 30,5 cm) with the longer dimension facing the food trough. Three birds were placed in each cage. Twelve treatments were randomly allocated to 36 pens, giving three replicates of 66 birds for each treatment.

Prior to the introduction of the twelve experimental diets at 22 weeks of age all the caged pullets received a layer ration containing 155 g crude protein (gN x 6,25/kg) and 37 g calcium/kg.

The trial commenced on the 17th May, 1979 when the birds were 22 weeks of age and continued for nine 28 d periods. Egg numbers, total egg mass and mortality were recorded daily. The body mass of 18 birds per replication was determined at the commencement of each 28 d period. Body mass of the same birds was measured on each occasion. From this data the mean body mass gain/d was calculated. Feed intake was measured weekly.

The data was analysed per 28 d period and in addition the three periods encompassing peak production were combined for a separate analysis.

## RESULTS AND DISCUSSION.

### *Preliminary trial.*

Similar to the findings in the isoleucine and methionine trials, egg production declined markedly when birds were switched from the commercial diet to the two dilution diets (Table 5.6). It will further be noted that the rate of lay of birds on unsupplemented diets continued to decline whereas the addition of lysine evoked a significant response.

TABLE 5.6: Percentage egg production response of layers on supplemented and unsupplemented dilution diets.

Diet	Weeks					
	1	2	3	4	5	6
4	83,6	71,8	65,8	64,4	64,0	57,8
4 + L*	83,9	71,8	69,1	64,8	68,5	67,2
12	82,9	71,4	66,5	67,9	63,3	60,5
12 + L*	83,8	74,1	70,0	66,8	71,8	70,8

\* L denotes Lysine.



Similarly it will be observed from Table 5.7 that birds on supplemented diets responded positively in respect of egg output (g/bird d) when compared to the unsupplemented controls.

TABLE 5.7: Egg output response (g/bird d) to unsupplemented and supplemented dilution diets.

Diet	Weeks			
	3	4	5	6
4	37,54	36,64	36,03	32,25
4 + L	39,42	37,60	40,15	39,41
12	37,94	38,51	35,41	33,55
12 + L	39,94	38,83	41,92	41,66

From these results it can therefore be concluded that lysine was the first limiting amino acid. Since lysine was the first limiting amino acid in the summit and dilution diets it was also the first limiting amino acid in the blended diets. Any improved performance by the feeding of the diets with increasing levels of lysine can thus only be ascribed to this amino acid since there can be no other amino acid which can become first limiting in this procedure.

#### RESULTS BY PERIOD (MAIN EXPERIMENT)

The following production parameters for each of the nine periods of the trial are presented in Tables 5.8 to 5.16: Rate of lay (egg number/100 bird d), egg mass (g/egg), egg output (g/bird d), food intake (g/bird d), food conversion efficiency (g egg produced/g feed consumed) and body mass gain (g/bird d).

In each table the main effects of lysine (L) and of energy (E) are shown, together with the interaction terms (L x E). Also, the standard errors of each mean (SEM) and the least significant differences (LSD) at  $P < 0,05$  and  $P < 0,01$  are indicated.

These same results are illustrated in Figures 5.1 to 5.6 where the effects of the four levels of dietary lysine concentrations



TABLE 5.8: Response to Lysine and Energy intake for period 1. (22 - 25 weeks of age)

5.8.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	73,70	76,93	70,18	73,61	73,60
2	73,67	73,86	74,59	68,15	72,56
3	69,88	74,84	71,01	70,74	71,62
Mean	72,41	75,21	71,93	70,83	72,60
	SEM		LSD (0,05)		LSD (0,01)
L	0,92		2,69		3,66
E	0,80		2,33		3,17
L x E	1,59		4,67		6,34

5.8.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	44,83	46,01	44,63	45,89	45,34
2	43,86	45,59	45,81	45,04	45,08
3	44,36	45,73	45,23	44,51	44,96
Mean	44,35	45,78	45,22	45,15	45,12
	SEM		LSD (0,05)		LSD (0,01)
L	0,266		0,78		1,06
E	0,231		0,676		0,92
L x E	0,461		1,35		1,84

5.8.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	33,42	35,71	31,85	34,04	33,76
2	32,84	34,07	34,57	31,24	33,18
3	31,35	34,55	32,57	31,73	32,55
Mean	32,54	34,78	33,00	32,34	33,16
	SEM		LSD (0,05)		LSD (0,01)
L	0,45		1,33		1,82
E	0,396		1,16		1,57
L x E	0,79		2,31		3,15

TABLE 5.8 (Continued)

5.8.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	112,91	115,26	117,79	122,56	117,15
2	109,97	112,72	115,39	121,90	114,99
3	106,10	111,29	114,97	118,81	112,79
Mean	109,66	113,09	116,05	121,12	114,98
	SEM		LSD (0,05)		LSD (0,01)
L	0,85		2,49		3,38
E	0,73		2,15		2,93
L x E	1,47		4,31		5,86

5.8.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,297	0,310	0,267	0,280	0,288
2	0,303	0,307	0,300	0,257	0,292
3	0,297	0,313	0,287	0,267	0,291
Mean	0,299	0,310	0,284	0,268	0,290
	SEM		LSD (0,05)		LSD (0,01)
L	0,0051		0,015		0,020
E	0,0044		0,013		0,018
L x E	0,0088		0,026		0,035

5.8.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	2,51	2,87	5,51	4,32	3,80
2	4,60	4,95	3,51	2,47	3,88
3	3,92	5,13	5,59	3,23	4,47
Mean	3,68	4,32	4,87	3,34	4,05
	SEM		LSD (0,05)		LSD (0,01)
L	0,58		1,70		2,32
E	0,50		1,47		2,00
L x E	1,01		2,95		4,01

TABLE 5.9: Response to Lysine and Energy intake during period 2. (26 - 29 weeks of age)

5.9.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	89,79	92,03	89,30	77,46	87,15
2	91,54	91,45	91,39	82,46	89,21
3	89,90	91,61	90,44	78,72	87,67
Mean	90,41	91,70	90,38	79,55	88,01
	SEM		LSD (0,05)		LSD (0,01)
L	0,47		1,37		1,86
E	0,41		1,19		1,61
L x E	0,81		2,37		3,22

5.9.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	49,70	50,3	49,97	48,76	49,68
2	49,15	50,27	49,96	48,94	49,58
3	49,13	50,40	49,96	48,19	49,42
Mean	49,32	50,32	49,96	48,63	49,56
	SEM		LSD (0,05)		LSD (0,01)
L	0,19		0,57		0,78
E	0,17		0,50		0,68
L x E	0,34		0,99		1,35

5.9.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	44,62	46,27	44,62	37,77	43,32
2	44,97	45,96	45,65	40,35	44,23
3	44,15	46,17	45,19	37,95	43,36
Mean	44,58	46,13	45,15	38,69	43,64
	SEM		LSD (0,05)		LSD (0,01)
L	0,29		0,84		1,14
E	0,25		0,74		1,01
L x E	0,49		1,45		1,98



TABLE 5.9 (Continued)

5.9.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	118,80	123,75	127,34	123,34	123,31
2	112,41	116,63	122,83	122,48	118,59
3	104,17	113,82	122,37	120,97	115,33
Mean	111,79	118,07	124,18	122,26	119,08
	SEM		LSD (0,05)		LSD (0,01)
L	1,01		2,97		4,04
E	0,88		2,57		3,50
L x E	1,75		5,14		6,99

5.9.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,377	0,373	0,350	0,303	0,351
2	0,400	0,393	0,370	0,330	0,373
3	0,423	0,407	0,370	0,313	0,378
Mean	0,400	0,391	0,363	0,316	0,368
	SEM		LSD (0,05)		LSD (0,01)
L	0,0034		0,010		0,013
E	0,0031		0,009		0,012
L x E	0,0058		0,017		0,023

5.9.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,54	0,49	- 0,79	- 2,64	- 0,60
2	0,42	0,54	- 0,63	- 0,12	0,05
3	- 0,48	1,05	0,07	- 1,63	- 0,24
Mean	0,16	0,69	- 0,45	- 1,46	- 0,26
	SEM		LSD (0,05)		LSD (0,01)
L	0,365		1,07		1,46
E	0,317		0,93		1,27
L x E	0,63		1,86		2,53

TABLE 5.10: Response to Lysine and Energy intake during period 3. (30 - 33 weeks of age)

5.10.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	85,96	88,22	86,97	65,61	81,69
2	86,70	87,34	86,28	69,75	82,52
3	89,62	88,15	85,97	77,89	85,41
Mean	87,43	87,91	86,41	71,08	83,21
	SEM		LSD (0,05)		LSD (0,01)
L	0,84		2,45		3,33
E	0,72		2,12		2,88
L x E	1,45		4,24		5,77

5.10.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	51,75	52,73	51,74	49,36	51,40
2	51,20	52,08	51,79	49,75	51,21
3	51,46	52,26	52,19	50,26	51,54
Mean	51,47	52,36	51,91	49,79	51,38
	SEM		LSD (0,05)		LSD (0,01)
L	0,188		0,55		0,74
E	0,161		0,473		0,64
L x E	0,322		0,946		1,28

5.10.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	44,49	46,52	45,00	32,39	42,10
2	44,38	45,48	44,69	34,71	42,31
3	46,13	46,06	44,87	39,18	44,06
Mean	45,00	46,02	44,85	35,42	42,82
	SEM		LSD (0,05)		LSD (0,01)
L	0,41		1,20		1,63
E	0,35		1,04		1,41
L x E	0,71		2,07		2,82

TABLE 5.10 (Continued)

5.10.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	120,92	123,91	126,68	111,86	120,84
2	115,68	119,00	125,67	114,62	118,74
3	114,69	118,22	121,36	116,75	117,75
Mean	117,10	120,37	124,57	114,41	119,11
	SEM		LSD (0,05)		LSD (0,01)
L	1,14		3,35		4,55
E	0,99		2,90		3,94
L x E	1,98		5,79		7,87

5.10.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,367	0,373	0,353	0,290	0,346
2	0,383	0,383	0,357	0,303	0,357
3	0,403	0,390	0,370	0,337	0,375
Mean	0,384	0,382	0,360	0,310	0,359
	SEM		LSD (0,05)		LSD (0,01)
L	0,0044		0,013		0,018
E	0,0038		0,011		0,015
L x E	0,0078		0,023		0,031

5.10.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	1,75	1,48	0,36	- 4,23	- 0,16
2	2,01	1,06	0,88	- 4,07	- 0,03
3	2,25	1,24	1,62	- 0,95	1,04
Mean	2,00	1,26	0,95	- 3,08	0,28
	SEM		LSD (0,05)		LSD (0,01)
L	0,56		1,64		2,23
E	0,48		1,42		1,93
L x E	0,97		2,84		3,86



TABLE 5.11: Response to Lysine and Energy intake during period 4. (34 - 37 weeks of age)

5.11.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	90,05	88,96	84,74	63,34	81,77
2	87,62	87,72	84,95	62,22	80,63
3	91,52	88,10	87,87	69,18	84,17
Mean	89,73	88,26	85,86	64,91	82,19
	SEM		LSD (0,05)		LSD (0,01)
L	1,22		3,58		4,87
E	1,06		3,10		4,21
L x E	2,12		6,20		8,43

5.11.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	54,26	55,00	53,34	50,96	53,39
2	54,08	54,53	53,91	50,86	53,35
3	53,99	54,91	54,29	51,23	53,60
Mean	54,11	54,81	53,84	51,02	53,45
	SEM		LSD (0,05)		LSD (0,01)
L	0,16		0,47		0,64
E	0,14		0,41		0,55
L x E	0,28		0,82		1,11

5.11.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	48,87	48,93	45,20	32,40	43,85
2	47,38	47,84	45,79	31,68	43,17
3	49,39	48,36	47,70	35,49	45,23
Mean	48,55	48,38	46,23	33,19	44,09
	SEM		LSD (0,05)		LSD (0,01)
L	0,69		2,01		2,73
E	0,59		1,74		2,37
L x E	1,19		3,48		4,74

TABLE 5.11 (Continued)

5.11.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	126,29	125,32	128,78	114,59	123,75
2	120,84	121,03	125,67	109,44	119,24
3	123,28	118,14	120,78	114,00	119,05
Mean	123,47	121,50	125,08	112,68	120,68
	SEM		LSD (0,05)		LSD (0,01)
L	1,03		3,02		4,11
E	0,89		2,62		3,56
L x E	1,78		5,23		7,11

5.11.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,390	0,387	0,353	0,287	0,354
2	0,393	0,397	0,367	0,290	0,362
3	0,403	0,410	0,393	0,313	0,380
Mean	0,396	0,398	0,371	0,297	0,365
	SEM		LSD (0,05)		LSD (0,01)
L	0,0055		0,016		0,022
E	0,0048		0,014		0,019
L x E	0,0096		0,028		0,038

5.11.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,91	0,11	1,36	- 1,12	0,31
2	- 0,51	1,44	0,72	- 1,97	- 0,08
3	2,93	2,23	1,26	- 1,17	1,31
Mean	1,11	1,26	1,11	- 1,42	0,52
	SEM		LSD (0,05)		LSD (0,01)
L	0,64		1,87		2,54
E	0,55		1,62		2,20
L x E	1,10		3,23		4,39

TABLE 5.12: Response to Lysine and Energy intake during period 5. (38 - 41 weeks of age)

5.12.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	86,82	86,25	83,37	63,17	79,90
2	89,19	86,58	82,42	63,96	80,54
3	90,95	85,26	83,34	63,51	80,77
Mean	88,99	86,03	83,04	63,55	80,40
	SEM		LSD (0,05)		LSD (0,01)
L	1,06		3,12		4,25
E	0,92		2,71		3,68
L x E	1,85		5,41		7,35

5.12.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	55,92	56,37	54,65	51,62	54,64
2	55,52	56,04	55,16	51,64	54,59
3	55,67	56,32	55,33	51,83	54,79
Mean	55,70	56,24	55,05	51,70	54,67
	SEM		LSD (0,05)		LSD (0,01)
L	0,201		0,59		0,80
E	0,174		0,51		0,69
L x E	0,348		1,02		1,39

5.12.3: Total egg mass produced (g/bird d)

Energy MJ/kg.	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	48,56	48,62	45,55	32,61	43,83
2	49,51	48,52	45,47	33,05	44,14
3	50,63	48,02	46,12	32,92	44,42
Mean	49,57	48,39	45,71	32,86	44,13
	SEM		LSD (0,05)		LSD (0,01)
L	0,58		1,69		2,30
E	0,50		1,46		1,99
L x E	1,00		2,93		3,98



TABLE 5.12 (Continued)

5.12.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	125,78	126,43	127,61	124,11	125,98
2	122,73	124,02	122,59	123,56	123,22
3	126,76	119,91	119,74	116,13	120,64
Mean	125,09	123,46	123,31	121,27	123,28
	SEM		LSD (0,05)		LSD (0,01)
L	1,57		4,60		6,26
E	1,36		3,99		5,42
L x E	2,72		7,97		10,84

5.12.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,387	0,383	0,360	0,267	0,349
2	0,403	0,393	0,370	0,267	0,358
3	0,403	0,400	0,383	0,287	0,368
Mean	0,398	0,392	0,371	0,273	0,359
	SEM		LSD (0,05)		LSD (0,01)
L	0,0048		0,014		0,019
E	0,0041		0,012		0,017
L x E	0,0082		0,024		0,033

5.12.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	1,78	1,41	- 0,41	0,49	0,82
2	2,12	1,75	1,87	- 2,33	0,85
3	2,18	0,98	1,08	- 0,37	0,97
Mean	2,03	1,38	0,85	- 0,74	0,88
	SEM		LSD (0,05)		LSD (0,01)
L	0,65		1,89		2,57
E	0,56		1,64		2,23
L x E	1,12		3,27		4,45

TABLE 5.13: Response to Lysine and Energy intake during period 6. (42 - 45 weeks of age)

5.13.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	82,94	84,12	81,59	66,00	78,66
2	86,48	83,02	81,77	64,06	78,83
3	87,22	82,88	82,49	63,07	78,91
Mean	85,55	83,34	81,95	64,37	78,80
	SEM		LSD (0,05)		LSD (0,01)
L	1,27		3,72		5,06
E	1,10		3,22		4,38
L x E	2,20		6,45		8,77

5.13.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	56,94	57,55	55,66	53,07	55,81
2	56,52	56,98	56,51	52,95	55,74
3	56,06	57,48	57,01	53,09	55,91
Mean	56,51	57,34	56,39	53,04	55,82
	SEM		LSD (0,05)		LSD (0,01)
L	0,239		0,70		0,96
E	0,208		0,61		0,83
L x E	0,416		1,22		1,65

5.13.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	47,24	48,41	45,41	35,06	44,03
2	48,87	47,31	46,21	33,92	44,08
3	48,89	47,63	47,02	33,47	44,25
Mean	48,33	47,78	46,21	34,15	44,12
	SEM		LSD (0,05)		LSD (0,01)
L	0,73		2,15		2,92
E	0,63		1,86		2,53
L x E	1,27		3,72		5,06

TABLE 5.13 (Continued)

5.13.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	124,42	124,43	129,29	123,82	125,49
2	124,26	120,89	126,04	118,92	122,53
3	125,40	118,91	120,09	115,17	119,89
Mean	124,69	121,41	125,14	119,30	122,64
	SEM		LSD (0,05)		LSD (0,01)
L	1,70		4,99		6,78
E	1,47		4,32		5,87
L x E	2,95		8,64		11,74

5.13.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,380	0,390	0,350	0,283	0,351
2	0,393	0,393	0,367	0,290	0,361
3	0,393	0,403	0,393	0,293	0,371
Mean	0,389	0,396	0,370	0,289	0,361
	SEM		LSD (0,05)		LSD (0,01)
L	0,0044		0,013		0,018
E	0,0038		0,011		0,015
L x E	0,0078		0,023		0,031

5.13.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,58	1,29	1,44	0,14	0,86
2	1,44	1,69	2,37	1,83	1,83
3	1,18	1,72	1,15	0,63	1,17
Mean	1,06	1,56	1,65	0,87	1,29
	SEM		LSD (0,05)		LSD (0,01)
L	0,43		1,26		1,71
E	0,37		1,09		1,48
L x E	0,74		2,18		2,96



TABLE 5.14: Response to Lysine and Energy intake during period 7. (46 - 49 weeks of age)

5.14.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	81,24	78,36	79,62	61,29	75,12
2	84,86	79,94	80,00	61,88	76,67
3	85,60	80,13	78,47	62,71	76,73
Mean	83,90	79,48	79,36	61,96	76,17
	SEM		LSD (0,05)		LSD (0,01)
L	1,24		3,63		4,93
E	1,07		3,14		4,27
L x E	2,14		6,28		8,54

5.14.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	57,82	58,07	56,82	54,12	56,71
2	57,73	57,89	57,65	53,87	56,78
3	57,81	58,17	58,00	54,63	57,15
Mean	57,78	58,05	57,49	54,20	56,88
	SEM		LSD (0,05)		LSD (0,01)
L	0,31		0,917		1,25
E	0,27		0,79		1,08
L x E	0,54		1,59		2,16

5.14.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	46,97	45,53	45,21	33,18	42,72
2	48,97	46,28	46,14	33,34	43,68
3	49,48	46,62	45,51	34,30	43,98
Mean	48,48	46,14	45,62	33,61	43,46
	SEM		LSD (0,05)		LSD (0,01)
L	0,78		2,28		3,10
E	0,67		1,97		2,68
L x E	1,35		3,95		5,36

TABLE 5.14 (Continued)

5.14.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	127,21	130,08	131,13	128,33	129,19
2	127,38	125,51	126,16	121,39	125,11
3	126,54	118,10	120,57	119,43	121,16
Mean	127,04	124,56	125,95	123,05	125,15
	SEM		LSD (0,05)		LSD (0,01)
L	1,88		5,51		7,48
E	1,63		4,77		6,48
L x E	3,26		9,54		12,97

5.14.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,370	0,350	0,347	0,260	0,332
2	0,383	0,370	0,367	0,270	0,348
3	0,393	0,397	0,377	0,287	0,363
Mean	0,382	0,372	0,363	0,272	0,348
	SEM		LSD (0,05)		LSD (0,01)
L	0,0034		0,010		0,014
E	0,0031		0,009		0,012
L x E	0,0061		0,018		0,024

5.14.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	1,01	- 0,18	0,07	0,75	0,41
2	1,11	0,80	- 0,72	- 0,33	0,22
3	1,03	2,57	2,43	3,06	2,27
Mean	1,05	1,06	0,59	1,16	0,97
	SEM		LSD (0,05)		LSD (0,01)
L	0,65		1,89		2,57
E	0,56		1,64		2,22
L x E	1,12		3,27		4,45

TABLE 5.15: Response to Lysine and Energy intake during period 8. (50 - 53 weeks of age)

5.15.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	76,06	74,16	74,81	61,24	71,57
2	78,23	72,46	73,40	61,74	71,46
3	82,52	73,84	76,43	63,52	74,08
Mean	78,94	73,49	74,88	62,17	72,37
	SEM		LSD (0,05)		LSD (0,01)
L	1,43		4,19		5,69
E	1,24		3,63		4,93
L x E	2,48		7,26		9,87

5.15.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	57,47	58,71	57,41	54,64	57,06
2	57,31	59,15	57,95	56,90	57,83
3	57,33	58,14	58,58	55,61	57,41
Mean	57,37	58,67	57,98	55,72	57,43
	SEM		LSD (0,05)		LSD (0,01)
L	0,42		1,22		1,66
E	0,36		1,06		1,44
L x E	0,72		2,12		2,88

5.15.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	43,71	43,51	42,92	33,46	40,90
2	44,80	42,80	42,49	35,03	41,28
3	47,31	42,96	44,76	35,38	42,60
Mean	45,27	43,09	43,39	34,63	41,59
	SEM		LSD (0,05)		LSD (0,01)
L	0,83		2,43		3,30
E	0,72		2,10		2,86
L x E	1,44		4,21		5,72



TABLE 5.15 (Continued)

5.15.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	124,20	129,20	131,07	125,88	127,59
2	126,04	123,12	125,17	124,07	124,60
2	126,89	119,45	121,67	119,82	121,96
Mean	125,71	123,92	125,97	123,25	124,71
	SEM		LSD (0,05)		LSD (0,01)
L	1,87		5,47		7,44
E	1,62		4,74		6,44
L x E	3,24		9,48		12,89

5.15.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,353	0,337	0,327	0,267	0,321
2	0,357	0,350	0,343	0,283	0,333
3	0,373	0,360	0,370	0,297	0,350
Mean	0,361	0,349	0,347	0,282	0,335
	SEM		LSD (0,05)		LSD (0,01)
L	0,0051		0,015		0,020
E	0,0044		0,013		0,017
L x E	0,0085		0,025		0,034

5.15.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,34	1,58	0,69	- 1,58	0,26
2	1,88	1,15	2,05	3,08	2,04
3	1,45	0,70	0,28	- 0,02	0,60
Mean	1,22	1,14	1,01	0,49	0,97
	SEM		LSD (0,05)		LSD (0,01)
L	1,02		2,98		4,06
E	0,88		2,58		3,51
L x E	1,76		5,17		7,03

TABLE 5.16: Response to Lysine and Energy intake during period 9. (53 - 56 weeks of age)

5.16.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	72,01	71,99	71,52	56,79	68,08
2	75,54	73,42	71,38	64,49	71,21
3	76,94	73,68	72,53	62,29	71,36
Mean	74,83	73,03	71,81	61,19	70,21
	SEM		LSD (0,05)		LSD (0,01)
L	0,85		2,49		3,38
E	0,73		2,15		2,93
L x E	1,47		4,31		5,86

5.16.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	58,11	58,25	57,25	54,87	57,12
2	58,49	58,25	58,10	55,24	57,52
3	58,38	58,79	58,53	55,48	57,80
Mean	58,33	58,43	57,96	55,20	57,48
	SEM		LSD (0,05)		LSD (0,01)
L	0,26		0,75		1,02
E	0,22		0,65		0,88
L x E	0,44		1,30		1,77

5.16.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	41,85	41,97	40,94	31,16	38,98
2	44,17	42,78	41,47	35,63	41,01
3	44,93	43,32	42,46	34,58	41,32
Mean	43,65	42,69	41,62	33,79	40,44
	SEM		LSD (0,05)		LSD (0,01)
L	0,54		1,58		2,15
E	0,47		1,37		1,86
L x E	0,93		2,73		3,71

TABLE 5.16 (Continued)

5.16.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	125,0	123,3	127,5	132,9	127,2
2	124,2	123,9	125,4	130,8	126,1
3	121,9	117,0	119,7	123,0	120,4
Mean	123,7	121,4	124,2	128,9	124,6
	SEM		LSD (0,05)		LSD (0,01)
L	2,04		5,97		8,12
E	1,77		5,19		7,05
L x E	3,53		10,35		14,07

5.16.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,333	0,347	0,330	0,237	0,312
2	0,357	0,348	0,333	0,273	0,328
3	0,373	0,370	0,357	0,287	0,347
Mean	0,354	0,354	0,340	0,266	0,329
	SEM		LSD (0,05)		LSD (0,01)
L	0,0055		0,016		0,022
E	0,0048		0,014		0,019
L x E	0,0092		0,027		0,037

5.16.6: Mean body mass change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	- 1,34	- 0,84	- 0,51	0,17	- 0,63
2	- 0,86	0,86	0,05	- 0,06	0,00
3	0,07	0,77	- 0,26	- 0,57	0,00
Mean	- 0,71	0,26	- 0,24	- 0,15	- 0,21
	SEM		LSD (0,05)		LSD (0,01)
L	0,83		2,43		3,30
E	0,72		2,10		2,86
L x E	1,43		4,20		5,71



have been plotted at each energy level for the nine periods of the trial.

The effects of dietary lysine only will be considered in the present discussion as the effects of energy on the production parameters measured will be discussed in Chapter 6.

*Egg production.*

A general downward trend in egg production was observed as the lysine levels of diets were reduced. As in the isoleucine and methionine trials significantly lower egg production levels were obtained on diets with inadequate lysine concentrations. In most periods of the experiment birds on the second dilution diets also produced at a significantly lower rate than those on the summit diets. Although egg production of birds on the first dilution diets was higher than on the second dilution diets, the difference was non significant for all periods.

Production of birds on the medium and high energy summit diets was considerably higher than that of birds at similar energy levels but lower dietary lysine concentrations. At the low dietary energy level birds on the summit diet did not show this superior performance. Egg production of birds on the second and lowest dilution diets at all energy levels was very similar. Egg production however, on the low energy summit diets was considerably lower than that on the higher energy summit diets.

The reason for this lowered production on the low energy summit diet can most probably be ascribed to the lower lysine intake on this diet. As will be shown later, birds on the high energy diets consumed considerably more lysine per day than did birds on the low energy diets. This increased intake was reflected in a higher percentage egg production. This contrasts with the results in the methionine trial (Chapter 4) where, in spite of

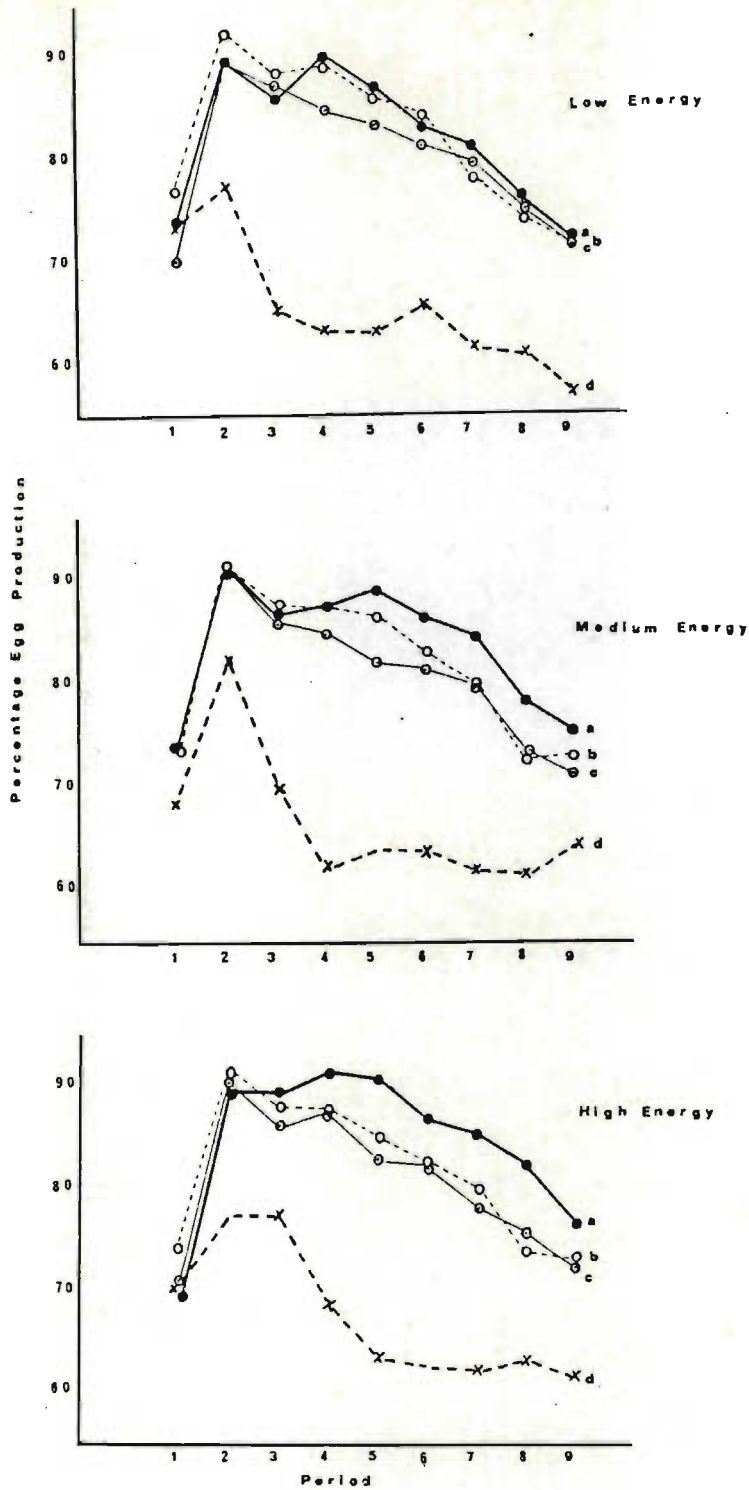


Figure 5.1 The effect of energy and lysine/energy ratio on percentage egg production during nine 28 d periods. (Refer to Table 5.4 for a description of the diets fed.)

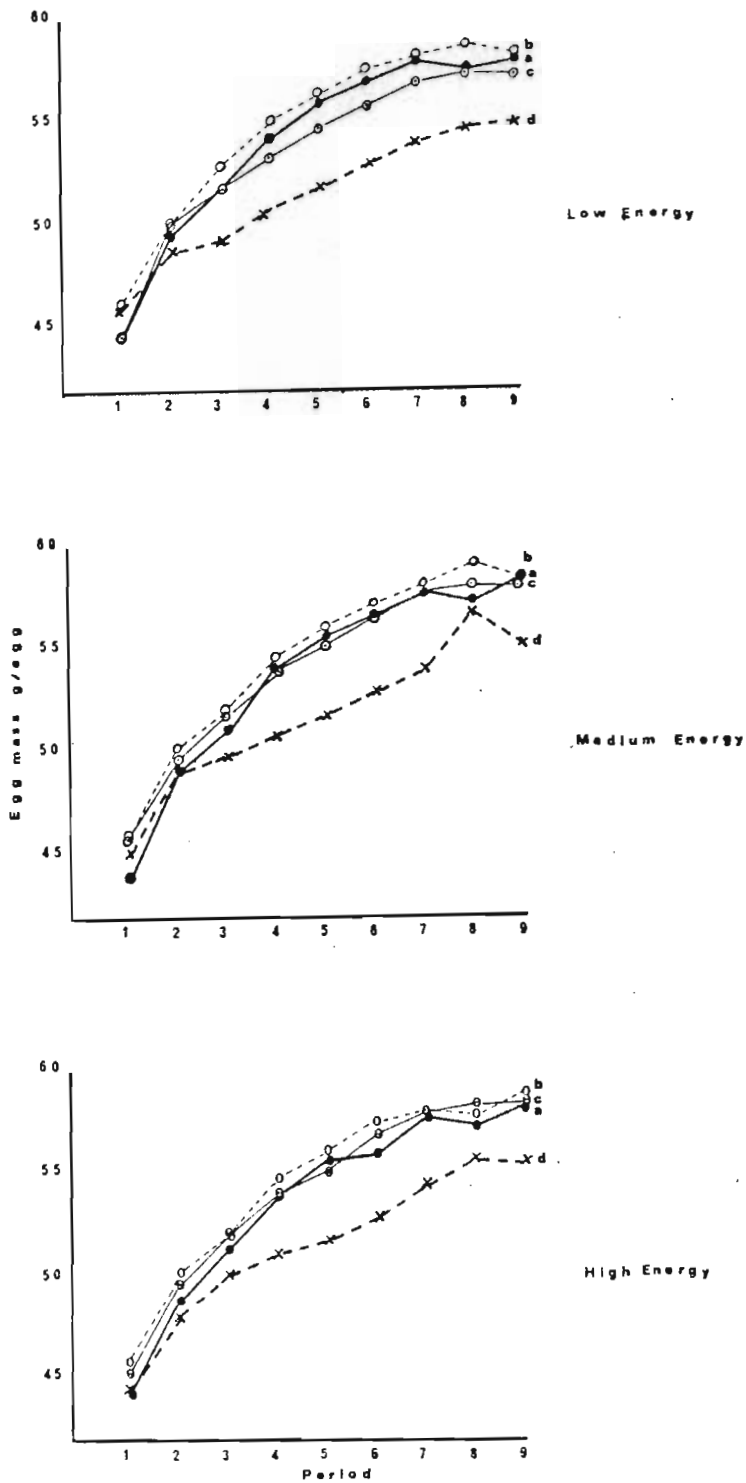


Figure 5.2 The effect of energy and lysine/energy ratio on egg mass (g/egg) during nine 28 d periods. (Refer to Table 5.4 for a description of the diets fed.)



a higher methionine intake on the high energy diets, percentage egg production did not improve correspondingly. Lysine therefore appears to have a more profound effect on percentage egg production than does methionine.

*Egg mass.*

Egg mass of birds on the lowest dilution diets was significantly lower than that of birds on the other treatments. In contrast to the observations from the methionine trial egg mass obtained from birds on the first dilution diets in this trial were higher than those on the summit diets. This was evident in all the periods and at all energy levels. Another deviation from the results of the methionine trial is that the range of egg mass values between the lowest dilution diet and the other diets was retained throughout the experiment.

As in the methionine trial feed intake on the lowest dilution diets increased during the latter stages of the trial which resulted in higher lysine intakes. However, the increased lysine intake did not improve egg mass to the same extent as did increased methionine intakes.

The lower egg mass results obtained on the lowest dilution diets at all three energy levels throughout the experiment implies that lysine cannot be ignored as a factor affecting this parameter.

*Egg output.*

Egg output on the first dilution diets was slightly higher than that of output on the summit diets. This pattern changed in the fourth period when the output on the summit diets exceeded that of the first dilution diets and this continued until the end of the experiment.

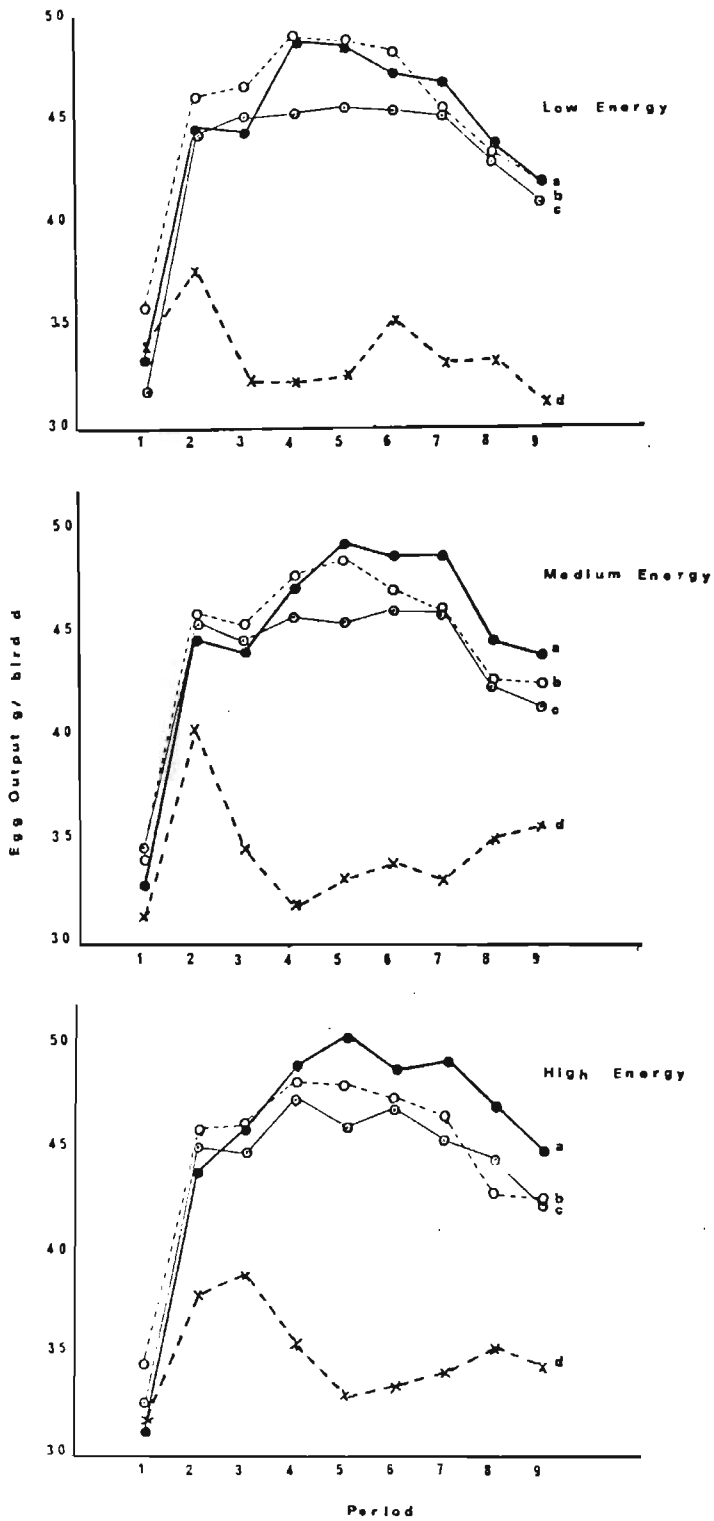


Figure 5.3 The effect of energy and lysine/energy ratio on egg output (g/bird) during nine 28 d periods. (Refer to Table 5.4 for a description of the diets fed.)

Further, it appears that lysine concentrations of 0,51, 0,54 and 0,57 percent in the 10,49, 11,09 and 11,7 MJ ME/kg diets respectively were inadequate as egg output from these birds was significantly lower than that from birds on the summit diets during periods four, five, seven and nine. Figure 5.3 illustrates that egg output from laying hens on the low energy summit diets was lower than the output from birds on summit diets at higher energy concentrations. This is explained by lower lysine intakes on lower energy diets when amino acids are related to dietary energy concentration.

#### *Food intake.*

Similar to findings in the methionine trial food intake on the lowest dilution diets was significantly lower than that of birds supplied diets containing adequate lysine. In the same manner food intake on the lowest dilution diets during the latter periods increased and at the end of the trial these intakes were higher than intakes on any of the other treatments.

Food intake of birds on diets with marginal lysine levels (second dilution diets) also increased in an effort to maintain production. Figure 5.4 also shows an abnormal increase in food intake of birds on the high energy summit diets during periods five to nine for which no logical explanation can be found.

#### *Food conversion efficiency.*

Food conversion efficiency of birds on the second and lowest dilution diets were significantly poorer in all periods than those fed diets with the two higher lysine concentrations. These results concur with those of the methionine trial for reasons previously explained. From Figure 5.5 it will also be observed that the food conversion efficiency of birds on the summit and first dilution diets were very similar throughout the trial.



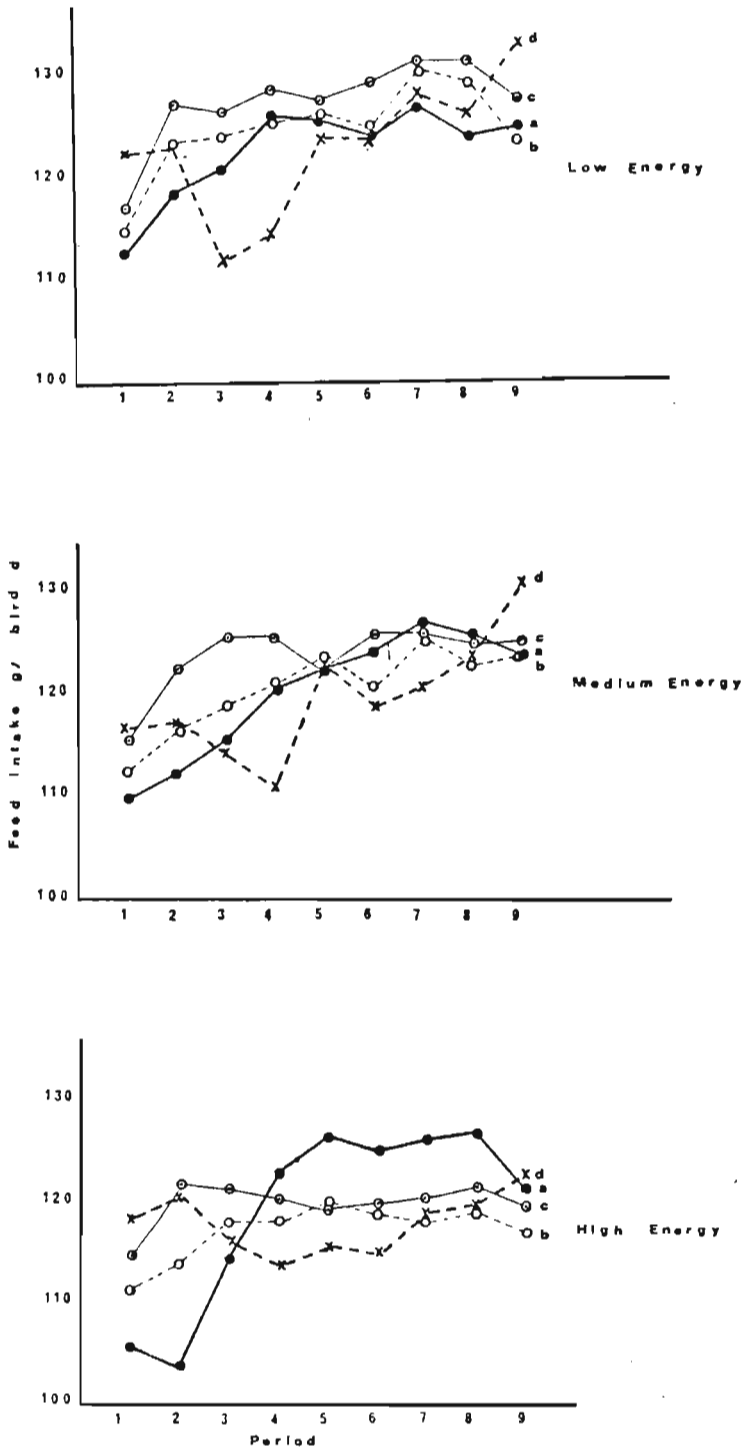


Figure 5.4 The effect of energy and lysine/energy ratio on food intake (g/bird d) during nine 28 d periods. (Refer to Table 5.4 for a description of the diets fed.)

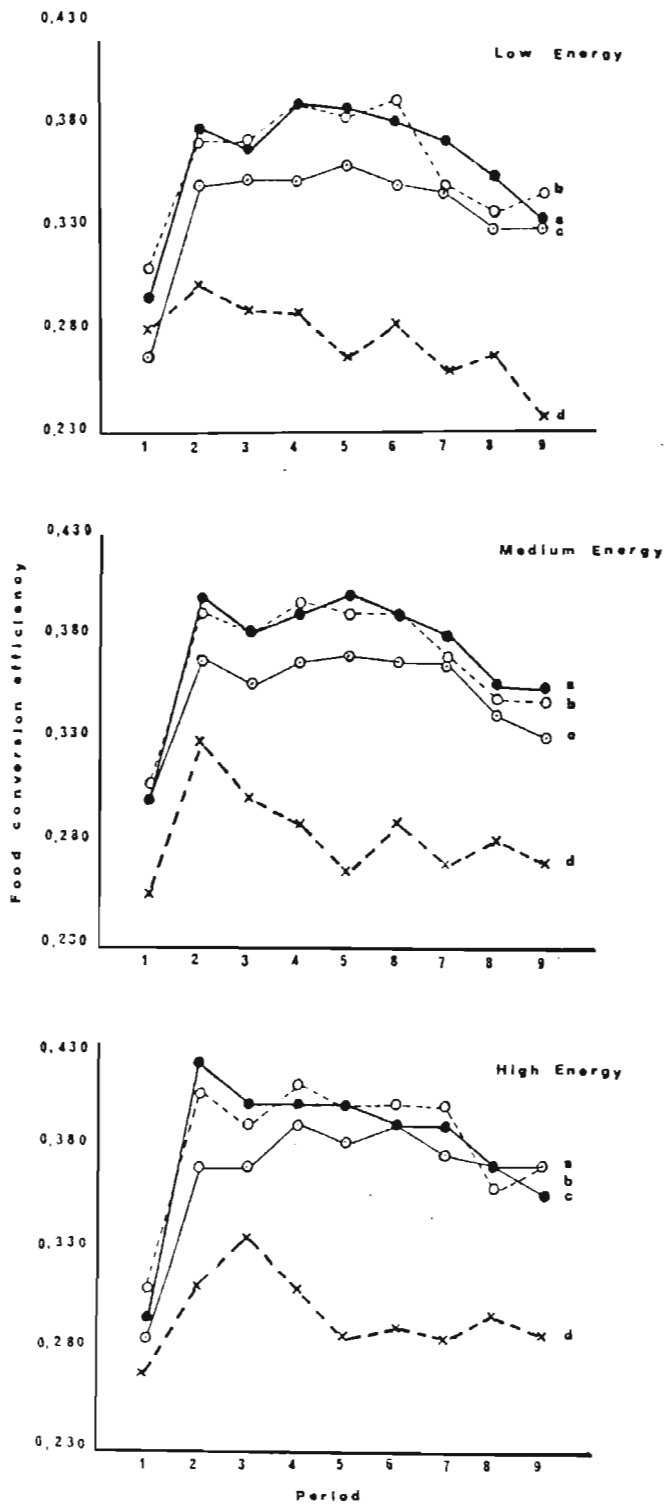


Figure 5.5 The effect of energy and lysine/energy ratio on food conversion efficiency (g egg/g food) during nine 28 d periods. (Refer to Table 5.4 for a description of the diets fed.)

*Body mass gain.*

The daily body mass increments, as in the isoleucine trial, were much higher in the first period than in any of the other periods. However, the mean daily increment of 4,05 g/bird d in all treatments was considerably lower than the 10,02 g/bird d found during the equivalent period in the isoleucine trial. This variation can probably be explained by the proportion of birds actually in production. As previously indicated most of the growth of a young pullet takes place before the first egg is laid. Since in this trial the production during the first period was 72,6 percent as compared to 23,08 percent in the isoleucine trial, it can be expected that body mass gain in the latter trial would be that much greater.

The daily body mass gains of laying birds on the lowest dilution diets during periods two, three, four and five were significantly lower than those of birds on the other treatments. During periods six to nine the body mass gain on this diet improved to such an extent that no significant difference in daily gain was evident between any of the treatments. This mass gain improvement during the latter periods of the trial can be ascribed to higher food intake on this diet as the trial progressed. The mass gain response of birds on the low energy second dilution diet (1C) was also more contained than that of birds on the second dilution diets at the two higher energy levels. (Figure 5.6). This response difference can be explained by the lower lysine intake on the lowest energy diet.

RESULTS DURING PERIOD OF MAXIMUM PRODUCTION (periods 4, 5 and 6, 34 - 45 weeks of age).

From Figure 5.3 it will be observed that maximum egg output was obtained between 34 and 45 weeks of age (periods 4, 5 and 6). For the same reasons indicated in Chapters 3 and 4, it was decided



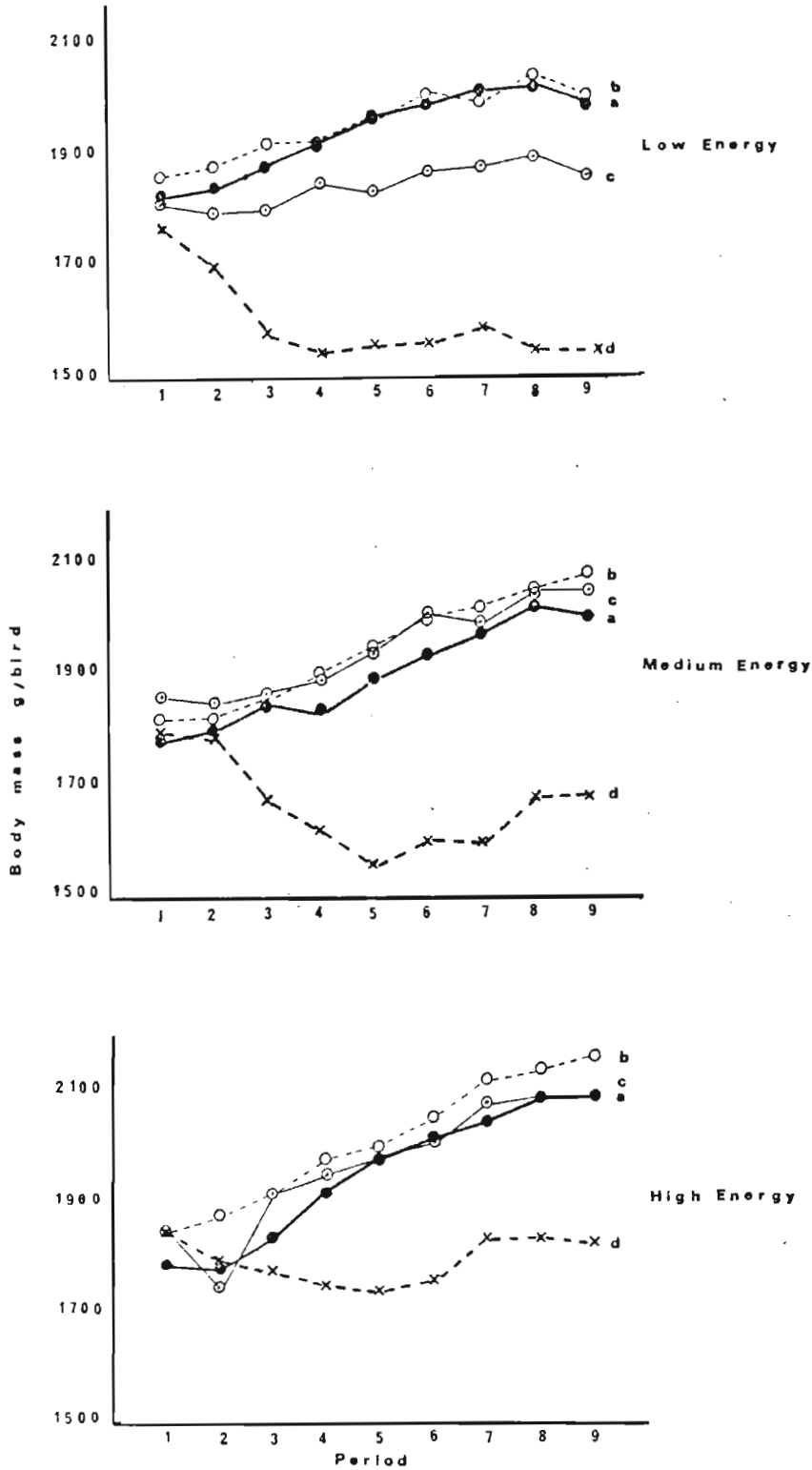


Figure 5.6 The effect of energy and lysine/energy ratio on body mass gain during nine 28 d periods. (Refer to Table 5.4 for a description of the diets fed.)

to combine the data of these periods in order to accurately assess the effect of lysine intake on the production parameters rate of lay (eggs/100 bird d), egg mass (g/egg), egg output (g/bird d), food intake (g/bird d), food conversion efficiency (g egg/g food) and mass gain(g/bird d).

The combined results of periods 4, 5 and 6 are presented in Table 5.17. The main effects of lysine/energy ratio (L) and of energy (E) are shown together with the interaction terms (L x E). Also the standard errors of each mean (SEM) and the least significant differences (L.S.D) at  $P < 0,05$  and  $P < 0,01$  are indicated. These same results are also illustrated in Figures 5.7 to 5.11 where lysine intake has been plotted against some of the production parameters mentioned above.

The effect of dietary lysine only will be considered in the present discussion as the effects of energy on the production parameters measured will be discussed in Chapter 6.

#### *Egg production.*

Egg production of laying hens on the summit, first, second and lowest dilution diets during the periods of peak production (four, five and six) was 88,09, 85,88, 83,62 and 64,28 percent respectively. This progressive decline in egg production was significant in all cases and resulted from respective mean lysine intakes of 1 070, 854, 672 and 447 mg/bird d.

The effect of lysine intake on percentage egg production is illustrated in Figure 5.7. The linear effect of adequate and marginally adequate intakes of lysine on egg production (600 mg or more) was computed, and this graph is included in Figure 5.7, together with the resultant equation. The slope of this line, which was highly significant indicates the increasing egg production resulting from higher intakes of lysine. Lysine is the only amino acid studied

TABLE 5.17: Response to Lysine and Energy intake during combined periods 4, 5 and 6.  
(34 - 45 weeks of age)

5.17.1: Rate of lay (eggs/100 bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	86,60	86,44	83,24	64,17	80,11
2	87,77	85,77	83,05	63,41	80,00
3	89,90	85,41	84,57	65,25	81,28
Mean	88,09	85,88	83,62	64,28	80,47
	SEM		LSD (0,05)		LSD (0,01)
L	0,711		2,00		2,65
E	0,615		1,73		2,30
L x E	1,23		3,47		4,59

5.17.2: Mean egg mass produced (g/egg)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	55,71	56,31	54,55	51,89	54,61
2	55,37	55,85	55,19	51,82	54,56
3	55,24	56,24	55,54	52,05	54,77
Mean	55,44	56,13	55,10	51,92	54,65
	SEM		LSD (0,05)		LSD (0,01)
L	0,226		0,636		0,840
E	0,196		0,551		0,730
L x E	0,391		1,10		1,46

5.17.3: Total egg mass produced (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	48,22	48,65	45,39	33,36	43,90
2	48,59	47,89	45,82	32,88	43,80
3	49,64	48,01	46,94	33,96	44,64
Mean	48,81	48,18	46,05	33,40	44,11
	SEM		LSD (0,05)		LSD (0,01)
L	0,363		1,02		1,35
E	0,316		0,89		1,17
L x E	0,629		1,77		2,35



TABLE 5.17 (Continued)

5.17.4: Mean daily food intake (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	125,49	125,39	128,56	120,84	125,07
2	122,61	121,98	124,77	117,30	121,67
3	125,14	118,99	120,21	115,10	119,86
Mean	124,42	122,12	124,51	117,75	122,20
	SEM		LSD (0,05)		LSD (0,01)
L	0,86		2,42		3,21
E	0,75		2,10		2,78
L x E	1,49		4,20		5,57

5.17.5: Food conversion efficiency (g egg/g feed)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	0,386	0,387	0,354	0,279	0,351
2	0,397	0,394	0,368	0,282	0,360
3	0,400	0,404	0,390	0,298	0,373
Mean	0,394	0,395	0,371	0,286	0,362
	SEM		LSD (0,05)		LSD (0,01)
L	0,0028		0,0079		0,0100
E	0,0024		0,0068		0,0090
L x E	0,0048		0,0136		0,0180

5.17.6: Mean body change (g/bird d)

Energy MJ/kg	Summit Diet (a)	1st Dilution (b)	2nd Dilution (c)	Dilution Diet (d)	Mean
1	1,09	0,94	0,80	- 0,17	0,66
2	1,02	1,62	1,65	- 0,83	0,87
3	2,10	1,64	1,16	- 0,30	1,15
Mean	1,40	1,40	1,20	- 0,43	0,89
	SEM		LSD (0,05)		LSD (0,01)
L	0,334		0,94		1,25
E	0,292		0,82		1,08
L x E	0,579		1,63		2,16

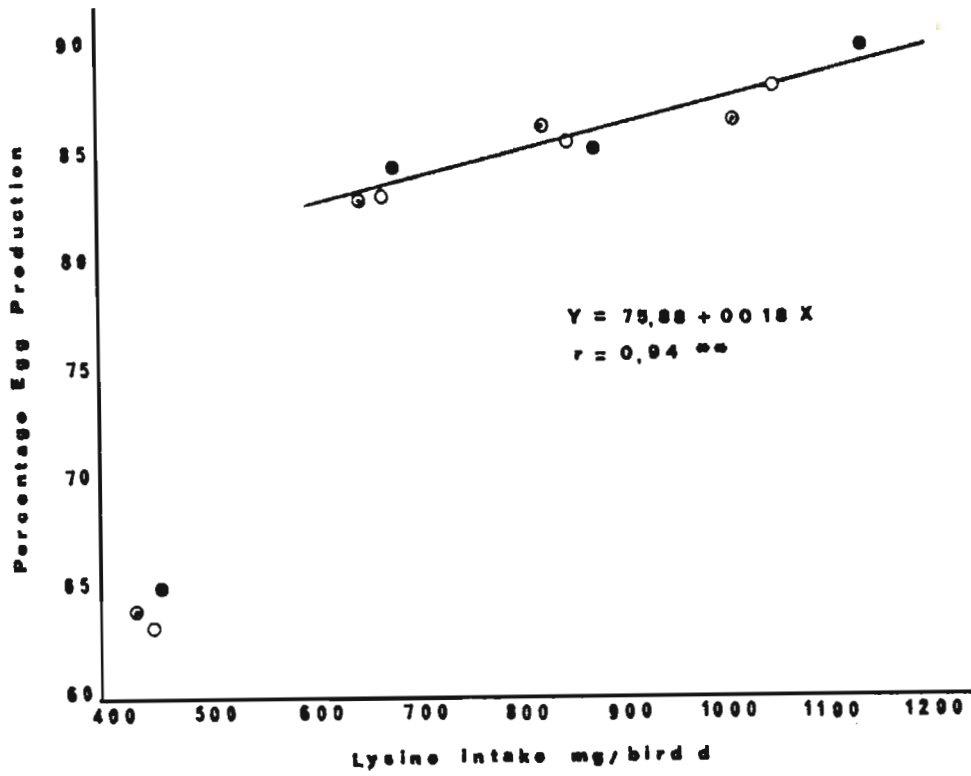


Figure 5.7 The relationship between lysine intake and percentage egg production. Regression equation fitted to data corresponding to an intake of more than 600 mg lysine/bird d.

in which a significant linear response in percentage egg production was observed.

It is interesting that percentage egg production continues to increase as lysine intake increases but at the expense of egg size which, as will be shown later, declined with increasing intakes of lysine. Egg mass output (g/bird d) therefore did not increase to the extent that might have been expected on perusal of the percentage egg production values.

#### *Egg mass.*

Egg mass from birds on the summit, first, second and lowest dilution diets during the peak production periods (four, five and six) were 55,44, 56,13, 55,10 and 51,92 g/egg respectively. These egg mass values corresponded to mean lysine intakes of 1 070, 854, 672 and 447 mg/bird d respectively.

The egg mass from layers on a 447 mg daily lysine intake was significantly inferior to that produced on all other treatments. Mean egg mass obtained from 854 mg lysine intake were significantly greater than that produced on intakes of 1 070 and 672 mg/bird d.

Using the combined data from periods four, five and six a regression equation was computed relating lysine intake to egg mass (g/egg). A curvilinear regression fitted the data very closely, and is as follows:

$$Y = 40,13 + 0,0351X - 0,0000193X^2$$

with a correlation coefficient of 0,822.

where  $Y$  = egg mass (g/egg)

and  $X$  = lysine intake (mg/bird d).

From the above mentioned equation it can be calculated that maximum



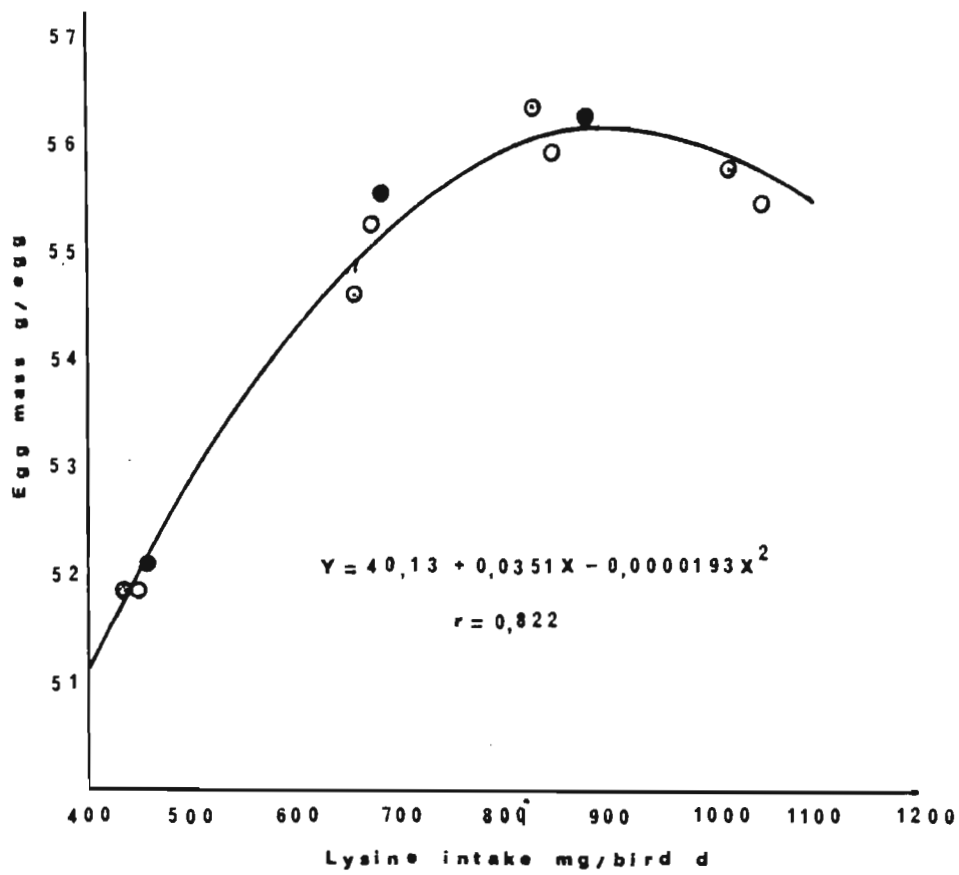


figure 5.8 The relationship between egg mass (g/egg) and lysine intake.

egg mass is obtained at a lysine intake of 909 mg/bird d.

Note the decline in egg mass corresponding to the continued increase in percentage egg production, resulting in a virtually constant daily egg mass output at the higher levels of lysine intake.

*Egg output.*

The Reading model (Fisher et al. 1973) described in Chapter 3 was used to analyse the combined egg output data obtained during periods four, five and six. As in the isoleucine trial the body mass changes of all birds were very small during these periods and so  $\Delta W$  was ignored and thus the coefficient for mass gain  $\underline{C}$  was eliminated from the calculations. The parameters of the resulting response curve (Figure 5.9) are:

$$\underline{a} = 10,65 \text{ mg lysine/g egg}$$

$$\underline{b} = 48,04 \text{ mg lysine/kg Wd}$$

$$\bar{E}_{\max} = 48,324 \text{ g egg/hen d}$$

$$W = 1,9 \text{ kg}$$

$$\text{s.d.E} = 10 \text{ g/hen d}$$

$$\text{s.d.W} = 0,20 \text{ kg}$$

$$\text{rEW} = 0,0$$

$$\text{residual sum of squares} = 5,58 \text{ (8 d.f.)}$$

$$\text{residual s.d.} = 0,84 \text{ g egg.}$$

From Figure 5.9 it will be observed that the data are tidy, and therefore the model gives a good fit in all cases.

It can also be concluded that the lysine requirement of an individual pullet in the early stages of lay is adequately represented (neglecting changes in body mass) by the equation

$$\text{Lysine req.} = 10,65 E_{\max} + 48,04W.$$

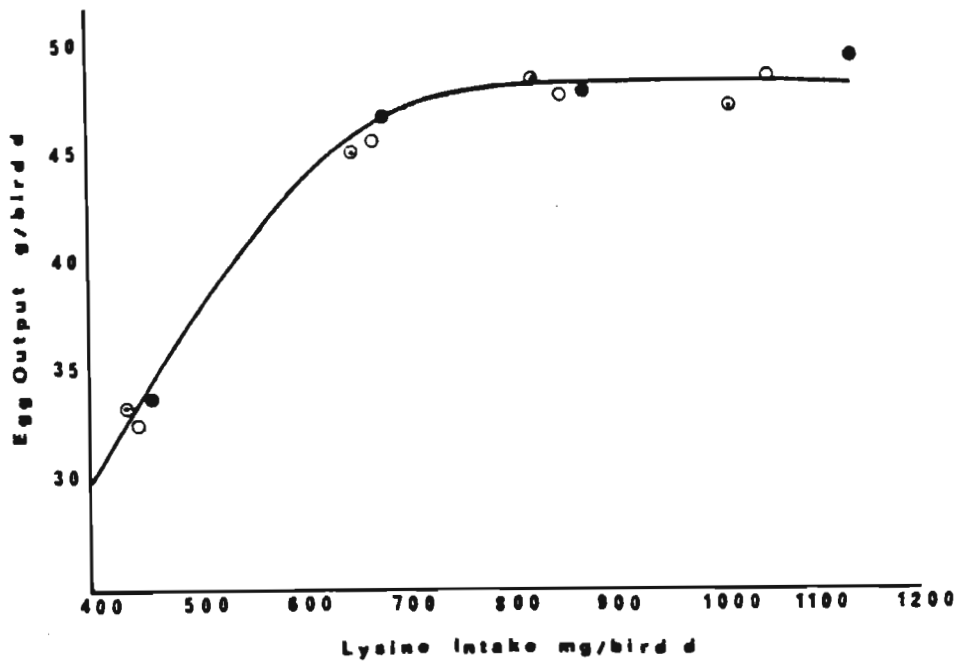


Figure 5.9 The relationship between egg output and lysine intake. The curve was fitted by the method of Curnow (1973) using the parameters described in the text.



This equation should not be used directly by substituting average values of  $E_{max}$  and  $W$  to predict the requirement of a flock, since it makes no allowance for the variations in yield and body mass which exist within the flock and the consequent curvilinear responses to be expected when diets of differing lysine concentration are fed to different groups of birds. Once curvilinear responses are assumed, a requirement can only usefully be defined in economic terms.

### *Optimum lysine intake*

In order to obtain estimates of optimum lysine intakes for laying flocks under different economic conditions, a table relating optimum intakes for various marginal costs of lysine and egg outputs was prepared (Table 5.18). The present marginal cost of lysine was calculated to be R3-80/kg and the price of eggs used was 60c/kg. Under these circumstances the optimum intake of lysine for a 1,9 kg bird producing 48,32 g of egg mass per day was 764 mg, corresponding to a  $k$  value of 0,0063.

TABLE 5.18. Optimum lysine intakes (mg/hen d) for flocks of young laying pullets as affected by the marginal cost of lysine and the value of egg output.

Marginal value of egg output (cents/kg)	Marginal cost of lysine (R/kg)		
	R3-80	R4-80	R5-80
	Lysine requirements, mg/hen d		
60	764	752	741
70	772	759	750
80	779	768	758
90	788	772	765
100	797	780	772
110	792	785	775

Evans et al. (1949) found a variation of 6,7 to 6,9 g lysine per 16 g nitrogen in the egg. The lysine content of 7,02 g/16 g

nitrogen in the egg reported by Fisher (1976) agrees substantially with that found by the above mentioned authors.

Using the lysine content suggested by Fisher (1976) and assuming that eggs contain 11,25 percent protein the calculated amount of lysine per gram of egg is 7,9 mg.

The coefficient for egg output in the equation of Pilbrow and Morris (1974) for an individual pullet in the early stages of lay is 9,5 mg/g egg. This indicates a net efficiency of lysine utilisation for egg production of 83 percent.

The coefficient for egg output in this study of 10,65 mg/g egg output for the combined periods 4, 5 and 6 is slightly higher than that found by Pilbrow and Morris. This egg output coefficient indicates a net efficiency of lysine utilisation for egg production of 74,2 percent.

Direct estimates of maintenance requirements in adult roosters range from 29 to 60 mg lysine/kg W (Leveille and Fisher, 1959; Kandatsu and Ishibashi, 1966). The coefficient of 48,04 mg/kg body mass found in this study is in close agreement with the value of 60 mg/kg found by Kandatsu and Ishibashi (1966) but is lower than the 90 mg/kg suggested by Pilbrow and Morris (1974).

The optimum requirement of 764 mg lysine/bird d found in this study is much lower than the 825 mg total lysine (750 mg available lysine) recommended by the A.R.C. (1975). It is however substantially higher than the 660 mg total lysine advocated by the N.R.C. (1977). The latter indicates that the requirement may be higher when wheat-based diets are fed.

Direct comparison of the estimates of optimal lysine intake shown in Table 5.18 with previous estimates of requirement would be misleading. The estimation of "requirement" by linear regression necessarily leads to lower estimates than the methods adopted

this paper. Pilbrow and Morris (1974) indicated that the fitting of straight lines will lead to an estimate of requirement which is greater than the "requirement of the average hen" but less than the "optimal dose", assuming the cost ratio  $k$  to be of the order of 0,004.

Should the 48,32 g egg output from a 1,9 kg layer with a daily mass gain of 1 g/bird d as found in this study be used in the equation of Thomas (as reported by Combs, 1968) the estimated available lysine requirement is 694 mg/hen d. This figure converted to total lysine (assuming 90 percent of total lysine is available) is equivalent to 763 mg. This is in close agreement with the optimum requirement of 764 mg/bird d determined in this study.

When the same production data used above are fitted to the "B-model" equation of Hurwitz and Bornstein (1973) and Wilgus (1976), the calculated requirements are 612 and 700 mg/bird d. Using the "model 2" equation of Smith (1978) the total lysine requirement for the same bird is 670 mg total lysine/bird d.

Using eight commercial stocks of layer-type hens, Pilbrow and Morris (1974) found the lysine requirement of an individual pullet in the early stages of lay adequately represented by the equation:

$$L = 9,5E + 90W$$

where  $L$  = mg available lysine per bird d

$E$  = grams of egg per bird d

and  $W$  = body mass in kilograms.

For prices of 40 pence/kg for eggs and 160 pence/kg for the marginal cost of supplying lysine, they found that the optimal lysine intakes for the eight stocks examined ranged from 820 to 920 mg available lysine per bird d.

Using the same  $k$ -value ( $k = 0,004$ ) in the present study the optimal



lysine intake for layers producing 48,32 g egg/bird d appears to be 793 mg total lysine/bird d. (Table 5.18.) The estimate of Pilbrow and Morris however is based on available lysine while estimates from this work is based on total lysine. If it is accepted that approximately 90 percent total lysine is available, 714 mg available lysine in the present study ( $k = 0,004$ ) should be compared to that found by Pilbrow and Morris. This discrepancy of at least 106 mg available lysine/bird d is probably due to the fact that the diets used by the latter authors contained 30 percent wheat.

Bray (1969) estimated the lysine requirement of a flock of White Leghorn pullets with a maximum egg output of 45,8 g/d to be 522 mg/bird d. This was substantially less than the estimate of 612 mg/bird d which would be predicted as the requirement for the average bird when using the equation presented by Pilbrow and Morris (1974). According to the latter authors there were several bases for speculation as to why the requirement found by Bray was comparatively low.

Firstly, different methods were used to formulate experimental diets. In their experiment, lysine intake was altered by varying the proportion of protein in the diet, whereas Bray used purified lysine added to diets of constant protein content (12 percent). Secondly, in Bray's experiment bias was introduced by analysing results for the whole of an 8-week experiment, including the initial period when birds fed on the deficient diets were losing body mass rapidly and when, presumably, a proportion of the lysine for egg production came from the depletion of body tissue. Thirdly, the basal diet used by Bray contained more lysine than the quantity estimated by microbiological assay.

Thomas (1966) used a method similar to that of Bray and obtained data which agreed well with those reported by Pilbrow and Morris. After careful analysis of all Bray's data Pilbrow and Morris (1974)

concluded that the third hypothesis was the most likely explanation for the discrepancy.

March and Biely (1972) found maximum egg mass output per hen day on wheat based diets when the diets supplied 800 - 850 mg of lysine per day. Similarly, Taylor et al. (1967) reported that 794 mg lysine per day was needed to support maximum egg output when wheat is used as a dietary component.

In contrast, Sell and Johnson (1974) and Gardiner and Dubetz (1977) found that 624 and 637 mg lysine per hen daily was needed to support egg production.

Summarising a number of reports Jensen et al. (1974) concluded that there appears to be a higher daily lysine requirement when hens are fed rations containing wheat. They indicated that it is possible that lysine is less available in wheat than in maize or that the amino acid balance in a wheat based ration results in a higher requirement for lysine.

In an effort to resolve this issue Morris (unpublished, 1980) made a direct comparison of responses to lysine in wheat-based and maize-based diets and found a significant separation of the response curves for the two types of diets (Figure 1.1, Chapter 1).

This work confirmed the idea of Jensen et al. (1974) that the effective requirement for lysine is higher when wheat is used as the major dietary component. Morris (unpublished, 1980) furthermore referred to studies conducted with chicks at Reading where it was shown that amino acid imbalance was responsible for the apparent unavailability of lysine in wheat. If care was taken to balance the diet by manipulating free amino acid levels, this effect could be avoided.

To determine more accurately the lysine requirement of layers Nathanael (1980) fed eight diets to layers that differed only

slightly in lysine content. Regression analyses of the data obtained and the fit of a polynomial equation to the data showed that maximum egg output occurred with average daily lysine intakes of 690 to 710 mg per hen d. He indicated that these findings corroborate the more recent estimates of lysine needs of hens fed maize-based rations (N.R.C., 1977; Latshaw, 1976; Wilgus, 1976; Hurwitz and Bornstein, 1977). It was thus concluded that research conducted within the last five years has yielded a relatively reliable quantitative lysine requirement (650 to 700 mg per hen daily) for laying hens fed maize-based diets.

Halloran and Almquist (1978) conducted a six-week test with Hy-Line W-36 hens that were 32 weeks of age at the start of the test. Daily total lysine intakes of the four different treatments were 940, 780, 720 and 660 mg per hen d.

The results showed that 660 mg of lysine per hen d resulted in statistically lower egg production and poorer feed conversion than the 720 mg level. However, it was not significantly different from the 780 or 940 mg level. With regard to feed intake, egg mass and body mass increases, there were no significant differences between the four groups. The authors therefore concluded that on the basis of total lysine analyses, the lysine requirement for layers was between 660 and 720 mg per hen per day.

Scott, et al. (1976) have also shown that the lysine requirement for 90 percent production is not greater than 720 mg per hen per day.

Chi and Speers (1976) found that the concentration of free lysine in the plasma of hens was increased by each increment of lysine in the diet, but the magnitude of the increase was very small until dietary lysine reached the level at which egg production was maximised. When higher levels of lysine were fed, there was a rapid and approximately linear increase in the plasma free



lysine level while egg production plateaued. Using a plasma lysine response curve Chi and Speers estimated the requirement of laying hens for dietary lysine to be 677 mg/hen daily. This value was similar to the lysine requirement they predicted when using egg production (687 mg/hen d) and nitrogen retention (664 mg/hen d) as response criteria.

As discussed above, review of research work on lysine requirements of laying hens covering the past five years, indicates a lysine requirement of 650 to 720 mg/bird d. Earlier requirement estimates cannot be strictly equated to the optimal requirement determined in this study. Using the equation computed in this work to determine the lysine requirement of an average individual pullet, the calculated daily requirement is 628 mg/bird d. This is considerably lower than the 764 mg/bird d optimal requirement. It can be expected that requirements determined by conventional methods will fall within the range of 628 to 674 mg/bird d which in fact is the case.

#### *Food intake.*

The combined food intake data for periods four, five and six are presented in Table 5.17.4. No significant difference in food intake was found between the summit, first and second dilution diets during periods of peak output. However, layers on the lowest dilution diets as in all the other amino acid trials showed a significantly lower food intake.

As in the isoleucine and methionine trials it was also found that energy concentration had a significant effect on food intake, independent of the lysine effect. Three separate graphs were therefore fitted (Figure 5.10) and it will be observed that the highest intake corresponded to the sub-optimal lysine intake of 656 mg/bird d.

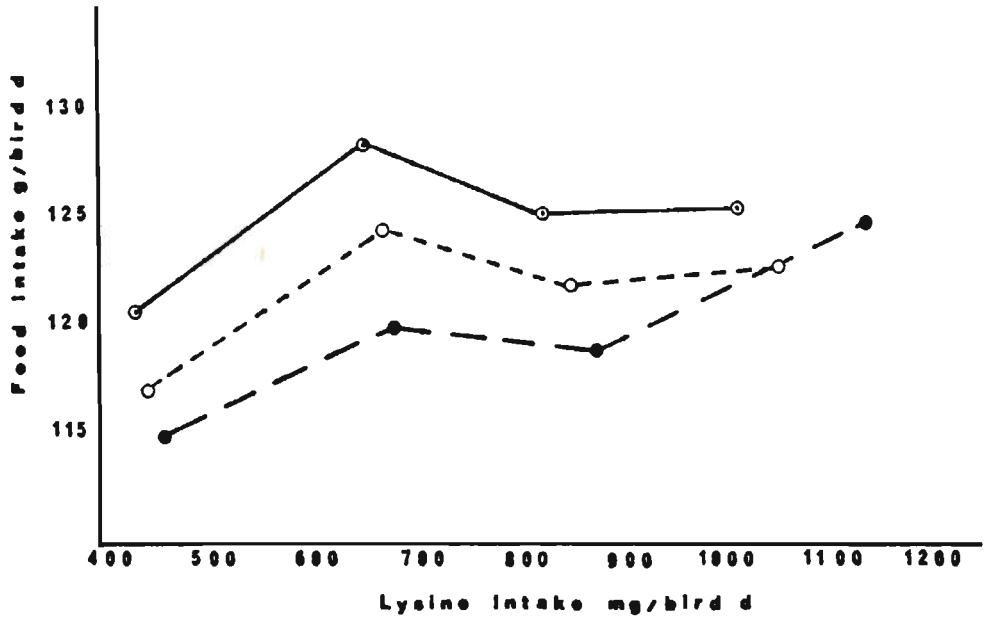


Figure 5.10 The relationship between food intake (g/bird d) and lysine intake (mg/bird d) when fed high energy diets (●—●), medium energy diets (○-----○) and low energy diets (○—○).

*Food conversion efficiency.*

The mean food conversion efficiency during the periods of peak production were 0,394, 0,395, 0,371 and 0,286 (Table 5.17.5) corresponding to mean lysine intakes of 1 070, 854, 672 and 447 mg/bird d. The food conversion efficiency of laying hens on the lower lysine intakes of 672 and 447 mg/bird d was significantly poorer than that of birds on the two higher mean lysine intakes.

Using the combined data from periods four, five and six, regression equations relating lysine intake to food conversion efficiency were computed for each energy level, as food conversion efficiency was not independent of energy concentration. The three equations are as follows:

Low energy (10,49 MJ ME/kg)

$$Y = 0,014 + 0,0008X - 0,00000043X^2$$

$$r = 0,938.$$

Medium energy

$$Y = 0,0112 + 0,0008X - 0,00000041X^2$$

$$r = 0,950.$$

High energy

$$Y = -0,0066 + 0,000884X - 0,00000046X^2$$

$$r = 0,913.$$

where Y = food conversion efficiency

and X = lysine intake (mg/bird d).

From these equations it was calculated that optimum food conversion efficiency is obtained from a lysine intake in excess of 930 mg/bird d. As in the case of methionine and isoleucine this study clearly shows that much higher lysine intakes are required for optimum feed conversion efficiency than for egg production.



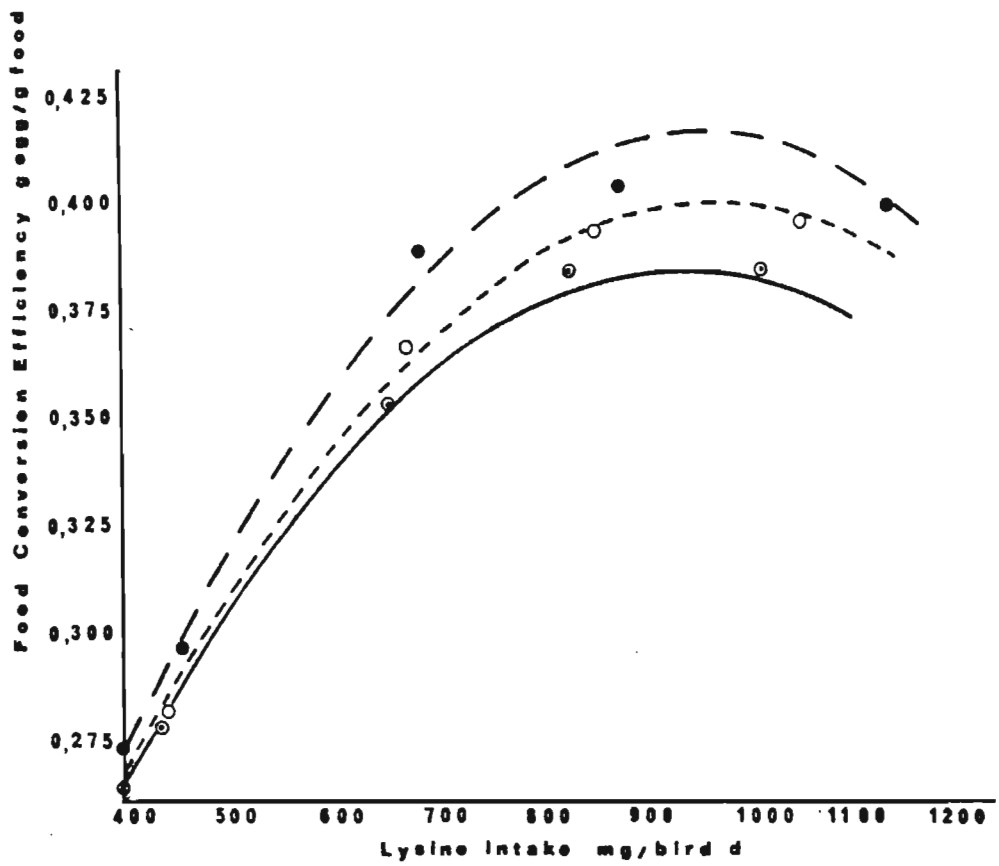


Figure 5.11 The relationship between food conversion efficiency and lysine intake when hens were fed the high energy diets (●—●), medium energy diets (○-----○) and low energy diets (○—○).

*Body mass gain.*

There was little difference in mean daily body mass gain during peak egg output periods between the three diets with the higher lysine levels. The mass increments of layers on the lowest dilution diets were however significantly lower ( $P < 0,01$ ) than those of birds on the three higher dietary lysine concentrations.

SUMMARY

1. A total of 2 376 laying hens were used in this study designed to determine the response of laying hens to increasing concentrations of lysine.
2. Using a dilution technique, four lysine levels at three different energy concentrations were each fed to three replicates of 66 birds.
3. At adequate and marginally adequate levels of lysine egg production continued to increase as intake of lysine increased, but this was at the expense of egg mass.
4. The lysine requirement of an individual pullet is represented (ignoring change in body mass) by the equation

$$\text{Lysine req.} = 10,65E_{\text{max}} + 48,04W.$$

5. Relating marginal cost of eggs and lysine it was determined that optimal egg output was achieved with a lysine intake of 764 mg/bird d.
6. A table is presented from which optimal response to lysine intake can be determined (2 kg layers producing 50 g egg mass/bird d) should the value of eggs or marginal cost of lysine change.

7. Maximum egg mass was achieved with a lysine intake of 909 mg/bird d.
8. Three separate graphs were fitted relating food intake to lysine intake due to the fact that energy concentration had a significant effect on food intake independent of lysine effect. The highest food intake corresponded to the sub-optimal lysine intake of 656 mg/bird d. It was concluded that with marginally sub-optimal levels of lysine in the diet food intake increased in an attempt to sustain production
9. Food intake of birds fed lysine deficient diets was significantly lower than that of birds fed adequate diets during the initial periods of the trial. Although food intake on the inadequate diets markedly increased towards the end of the trial, egg production nevertheless remained at a low level.
10. Three regression equations were computed relating food conversion efficiency to lysine intake due to the fact that energy had a profound effect on this parameter independent of the lysine effect. From these equations it was calculated that optimum food conversion efficiency is obtained with a lysine intake in excess of 930 mg/bird d. This is higher than the requirement for both optimal egg output and egg mass.
11. Body mass gain of birds on inadequate lysine diets were significantly poorer than that of birds receiving diets with adequate lysine levels.
12. The utilisation of lysine was not affected by dietary energy concentration.



## CHAPTER 6

### RESPONSE OF LAYING HENS TO DIETARY ENERGY CONCENTRATIONS.

#### INTRODUCTION.

Nutrient requirements expressed as percentages have been shown to differ because of variations in food intake due to such factors as breed (Sharpe and Morris, 1965, Harms and Waldroup, 1962); environmental and seasonal temperature (Bray and Gesell, 1961, Bray and Morrissey, 1962, Reid and Weber, 1973) and dietary energy content (Smith, Payne and Lewis, 1963). These differences in apparent requirement do not reflect changes in nutrient utilisation - the main reason why nutrient requirements expressed as a percentage are misleading when used in formulating diets to be used among different breeds or in different environments.

This study on dietary energy concentrations in conjunction with the studies on amino acid responses had a two-fold purpose: to determine the effects of energy on the production parameters, egg production, egg mass, egg output, food intake, food conversion efficiency and body mass gain; and to investigate whether an interaction exists between those factors that determine food intake (in this case, energy concentration) and the utilisation of essential nutrients.

Although some reports indicate that laying hens maintain a constant energy intake when diets of widely different energy levels are fed (Hill, 1962), the reduction in food intake which occurs at high dietary energy concentrations is generally insufficient to maintain a constant energy consumption. Energy intake and body fat deposition tend therefore to be greater at higher dietary energy concentrations (Morris, 1968, De Groote, 1972). There may be some increase

in egg mass but there is usually no increase in rate of lay as energy intake is increased (Morris 1969).

Aside from its role in regulating food intake, energy concentration materially affects the cost of a layer diet. As the energy concentration of the diet is increased voluntary intake of the food declines and this necessitates an increase in dietary amino acid concentrations. Optimum daily intakes of lysine, methionine and isoleucine can be determined by means of the response curves presented in earlier Chapters, but it is equally important to be able to determine optimum energy concentrations for economic feeding of laying hens. In order to determine this optimum dietary energy concentration, it is essential to know whether dietary energy intake and the interaction between energy intake and amino acid intake affects laying performance.

Previous sections of this thesis have dealt with the effects of amino acid intake on laying performance. Attention is here directed towards the effects of energy intake on performance characteristics, whereafter general recommendations can be made regarding the determination of the most economic nutrient concentrations in diets for laying hens.

#### PROCEDURE.

In order to determine the effects of energy intake on the different production parameters, the response to energy during the periods of peak production in the three experiments were combined. As indicated previously the mean performance during the period of maximum egg output is most appropriate for determining the effects of nutrient intake on production parameters.

In the isoleucine and lysine trials, performance was maximal on the summit and first dilution diets, so preference was given to

these diets in determining the response to energy intake as not only do they ensure optimum production but also they would exclude the possibility of any amino acid deficiencies. The other dilution diets were not considered because of marginal and inadequate supplies of amino acids which may have affected certain production parameters.

The first dilution diet at the 10,49 MJ ME/kg level in the methionine trial gave results that were inconsistent and consequently only results of the summit diets were used in this instance.

Regression analyses were performed on the relevant data already presented in the previous chapters. A preliminary investigation on the effect of energy on the different production parameters showed similar trends for all three amino acids studied. The results of the three trials were therefore combined in order to more accurately express performance in terms of energy intake.

## RESULTS.

Dietary energy concentration had no significant effect on rate of lay, egg mass, egg output or mass gain, the relevant regression coefficients and their respective standard errors being presented in Table 6.1. Food intake, food conversion efficiency and energy intake were significantly affected by dietary energy concentration as can be seen by the results in Table 6.1.

The effects of dietary energy concentration on egg mass output, food intake, food conversion efficiency and energy intake are depicted graphically in Figure 6.1.



TABLE 6.1: Effect of dietary energy concentration on different production parameters.

Production parameter	Constant term	Regression coefficient (b)	SE (b)
Egg production	78,33	0,804	2,015 <sup>NS</sup>
Egg mass	58,96	-0,165	0,735 <sup>NS</sup>
Egg output	46,30	0,317	0,975 <sup>NS</sup>
Food intake	163,36	-4,058	2,504 <sup>**</sup>
Food conversion efficiency	0,22	0,019	0,008 <sup>**</sup>
Mass gain	-2,49	0,352	1,161 <sup>NS</sup>
Energy intake	0,49	0,074	0,143 <sup>**</sup>

<sup>\*\*</sup> Denotes significance at  $P < 0,01$ .

## DISCUSSION.

The fact that dietary energy concentration had no significant effect on the output of eggs (neither rate of production nor egg mass) indicates that diets containing energy levels varying from 10,3 to 13,39 MJ ME/kg will not alter production providing that amino acids are not limiting. These findings are contrary to those of De Groote (1972), Sherwood et al. (1978) and Reid et al. (1978). De Groote found an increase in mean egg mass (g/egg) of 1,4 g when the metabolisable energy content of the diet was increased from 10,46 to 13,39 MJ ME/kg. He concluded that the increased egg mass was the result of the additive effect of increased metabolisable energy intake and increased linoleic acid consumption.

Reid et al. (1978) found a correlation of 0,95 between metabolisable energy consumption and egg mass. Their data indicate that for each

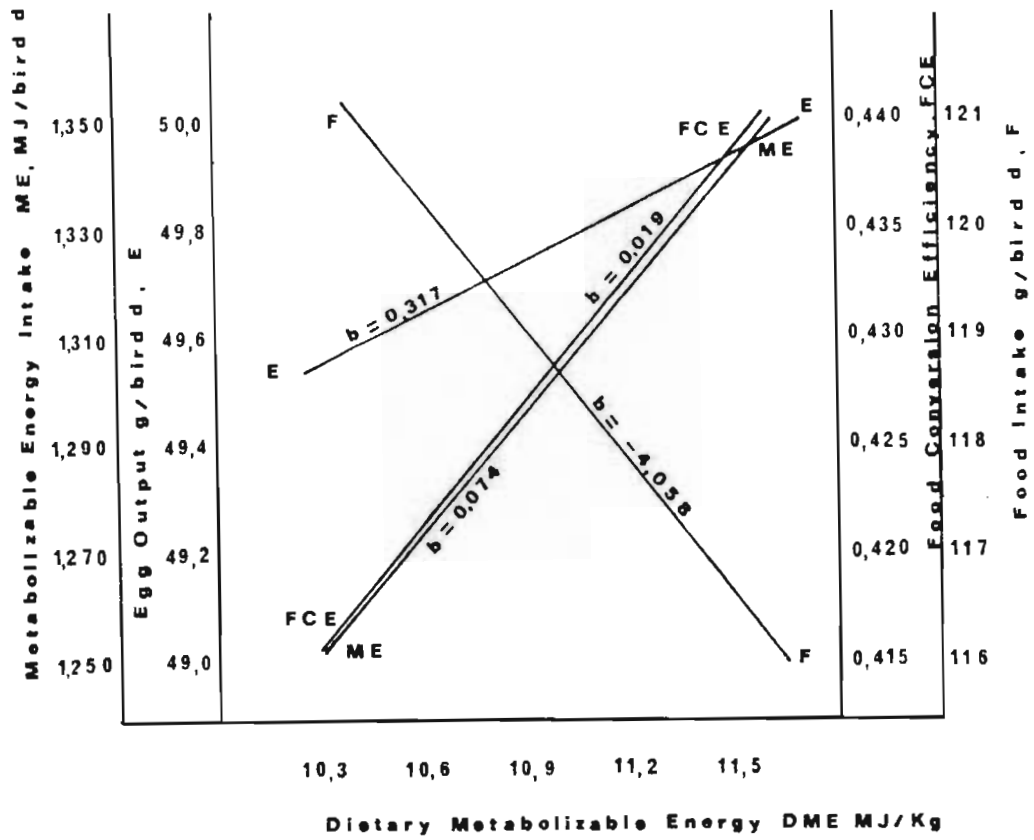


Fig 6.1 Pooled linear regression coefficients of layer performance characteristics on dietary metabolisable energy level during periods of peak production.

4,184 KJ metabolisable energy consumed, egg mass improved by 0,056 g/egg.

Sherwood et al. (1978) found a significantly lower egg mass (57,7 g) on a diet containing 10,59 MJ ME/kg compared to egg masses (59,7 and 59,1 g) obtained on diets containing 11,28 and 11,97 MJ ME/kg respectively. They attributed the decline in egg mass to decreased energy intake on the 10,59 MJ ME/kg diet.

Edwards and Morris (1967) in two short term experiments found that the direct substitution of maize for wheat in a laying diet led to a small but significant increase in egg mass. They further showed that the addition of 2,5 percent and 5 percent maize oil to a 55 percent wheat diet resulted in increases in mean egg mass of 0,7 g and 1,3 g respectively. They concluded that maize had an effect on egg mass which is not attributable to differences in dietary energy normally associated with the substitution of one cereal for another in practical diets.

Although linoleic acid has an important effect on egg mass, the present study would indicate that low amino acid intakes in combination with low dietary energy concentrations was probably of greater importance in affecting egg mass. Marginal linoleic acid intakes seemed to have some effect on egg mass in certain treatments. This can be seen from the results shown in Tables 6.2, 6.3 and 6.4 where the amino acid, kilojoules metabolisable energy and linoleic acid intakes are shown, together with the mean egg mass resulting from the feeding of the summit and first dilution diets in the three experiments respectively. It is only in the case of the lowest energy diets in the isoleucine trial where the possibility existed of a marginal deficiency of linoleic acid. In the case of the low energy, first dilution treatment, the low egg mass could be due to a combination of linoleic acid and isoleucine deficiencies, as maximum egg size was found to result from an intake of 870 mg isoleucine per day, and intake in this treatment was only 776 mg/day.



TABLE 6.2: Mean egg mass (g/egg) resulting from the feeding of summit and first dilution diets - Isoleucine trial.

Diet	Egg mass	KJ metabo- lisable energy intake	Isoleucine intake (mg/bird d)	Linoleic acid intake (mg/bird d)
Summit diet (10,3 MJ ME/kg)	58,37	1 222	925	1 541
Summit diet (10,9 MJ ME/kg)	58,72	1 251	963	2 259
Summit diet (11,5 MJ ME/kg)	59,77	1 243	973	2 853
First dilution diet (10,5 MJ ME/kg)	58,05	1 213	776	1 462
First dilution diet (10,9 MJ ME/kg)	58,99	1 276	831	2 045
First dilution diet (11,5 MJ ME/kg)	59,14	1 264	834	2 479

TABLE 6.3: Mean egg mass (g/egg) resulting from the feeding of summit and first dilution diets - Methionine trial.

Diet	Egg mass	KJ metabo- lisable energy intake	Methionine intake (mg/bird d)	Linoleic acid intake (mg/bird d)
Summit diet (10,49 MJ ME/kg)	56,37	1 272	492	3 000
Summit diet (11,09 MJ ME/kg)	56,38	1 335	516	2 742
Summit diet (11,70 MJ ME/kg)	56,66	1 318	511	2 351
First dilution diet (10,49 MJ ME/kg)	56,37	1 243	392	2 404
First dilution diet (11,09 MJ ME/kg)	56,54	1 318	415	2 488
First dilution diet (11,7 MJ ME/kg)	56,45	1 335	421	2 471

TABLE 6.4: Mean egg mass (g/egg) resulting from the feeding of summit and first dilution diets - Lysine trial.

Diet	Egg mass	KJ metabo- lisable energy intake	Lysine intake (mg/bird d)	Linoleic acid intake (mg/bird d)
Summit diet (10,49 MJ ME/kg)	55,71	1 314	1 017	2 271
Summit diet (11,09 MJ ME/kg)	55,37	1,360	1 055	1 931
Summit diet (11,7 MJ ME/kg)	55,24	1 464	1 139	1 677
First dilution diet (10,49 MJ ME/kg)	56,31	1 314	828	2 325
First dilution diet (11,09 MJ ME/kg)	55,85	1 351	854	2 049
First dilution diet (11,7 MJ ME/kg)	56,24	1 393	881	1 792

The most probable explanation for the fact that no response was obtained in egg mass to an increase in dietary energy concentration is that in the diets used to determine this effect, amino acid concentrations were in excess of the requirement for maximum egg mass. In previous studies on this subject (De Groote, 1972, Sherwood et al, 1978, Reid et al. 1978) amino acids were not provided in excess, leading to lower intakes of amino acids than required for maximum egg mass when low energy diets were fed. It can be concluded that egg mass is directly affected by a combination of amino acid and linoleic acid intakes rather than energy intake.

Food consumption declined significantly with increased dietary energy concentrations. This trend of reduced consumption at higher energy levels is consistent with that found by many other workers (Jones et al. 1976, De Groote, 1972, Jackson et al. 1969 and Morris, 1968).

Food conversion efficiency improved significantly as the density of the diet increased. This improvement is due fundamentally to a decreased food intake at high dietary energy concentrations, as egg output was not affected by changes in nutrient density.

Dietary energy intakes increased significantly as the energy concentration of the diet escalated. This is consistent with earlier reports that the reduction in food intake which occurs at high dietary energy concentrations is generally insufficient to maintain a constant energy consumption resulting in greater energy intakes at higher energy concentrations (Morris, 1968, Jackson et al. 1969). Morris (1968) has shown that heavy breeds of birds are less able to control energy intake than are light breeds. He suggested that light breeds (characteristic energy intake of 1 339 KJ/bird d) would increase their energy intake by 2,83 KJ for each 100 KJ increase in nutrient density. De Groote (1972) published a figure of 3,14 KJ extra intake for a 100 KJ increase in nutrient density and Janssen (1968) found this coefficient to be 2,61 KJ/100 KJ energy concentration. In these trials the increase in energy intake with higher energy concentrations amounted to 7,4 KJ/100 KJ energy concentration. The difference could be attributed to the low range of energy concentrations studied.

A consequence of the increase in energy consumption at higher energy concentrations is the deposition of this energy in the form of body fat. Although analysis of mass gain on a daily basis showed no significant differences between treatments, it is nevertheless evident from Figure 6,2 that at the end of the trials, higher body mass yields were obtained by layers receiving diets with high energy concentrations. The coefficient relating body mass gain to energy concentration of 0,352 is very similar to the value of 38,85 percent published by De Groote (1972). This change in body mass has an important bearing on the definition of the most economic dietary energy concentration to be used.

In an effort to determine an accurate method for predicting food



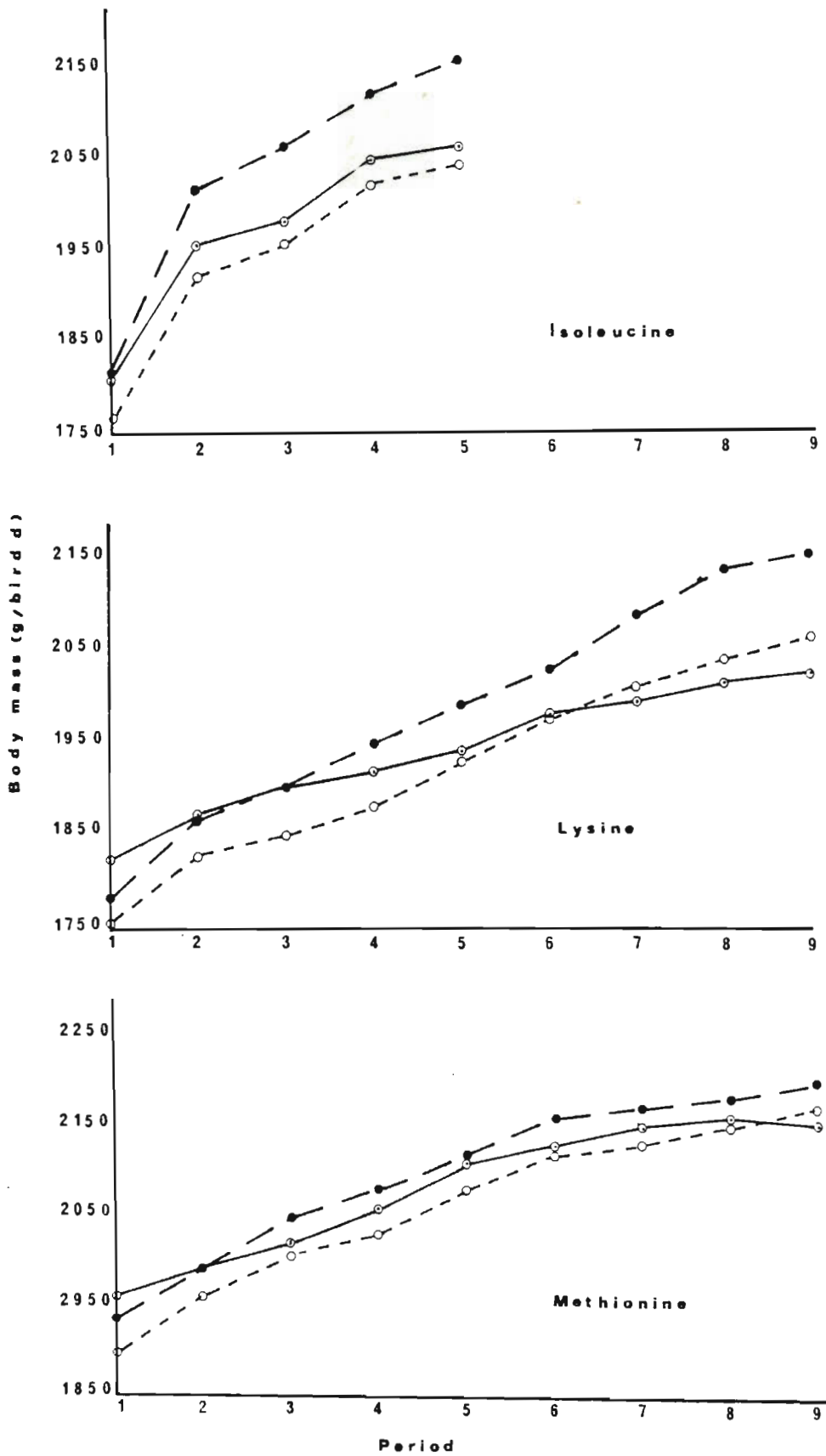


Figure 6.2 The effect of high energy (●—●), medium energy (○—○) and low energy (○-----○) diets on body mass (g/bird) per period.

intake, the formula proposed by Morris (1968) was tested against the results obtained in this study. This formula can only be applied if the characteristic energy consumption of the laying strain is known on a diet containing 11,3 MJ ME/kg.

The characteristic energy consumption of the Amberlink strain used in the lysine and methionine trials at the mean temperatures (15,28 and 15,66 °C respectively) which prevailed at peak production periods in these trials is 1 339 KJ ME/bird d on diets containing 11,3 MJ ME/kg. Similarly the Ross strain used in the isoleucine trial had a characteristic energy consumption of 1 255 KJ/bird d at temperatures recorded at peak production of the trial. (Mean temperature 21,22 °C.) The actual energy and food intake figures obtained in this study compared to calculated figures using the formula suggested by Morris are presented in Table 6.5.

TABLE 6.5: Predicted and actual feed intake during periods of peak production.

Dietary Energy Concentration (MJ ME/kg)	Predicted* Intake		Actual intake	
	KJ ME/bird d	Food intake (g/bird d)	KJ ME/bird d	Food intake (g/bird d)
First dilution isoleucine diets				
10,3	1 238	120,17	1 211	117,59
10,9	1 248	114,5	1 275	116,98
11,5	1 259	109,4	1 265	110,02
First dilution lysine diet				
10,49	1 316	125,45	1 315	125,39
11,09	1 333	120,22	1 353	121,98
11,7	1 350	115,42	1 392	118,99
Summit methio- nine diets				
10,49	1 316	125,45	1 274	121,46
11,09	1 333	120,22	1 334	120,27
11,7	1 350	115,42	1 316	112,50

\* Morris, 1968)

Table 6.5 clearly shows the extent to which energy or food intake can be expected to change for a given change in dietary energy. The data from this study thus demonstrates the reliability of using the Morris (1968) formula for predicting the energy or food intake of layers.

#### PREDICTION EQUATIONS.

In Chapter 2 a number of equations put forward by various workers to determine energy intake of layers, were presented.

Data obtained in this study was used to determine the accuracy of these equations in predicting the energy intake of layers. It is known that sub-optimal and inadequate diets have a marked effect on food or energy intake. For comparative purposes therefore the first dilution diets which provided adequate amino acid intakes at respective energy levels of 11,5 MJ ME/kg in the isoleucine trial and 11,09 MJ ME/kg in the methionine and lysine trials were used to assess the accuracy of these prediction equations.

The energy intake figures derived from the majority of the equations were far higher than the actual intake figures found in this investigation. Only the equations produced by Emmans (1974) for brown laying strains, Gous et al. (1978) and Hurwitz and Bornstein (1977) agreed reasonably with the actual energy intake observed. (Tables 6.6, 6.7, 6.8.)

TABLE 6.6: Actual and Predicted energy intake in Isoleucine trial.

Period	Actual Energy intake determined in study. KJ/bird d	Emmans (1974) equation	Gous et al. (1974) equation	Hurwitz and Bornstein (1977) equation
1	1 218	1 201	1 238	1 113
2	1 276	1 339	1 360	1 351
3	1 272	1 368	1 443	1 393
4	1 289	1 318	1 448	1 406
5	1 234	1 293	1 410	1 402



TABLE 6.7: Actual and Predicted energy intake in Methionine trial.

Period	Actual energy intake determined by study. KJ/bird d	Emmans (1974) equation	Gous et al. (1974) equation	Hurwitz and Bornstein (1977) equation
1	1 176	1 188	1 218	1 272
2	1 251	1 280	1 247	1 343
3	1 301	1 335	1 264	1 368
4	1 335	1 368	1 314	1 372
5	1 314	1 469	1 347	1 389
6	1 414	1 498	1 343	1 389
7	1 360	1 485	1 318	1 389
8	1 331	1 435	1 297	1 377
9	1 364	1 377	1 297	1 364

TABLE 6.8: Actual and Predicted energy intake in Lysine trial.

Period	Actual energy intake determined by study. KJ/bird d	Emmans (1974) equation	Gous et al. (1978) equation	Hurwitz and Bornstein (1977) equation
1	1 251	1 225	1 088	1 180
2	1 293	1 301	1 247	1 259
3	1 318	1 305	1 264	1 268
4	1 343	1 318	1 314	1 301
5	1 377	1 326	1 347	1 322
6	1 339	1 310	1 343	1 326
7	1 393	1 276	1 318	1 322
8	1 364	1 255	1 297	1 310
9	1 372	1 234	1 297	1 318

During certain periods close agreement between actual and calculated intakes is evident but deviations are obvious during other periods. It would therefore appear that the use of prediction equations is less reliable than actual intake determinations. Equations produced by Emmans, Gous and Hurwitz and Bornstein can however be used when actual information regarding intake is lacking, the formula

of Emmans (1974) being the most accurate predictor of food intake during the period of maximum egg output. A knowledge of intake during this period is most important in determining optimal nutrient concentration in a laying diet, and known values of food intake for particular flocks in defined environments is a prerequisite for ensuring accurate estimates of optimal nutrient concentrations in diets of layers for the entire laying period.

#### SUMMARY.

The effect of dietary metabolisable energy during the peak production periods on layer performance, combining the three major amino acid studies may be summarised as follows:

1. Dietary energy concentration had no significant effect on rate of lay, egg mass or egg output.
2. No response in egg mass to increased dietary energy concentration was observed in this study, probably due to the fact that diets at different energy levels provided adequate amino acids.
3. Food consumption declined significantly with increased dietary energy concentration.
4. Food conversion efficiency improved significantly as the density of the diet increased. This improvement was attributed to decreased food intake at higher dietary energy concentrations, as egg output was not affected by changes in nutrient density.
5. Energy intake increased significantly as the energy concentration of the diet escalated.
6. A consequence of increased energy consumption at higher energy concentrations is the deposition of this excess energy in the form of body fat. Although analyses of mass gain on a daily



basis showed no significant differences between treatments of different dietary energy levels, it was nevertheless evident that at the end of the trials, the body mass of layers on the higher energy diets was greater than that on the lower energy diets.

7. It was established that when the energy consumption by layers fed a diet containing 11,3 MJ ME/kg is known, energy intake at other dietary energy concentrations can be accurately predicted by using the equation produced by Morris (1968).
8. Of the numerous equations proposed by various workers to predict the energy intake of layers, the one produced by Emmans (1974) was found to be the most accurate predictor of energy intake during periods of maximum egg output in these studies.



## GENERAL DISCUSSION

Protein and energy sources constitute the greatest volume and cost of all livestock diets. Cloete (1980) and Griessel (1979) have indicated that there will be a rapidly increasing demand for livestock produce in South Africa during the next two decades which in turn will increase the demand for more protein and energy for livestock diets.

Lochner (1980) reported that fish resources have been exploited to their maximum potential in South African waters and earnest attention will have to be given to adequate conservation of this strategic protein material. It is evident that in order to meet the demand for protein in future livestock diets, the production of protein of plant origin will have to be expanded and alternate sources will have to be found.

Unfortunately less than ten percent of the present land surface in South Africa has climatic conditions suitable for crop production. Even in those areas with sufficient rainfall the soils are invariably shallow, infertile and prone to erosion. The problems are further compounded by erratic rainfall and climatic extremes. Such factors will make it particularly difficult to keep pace with the protein needs of the rapidly expanding livestock industry. The Minister of Agriculture and Fisheries, being aware of this situation, established a Protein Advisory Committee to advise him inter alia on the more efficient utilisation of available protein sources, to monitor supply and demand conditions on a regular basis, and the development and promotion of alternative protein materials.

The looming protein shortage in South Africa which will become particularly prevalent during the next two decades poses serious problems with regard to the supply and efficient utilisation of

raw materials. This study has attempted to contribute some answers to the improved utilisation of protein materials in layer diets.

When practical layer diets are formulated in South Africa it becomes apparent that the cost of these diets is largely dependent on the levels specified for the three limiting essential amino acids, lysine, methionine and isoleucine. An improved understanding of the response of layers to various intakes of these amino acids was therefore considered necessary to determine the most economic utilisation of proteinaceous materials.

A review of the literature indicated that the most suitable technique of determining the response of layers to varying amino acid intakes is that developed by Fisher and Morris (1970) or the revision put forward by Pilbrow and Morris (1974). These workers realised the shortcomings of the historical techniques of determining the amino acid requirements of laying hens and developed this pioneering technique which considered not only biological but also economic parameters in diet formulation. One of the many advantages of this technique, as discussed in Chapter 1, is that response curves are obtained from which improved performance value is measured against the cost of increased amino acid input. As an economically justifiable decision can only be taken when these two parameters are measured it was decided to use this technique in this study.

As energy is also an important cost factor in the formulation of layer diets it was decided to investigate each of the three amino acids at three energy levels. It is also important to prove that no interaction exists between those factors that determine food intake and the utilisation of nutrients by the laying hen. To this end the series of experiments reported here were designed in such a way as to show the effect of energy concentration (as a modifier of food intake) on the utilisation of isoleucine, lysine and methionine.



The results of the three trials showed that the isoleucine, methionine and lysine requirements of individual pullets are adequately represented (ignoring change in body mass) by the following equations:

$$\text{Isoleucine req.} = 9,076E_{\text{max}} + 48,474W$$

$$\text{Lysine req.} = 10,65E_{\text{max}} + 48,04W$$

$$\text{Methionine req} = 3,5E_{\text{max}} + 43,55W.$$

It was however emphasised that these equations should not be used directly by substituting average values of  $E_{\text{max}}$  and  $W$  to predict the requirements of a flock, since it makes no allowance for the variations in yield and body mass which exist within flocks and the consequent curvilinear responses to be expected when diets of differing amino acid concentration are fed to different groups of birds.

It was calculated that at the present marginal costs of isoleucine, methionine and lysine together with the current egg prices in South Africa, the optimal intake of the above mentioned amino acids are 651, 337 and 764 mg/bird d respectively. Because the cost ratio will no doubt change in future, tables relating optimum intake for various price structures were prepared and presented in Tables 3.14, 4.19 and 5.18.

These optimal amino acid requirements were determined for the periods of peak output as egg production is normally distributed during these periods. It was also assumed that the requirement does not alter for the rest of the laying cycle. It is accepted that egg output declines with age but it is also realised that the mean egg output is that of a population which represents both birds that are in full lay and in pause.

The presence of increasing numbers of non-productive birds in older flocks, which consume amino acids but do not contribute to flock output, was not considered a valid reason to reduce the



amino acid supply to older birds which are still in full lay.

The theoretical argument behind phase feeding that production declines with age and therefore amino acid requirements must also decline, is considered unsound because it is founded on the assumption that the amount of amino acids required per unit of egg output remains constant. Figure 1,4 in Chapter 1 supports the contention that phase feeding is incorrect in that the efficiency of amino acid utilisation declines as the production cycle progresses. Fisher (1970) cited by A.R.C. (1975) has shown that about half the decline in efficiency of methionine utilisation which occurs during the layer year is due to the presence of poor producing birds in the flock. He postulated that the other half of this decline presumably reflects some real change in the metabolic efficiency of the ageing bird. Subsequent work by Wethli and Morris (1978) clearly indicates that the poorer utilisation must not be sought in age. From their work a rest period of force moulted birds resulted in a utilisation equivalent to that of young birds from which it can possibly be concluded that the oviduct requires a restorative period to return to its optimal physiological efficiency.

Direct comparison of the estimates of optimal amino acids shown in Tables 3.14, 4.19 and 5.18 with previous estimates of requirement would be misleading. It was indicated in Chapter 1 that the estimation of requirement by linear regression, as used by many workers, necessarily leads to lower estimates than the methods adopted in this paper. It is however of interest to note that optimal isoleucine requirement of 651 mg/bird d determined in this study is substantially lower than the 850 mg/bird advocated by Scott et al (1976). This large discrepancy was attributed to different isoleucine values assigned to raw materials. This can in turn be ascribed to hydrolysis time, use of different hydrolytic agents or equipment used for amino acid assay. The pitfalls of following the procedure of using the raw material matrix value



from one source and yet using the requirement standards from another source, is emphasised.

Using the equations determined in this study in respect of lysine and methionine requirements for individual birds, lower estimates than those reported during the past five years were obtained. As expected the optimal levels were higher than those reported. Lysine was an exception in that estimates in this study were lower than those reported for diets based on wheat. Reasons for this anomaly were given in Chapter 5.

It was found in all the trials that the amino acid requirements for optimal food conversion efficiency were considerably higher than requirements for optimal egg output. Some researchers have used this parameter as a criterion to assess amino acid requirements. However, it is felt that when food conversion efficiency is used to determine requirements, diet cost can under certain circumstances not only be uneconomic, but also lead to unnecessary waste of valuable protein. The Reading model used in this study is considered to be a superior measure since it determines the economically optimum amino acid intake taking into account the cost of the amino acid input and the value of the output.

There was a definite tendency for food intake to increase in all the trials where diets contained marginal levels of the first limiting amino acid above intakes on diets with adequate amino acid levels. This observation concurs with the findings of other workers who indicated that food intake is not independent of dietary amino acid content (Fisher and Morris, 1967, Harms, Damron and Waldroup, 1967 and Pilbrow and Morris, 1974). With marginally sub-optimal levels of amino acids in the diet food intake is increased in an effort to sustain production. It was therefore not surprising that the food conversion efficiency of birds receiving marginally sub-optimal amino acid diets was significantly poorer than that of birds fed adequate diets.

This work also showed that at given amino acid intakes egg output was equal irrespective of the dietary energy used. It was therefore concluded that relatively low energy diets can be used economically, provided that sufficient amino acids are supplied in the diet. This is contrary to the generally accepted belief that the feeding of high energy diets result in better performance. A possible explanation for this misconception is that an inadequate amino acid intake is often obtained at lower energy levels when these two nutrients are strictly related.

Significantly superior food conversion efficiencies were observed for birds fed the higher energy diets. Since it has been previously indicated that egg output is not affected by dietary energy concentration, provided amino acid intake is adequate, it must be concluded that the improved food conversion efficiency can be ascribed to reduced food intake.

In both the lysine and methionine trials conducted over nine 28 d periods food intake on the lowest dilution diets was significantly lower initially than intake on the adequate amino acid diets. A long term adaptation was evident in that at the end of the trials intakes by birds on the former diets was significantly higher. In the isoleucine trial a significantly lower food intake at the end of the fifth 28 d period by birds on the lowest dilution diets was still evident. Whether food intake would have increased had the trial been extended is open to speculation.

It is of interest to note that although food intake increased progressively on the inadequate lowest dilution diets as the trial progressed, egg production nevertheless remained at a low level. It must therefore be concluded that inadequate amino acid diets affect egg output directly. This is contrary to the suggestion of Filmer (1974) who indicated that with severely deficient amino acid diets egg output falls, the demand for nutrients diminishes and therefore food intake falls in consequence.



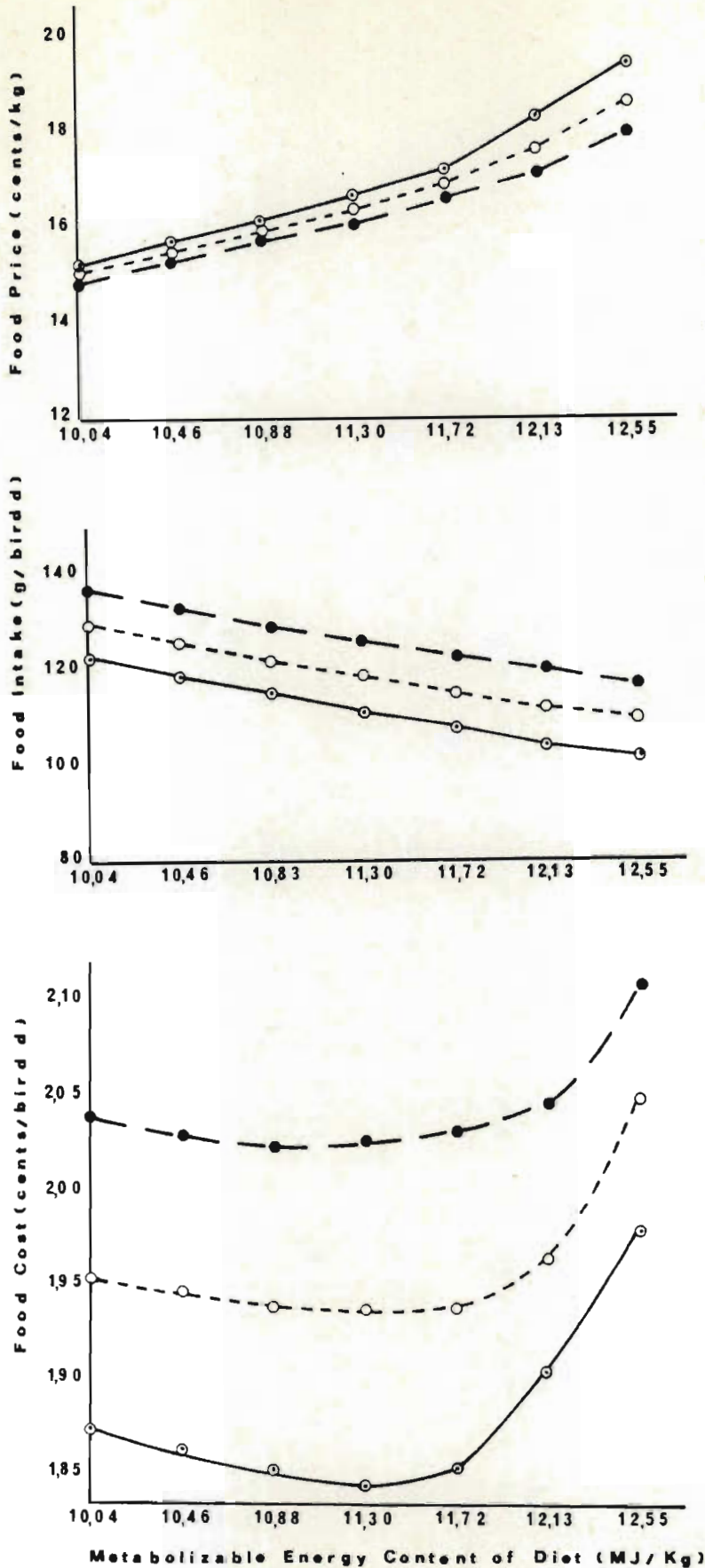


Fig 7.1 The effect of nutrient density on diet cost, food intake and food cost/bird d for laying strains with characteristic energy intakes of 1 255 (○—○), 1 339 (○----○), and 1 423 (●—●) KJ/bird d.

It has been shown in Chapter 6 that if the characteristic energy intake of birds on a diet containing 11,3 MJ ME/kg is known, the energy intake on other dietary energy concentrations can be accurately determined using the equation suggested by Morris (1968). This equation was therefore used to calculate the energy and equivalent food intake of birds receiving diets with different dietary energy concentrations. In order to ensure optimal intake of the amino acids investigated, specifications have been drawn up which are presented in Tables 7.1 to 7.7.

When setting these specifications consideration was also given to optimal tryptophan levels. The present marginal cost of tryptophan in South Africa is R35/kg when supplied in the form of protein. At the present egg price of 60 cents/kg it represents a "k-value" of 0,0583. This value corresponds to an optimal available tryptophan intake of 165 mg/bird d or 183 mg total tryptophan per bird d for a 2 kg bird with an egg output of 50 g/bird d (Morris and Wethli, 1978). Further constraints specified were 550 mg sulphur amino acids (TSAA), 400 mg available phosphorus, 700 mg total phosphorus and 4 g calcium/bird d. The diets were formulated on a least cost basis and the costs are also presented in the Tables.

Figure 7.1 illustrates the escalating cost related to increasing nutrient densities of diets for layers with characteristic energy intakes of 1 255, 1 339 and 1 423 KJ ME per bird d. Dietary costs for birds with lower energy intakes are higher than those of birds with higher energy intakes. The reason for this is that a higher inclusion of essential nutrients is necessary in diets for layers with characteristically low energy intakes. It is also evident from Figure 7.1 that food intake declines with a progressive increase in dietary nutrient density. Using current South African ingredient prices the optimal dietary energy concentration for layers with a characteristic energy intake of 1 255 KJ/bird d appears to be 11,3 MJ ME/kg. When the characteristic energy intakes of birds are 1 339 and 1 423 KJ/bird d, the food cost/bird d within the energy range of 10,46 to 11,72 MJ ME/kg is very



TABLE 7.1: Dietary specifications and cost of diets containing 10,04 MJ ME/kg for layers with different energy intakes.

Characteristic energy intake on diet containing 11,3 MJ ME/kg (KJ/bird d)	1 255	1 297	1 339	1 381	1 423
Calculated* food intake on 10,04 MJ ME/kg diet (g/bird d)	123	126	130	133	137
Methionine (g/kg)	2,76	2,70	2,61	2,55	2,48
Sulphur amino acids (TSAA) (g/kg)	4,47	4,37	4,23	4,14	4,01
Lysine (g/kg)	6,21	6,06	5,88	5,74	5,58
Tryptophan (g/kg)	1,48	1,45	1,41	1,38	1,34
Isoleucine (g/kg)	5,28	5,16	5,00	4,89	4,74
Calcium (g/kg)	32,5	31,8	30,8	30,1	29,2
Total phosphorus (g/kg)	5,70	5,56	5,38	5,26	5,11
Available phosphorus (g/kg)	3,25	3,17	3,08	3,01	2,92
Food cost (cents/kg)	15,24	15,15	15,06	14,96	14,89
Food cost/bird d (cents)	1,875	1,909	1,957	1,990	2,04

\* Morris (1968)

TABLE 7.2: Dietary specifications and costs of diets containing 10,46 MJ ME/kg for layers with different characteristic energy intakes.

Characteristic energy intake on diet containing 11,3 MJ ME/kg (KJ/bird d)	1 255	1 297	1 339	1 381	1 423
Calculated* food intake on 10,46 MJ ME/kg diet (g/birdd)	119	122	126	129	133
Methionine (g/kg)	2,86	2,79	2,70	2,64	2,56
Sulphur amino acids (TSAA) (g/kg)	4,62	4,51	4,37	4,26	4,14
Lysine (g/kg)	6,42	6,26	6,06	5,92	5,74
Tryptophan (g/kg)	1,54	1,50	1,45	1,42	1,38
Isoleucine (g/kg)	5,46	5,33	5,16	5,04	4,89
Calcium (g/kg)	33,6	32,8	31,8	31,0	30,1
Total phosphorus (g/kg)	5,88	5,74	5,56	5,43	5,26
Available phosphorus (g/kg)	3,36	3,28	3,17	3,10	3,01
Food cost (cents/kg)	15,69	15,59	15,46	15,38	15,28
Food cost/bird d (cents)	1,867	1,902	1,948	1,984	2,033

\* Morris (1968)



TABLE 7.3: Dietary specifications and costs of diets containing 10,88 MJ ME/kg for layers with different characteristic energy intakes.

Characteristic energy intake on diet containing 11,3 MJ ME/kg (KJ/bird d)	1 255	1 297	1 339	1 381	1 423
Calculated* food intake on 10,88 MJ/kg diet (g/bird d)	115	118	122	126	129
Methionine (g/kg)	2,96	2,88	2,79	2,70	2,64
Sulphur amino acids (TSAA) (g/kg)	4,78	4,66	4,51	4,37	4,26
Lysine (g/kg)	6,64	6,47	6,26	6,06	5,92
Tryptophan (g/kg)	1,59	1,55	1,50	1,45	1,42
Isoleucine (g/kg)	5,66	5,51	5,33	5,16	5,04
Calcium (g/kg)	34,8	33,9	32,8	31,7	31,0
Total phosphorus (g/kg)	6,09	5,92	5,70	5,56	5,43
Available phosphorus (g/kg)	3,48	3,39	3,28	3,17	3,10
Food cost (cents/kg)	16,15	16,04	15,91	15,79	15,70
Food cost/bird d (cents)	1,857	1,893	1,941	1,999	2,025

\* Morris (1968)

TABLE 7.4: Dietary specifications and costs of diets containing 11,3 MJ ME/kg for layers with different characteristic energy intakes.

Characteristic energy intake on diet containing 11,3 MJ ME/kg (KJ/bird d)	1 255	1 297	1 339	1 381	1 423
Calculated* food intake on 11,3 MJ ME/kg diet (g/bird d)	111	115	119	122	126
Methionine (g/kg)	3,06	2,96	2,86	2,79	2,70
Sulphur amino acids (TSAA) (g/kg)	4,95	4,78	4,62	4,51	4,37
Lysine (g/kg)	6,88	6,64	6,42	6,26	6,06
Tryptophan (g/kg)	1,65	1,59	1,54	1,50	1,45
Isoleucine (g/kg)	5,86	5,66	5,46	5,33	5,15
Calcium (g/kg)	36,0	34,8	33,6	32,8	31,7
Total phosphorus (g/kg)	6,30	6,09	5,88	5,74	5,56
Available phosphorus (g/kg)	3,60	3,48	3,36	3,28	3,17
Food cost (cents/kg)	16,64	16,49	16,32	16,24	16,11
Food cost/bird d (cents)	1,847	1,896	1,942	1,981	2,030

\* Morris (1968)

TABLE 7.5: Dietary specifications and cost diets containing 11,72 MJ ME/kg for layers with different characteristic energy intakes.

Characteristic energy intake on diet containing 11.3 MJ ME/kg (KJ/bird d)	1 255	1 297	1 339	1 381	1 423
Calculated* food intake on 11,72 MJ ME/kg diet (g/bird d)	108	111	115	119	123
Methionine (g/kg)	3,15	3,06	2,96	2,86	2,76
Sulphur amino acids (TSAA) (g/kg)	5,09	4,95	4,78	4,62	4,47
Lysine (g/kg)	7,07	6,88	6,64	6,42	6,21
Tryptophan (g/kg)	1,69	1,65	1,59	1,54	1,48
Isoleucine (g/kg)	6,02	5,86	5,66	5,46	5,28
Calcium (g/kg)	37,0	36,0	34,8	33,6	32,5
Total phosphorus (g/kg)	6,48	6,31	6,09	5,88	5,69
Available phosphorus (g/kg)	3,70	3,60	3,48	3,36	3,25
Food cost (cents/kg)	17,18	17,02	16,86	16,72	16,55
Food cost/bird d (cents)	1,855	1,889	1,939	1,989	2,036

\* Morris (1968).

TABLE 7.6: Dietary specifications and cost diets containing 12,13 MJ ME/kg for layers with different characteristic energy intakes.

Characteristic energy intake on diet containing 11,3 MJ ME/kg (KJ/bird d)	1 255	1 297	1 339	1 381	1 423
Calculated* Food intake on 12,13 MJ ME/kg diet (g/bird d)	104	109	112	116	120
Methionine (g/kg)	3,27	3,12	3,04	2,93	2,83
Sulphur amino acids (TSAA) (g/kg)	5,29	5,05	4,91	4,74	4,58
Lysine (g/kg)	7,35	7,01	6,82	6,59	6,37
Tryptophan (g/kg)	1,76	1,68	1,63	1,58	1,53
Isoleucine (g/kg)	6,25	5,96	5,80	5,60	5,42
Calcium (g/kg)	38,5	36,7	35,7	34,5	33,3
Total phosphorus (g/kg)	6,73	6,42	6,25	6,03	5,83
Available phosphorus (g/kg)	3,85	3,67	3,57	3,45	3,30
Food cost (cents/kg)	18,32	17,78	17,55	17,25	17,06
Food cost/bird d (cents)	1,905	1,937	1,966	2,001	2,047

\* Morris (1968)



TABLE 7.7: Dietary specifications and cost diets containing 12,55 MJ ME/kg for layers with different characteristic energy intakes.

Characteristic energy intake on diet containing 11,3 MJ ME/kg (KJ/bird d)	1 255	1 297	1 339	1 381	1 423
Calculated* Food intake on 12,55 MJ ME/kg diet (g/bird d)	102	106	110	113	117
Methionine (g/kg)	3,33	3,21	3,09	3,01	2,91
Sulphur amino acids (TSAA) (g/kg)	5,39	5,19	5,00	4,87	4,70
Lysine (g/kg)	7,49	7,21	6,95	6,76	6,53
Tryptophan (g/kg)	1,79	1,73	1,66	1,62	1,56
Isoleucine (g/kg)	6,37	6,13	5,91	5,75	5,56
Calcium (g/kg)	39,2	37,7	36,4	35,4	34,2
Total phosphorus (g/kg)	6,86	6,60	6,36	6,19	5,98
Available phosphorus (g/kg)	3,92	3,77	3,64	3,54	3,42
Food cost (cents/kg)	19,43	19,04	18,62	18,38	18,02
Food cost/bird d (cents)	1,982	2,018	2,049	2,076	2,108

\* Morris (1968)



similar. Since this study showed that equal performance is achieved by birds on diets ranging between the above dietary energy levels it can therefore be concluded that any diet within this energy range can be used with equal economic benefit.

In Chapter 6 attention has however been drawn to the increased body mass of birds receiving higher energy diets at the termination of the trials. This increased body mass was ascribed to "over-consumption" of energy on higher energy diets resulting in fat deposition. When higher prices are obtained for cull birds in terms of cents/kg body mass or when a better price is obtained for heavier culls per se, the choice of the optimal energy concentration will tend towards diets with higher energy concentrations.

Contrary to many previous reports dietary energy intake appeared to have no effect on egg mass provided that amino acid intake was adequate. In both the summit and first dilution diets, which provided adequate intakes of all essential amino acids, no significant egg mass (g/egg) differences at the three energy levels were observed in the methionine and lysine trials. The reason for this opposing finding can probably be attributed not only to adequate amino acid intakes but also to a sufficient linoleic acid intake. In the isoleucine trial marginal intakes of linoleic acid on some treatments probably contributed towards the lower egg mass found on the low energy diets.

In formulating diets, amino acid concentrations are usually related to dietary energy content. Under these circumstances energy intake on low energy diets is usually lower than that on high energy diets and therefore amino acid intake will be correspondingly lower. As shown in this study the amino acid requirement for maximum egg mass (g/egg) is far greater than that for optimal egg output. It can therefore be concluded that the low energy intake on low energy diets disguises the concurrently reduced essential amino acid intake which appears to be the actual cause of reduced egg mass. It must be appreciated that fifty percent



of egg dry matter is composed of protein and that adequate amino acid supply is critical for this protein synthesis. When the supply of either one or several amino acids is low, egg protein with an altered amino acid composition will not be synthesised. Instead, under conditions of mild deficiency, the quantity of protein synthesised may be decreased, and in severe dietary deficiency egg protein synthesis may essentially cease (Scott et al, 1976). This has the effect of reducing egg size or completely stopping egg production. It can in fact be stated that reduced egg size is often the only consequence observed in a marginal protein or amino acid deficiency.

Amino acid concentrations in diets for birds with a low food intake must of necessity be higher than concentrations in diets for birds with a high food intake to ensure equal amino acid consumption. The dietary cost difference at the same energy concentration is shown in Tables 7.1 to 7.7. The specifications in Tables 7.1 to 7.7 can thus also be used to advantage when intake varies due to seasonal temperature fluctuations. During colder weather as experienced in winter energy needs are increased for maintenance with a consequent increase of food intake. In this process consumption of all essential nutrients increases unless adjustments are made. Without changes to the diet amino acid intake will therefore increase above levels needed to support optimal egg production and this will increase feeding costs unnecessarily. During summer on the other hand, energy needs of the layer are reduced and appetite declines. This results in a reduction of food intake and a reduction in critical nutrient consumption for the maintenance of egg production. Thus egg production will decline should the diet not be altered to provide more amino acids, minerals and vitamins. In this case egg production suffers, and as a result food cost/kg eggs produced will escalate.

It is evident from this study that when amino acids are related in a fixed ratio to energy concentration, amino acid intake on

low energy diets is lower than on high energy diets. Although at a given ratio amino acid intake may be adequate on high energy diets, it may not necessarily be so for low energy diets.

Expressing amino acids to energy in the form of a ratio cannot therefore be recommended.

In conclusion, the looming protein shortage in the South African context warrants the adoption of the techniques used in this study for developing models that could be used to determine optimum nutrient requirements not only for poultry but also for other classes of monogastric animals.

More attention must be directed toward the possibility of adjusting the level of each nutrient so as to maximise profit margins rather than attempting to achieve maximum levels of production. This implies abandoning the idea of a "fixed requirement" for a nutrient and replacing it by data relating the cost of nutrient inputs to the value of outputs.



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