# Temperature and dietary lysine levels for laying broiler breeder hens

## Thesis

UNRESTRICTED.

# TEMPERATURE AND DIETARY LYSINE LEVELS FOR LAYING BROILER BREEDER HENS

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# Abstract

The specific objectives of this project were; to examine and explain the response of laying broiler breeders to different constant and cycling ambient temperatures, to different dietary concentrations of lysine and the temperature x dietary lysine interactions. A quantitative literature review indicated that there was a curvilinear ($P<0.001$) response in most egg production variables by egg-laying strains kept in different ambient temperatures. A second quantitative evaluation of literature indicated that increases in dietary amino acid concentrations given to egg-laying strains of hens gave small increases ($P<0.001$) in egg laying performance until an asymptote was reached. Little information is available on these responses in laying meat-line (broiler breeder) hens. Four feeding experiments were performed to examine and explain the response of laying broiler breeders to different constant and cycling temperatures, to different dietary concentrations of lysine and the temperature x lysine interactions. Two experiments used small flocks of floor-housed breeders in which egg production and egg composition, hatchability and chick quality variables were examined. Two further experiments were conducted in which breeder hens were caged individually and egg production, egg composition, blood physiology and carcass composition variables were examined. The egg production (egg mass output and egg weight) response of broiler breeder hens to different ambient temperatures was similar to that previously demonstrated for egg-laying strain birds, but there were no ($P>0.05$) effects on hatchability or chick quality. Increasing dietary lysine concentrations tended ($P<0.1$) to give a curvilinear response in egg mass outputs although the shape of the response curve was different to that of egg-laying strains. Different dietary lysine concentrations did not affect ($P>0.05$) hatchability or chick quality. Increasing dietary lysine increased ($P=0.044$) blood lysine concentration and increased ($P=0.026$) blood haematocrit values in birds kept at 21°C but not in those kept at 32°C. There was a similar temperature x lysine interaction ($P= 0.031$) in carcass protein composition and a trend ($P<0.1$) for this same interaction in body weight change of the hens in two experiments.

# Declaration

I hereby declare that this thesis has been composed by myself and has not previously been submitted for any other degree. The work described herein is my own and all work of other authors is duly acknowledged.

AL-saffar, A.A.

# Acknowledgments

I am very grateful to my supervisor, Dr S.P. Rose, for receiving me at Harper Adams University College and giving me the opportunity of conducting the experiments described in this thesis. I thank him for supervising the work and for his constant encouragement throughout the course of this study. Special thanks for his support, the valuable discussions and constructive criticism during the preparation of this manuscript. Also, I have to thank him for his patience, guidance and help during the writing of the thesis. I am also grateful to my second supervisor, Dr G. Scott, for his support during the work and to staff at University of Reading for statistical advice.

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Finally, the author thanks Aviagen Ltd. and Dr. Dinah Burgess for the supply of birds for the experimental work and helpful advice on the research project.

# Dedication

I dedicate this work to ALQAIM (AS); to my father and mother for their love, spiritual support, encouragement and tender prayers; and to sisters and brothers who have given much time to inspiring and supporting me throughout my academic life. I also dedicate it to my dear wife, who helped me in every possible way, for her suffering, loneliness enduring patience, support, understanding and sacrifices. This work is also dedicated to my sons.

I like also to dedicate this work to my country as represented by Kuwait Institute for Scientific Research that provided financial support for this degree.

# Publications arising from this thesis

- **AL-SAFFAR, A.A. and ROSE, S.P.** (2001) Effect of temperature on the productive performance of laying hens. *Proceeding of World Poultry Science Association,* York, pp 14 -15.

- **AL-SAFFAR, A.A. and ROSE, S.P.** (2002) Effects of ambient temperatures on the egg laying characteristics of hens. *World's Poultry Science Journal* (in press).

- **AL-SAFFAR, A.A. and ROSE, S.P.** (2002) A quantitative evaluation of the response of laying hens to dietary amino acids. *World's Poultry Science Journal* (in press).

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# 1. General Introduction

The countries of the Arabian Gulf (Bahrain, Kuwait, Saudi Arabia, Qatar, Oman and United Arab Emirates) a have high chicken meat consumption (31.7kg/person/year). Although these countries are still among the leading importers of chicken meat, there has been a recent trend to increase national production. In the past five years, chicken meat production in the region has increased by 33% compared to the overall world chicken meat production increase of 18.5%.

Not only has there been an increase in commercial chicken production in the Arabian Gulf countries but also there has been an increase in the production of hatching eggs to supply this industry. Hatching eggs can be imported to the region but national production gives a number of advantages of vertical integration to the poultry meat production companies.

Management of broiler breeder flocks in Arabian Gulf countries has some major problems: First, although cereals can be produced in the region, a large proportion of protein concentrates must be imported to be included in the feeds. Proteins and particularly limiting amino acids therefore have a relatively high unit price. Second, there are very high ambient temperatures in many of the main poultry producing areas of the region that must be overcome and understood to enable efficient hatching egg production.

Hatching egg production in the Arabian Gulf is almost always undertaken in controlled environment buildings. These buildings will incorporate cooling equipment but still optimum environmental temperatures cannot always be maintained. The laying birds will

need to endure high ambient temperatures during daylight hours for large parts of the year. Hatching egg producers need information to be able to predict and understand the effects on the birds of these high temperatures in order to be able to evaluate the economic efficiency of further investments in new sites or in further investment in cooling equipment for existing sites.

There are sufficient data that describe the response to temperature of commercial egg laying strains of hens, although there is a lack of a suitable summary of these effects. However, there is a possibility that the responses of laying broiler breeders could be substantially different to that of commercial egg layers: Broiler breeders are restrictedly fed yet have a body weight that is over twice that of birds from commercial egg laying strains. There is a need to examine the responses of broiler breeders to different temperatures.

The balance of amino acids in poultry diets is an important criterion that affects economic viability of a poultry enterprise and lysine is one of the first limiting amino acids in broiler breeder diets. Morris (1983) showed that if flocks of laying hens are given increased dietary concentrations of a limiting amino acid then there is a smooth response curve where egg mass output gradually reaches an asymptote. Knowledge of the exact shape of the response curve in egg output of laying poultry to varying levels of an amino acid is important in formulating diets that optimize the economic efficiency of poultry production systems. There were sufficient published data that described this response to the major limiting amino acids in commercial egg-laying strains, although there is a lack of a good summary of these effects. Additionally there is a lack of studies in broiler breeders and it is possible that this strain of chicken may have a different response to amino acid supply to the commercial egg laying strains.

Observations of commercial broiler breeder flocks has suggested that the response to dietary amino acids may be variable. This variability could be related to the ambient temperatures of the birds. Amino acid imbalance results in excess amino acids being oxidised. This metabolic process is relatively inefficient and results in a high heat output (Emmans 1994). The response of birds at high temperatures may differ because of their reduced energy requirements and their inability to dissipate excess body heat. There is a need to examine whether the ideal balance of essential amino acids is different at different ambient temperatures. Many practical broiler breeder feeds are limiting in lysine, so this amino acid may be the most appropriate amino acid to examine first.

The specific objectives of this project were;

- To examine and explain the response of laying broiler breeders to different constant and cycling ambient temperatures.
- To examine and explain the response of laying broiler breeders to different dietary concentrations of lysine.
- To examine and explain the temperature x dietary lysine interactions in the response of broiler breeders.

# Chapter 2

# 2. Literature

This thesis examines the response of laying broiler breeders to dietary lysine supply, varying ambient temperatures and their interaction. The literature review therefore examines the physiological responses of poultry to dietary lysine and to different environmental temperatures. There is a lack of published information on the responses of broiler breeders to these variables. Conversely, there are sufficient data on the responses of laying hens from commercial egg laying strains. However, these data have not been summarized to allow effective conclusions to be made. This literature review therefore includes quantitative analyses of all published literature on the responses of laying hens to dietary amino acid supply and to ambient temperature in order to summarize the available data.

## 2.1 Protein and amino acids

This section is not intended as a review of current research on amino acid biochemistry, as that is outside the scope of this project. It is intended as a background for amino acid nutrition. All facts that are not specifically referenced come from Sturkie (1976), Scott *et al.* (1982) and Stryer (1995), and these texts provide a more in-depth coverage of the subject.

### 2.1.1 Protein structures

The name protein is taken from the Greek *proteios*, which means *first*. Proteins are found in all living cells. They are the principal materials of skin, muscle, tendons, nerves, and blood; of enzymes, antibodies, and many hormones.

Chemically, proteins are high polymers. They are polyamides, and the monomers from which they are derived are the α-amino carboxylic acids. A single protein molecule contains hundreds or even thousands of amino acid units; these units can be of over twenty different kinds. The number of different combinations of amino acids in complex protein molecules is almost infinite. It is likely that tens of thousands of different proteins are required to make up an animal body, and this set of proteins is not identical with the set required by an animal of a different kind.

All proteins contain the chemical elements; carbon, hydrogen, oxygen and nitrogen, practically all contain sulphur. The elementary composition of a typical protein is approximately as follows: Carbon 53%, hydrogen 7%, nitrogen 22%, oxygen 22% and sulphur 2% (Ewing, 1963).

### 2.1.2 Amino acid biochemistry

Individual protein types have precise concentrations of specific amino acids that are linked together in a pre-determined sequence. Protein synthesis may therefore only take place if all the component amino acids are present. Birds are not able to synthesize some amino acids (or cannot synthesize at a rate that is sufficient to meet their high

production requirements). These amino acids must therefore be present in the diet, either as part of proteins or as free amino acids. The so-called 'essential' amino acids are listed in *Table 2.1.*

The essential amino acids may be subdivided into three categories:

- those which are strictly essential because they cannot be synthesized, even from intermediary metabolites particularly 2-oxo precursors. These are lysine and threonine for which the transaminases are absent.
- those which may be synthesized from their respective 2-oxo precursors, but at a rate that is insufficient; these are leucine, valine and isoleucine.
- those that may be synthesized within the general metabolic process but at a rate far too low to meet the requirements of the bird. These are arginine and histidine. The former may he synthesized from glutamate through utilizing a metabolic pathway involving N acetyl derivatives, or during the urea cycle in mammals.

The degree to which the diet supplies the 'ideal protein' has a major influence on bird performance. If one amino acid is not present in the correct proportion, then performance will be reduced to the level of that deficient amino acid (which is termed 'first limiting'). The body is unable to store amino acids provided in excess of requirements, which means that an amino acid supplied over this is not utilized. Therefore an amino acid imbalance may well result in increased excretion of nitrogen. This may have serious implications for carcass quality, as such a situation is associated with increased incidence of breast blisters and hock burns.

**Table 2.1** Natural amino acids

| **Essential** | **Non-Essential** |
|---|---|
| Arginine | Alanine |
| Cystine* | Aspartic acid |
| Histidine | Asparagine |
| Isoleucine | Glutamic acid |
| Leucine | Glutamine |
| Lysine | Hydroxyproline |
| Methionine | Glycine |
| Phenylalanine | Serine |
| Threonine | Proline |
| Tryptophan | |
| Tyrosine* | |
| Valine | |

*Tyrosine is synthesized from phenylalanine and cystine is synthesized from methionine

Although non-essential amino acids need not be provided in the diet, there is a requirement to fulfill general metabolic requirements (including transaminase reactions). A ratio of essential to non-essential amino acids of approximately 0.55:0.45 has been proposed.

Amino acids consist of an amino group ($NH_2$), a carboxyl group (COOH), a hydrogen atom and a distinctive side chain bonded to the α-carbon atom.

| | $NH_2$ | $NH_3^+$ |
|---|---|---|
| **R= Side chain** | H – C – COOH | H – C – $COO^-$ |
| | R | R |
| | Un-ionized form | Dipolar ion (*zwitterionic form*) |

### 2.1.2.1 Classification of amino acids

The twenty amino acids can be classed into six groups according to their side chains:

1. *Aliphatic amino acids:* glycine, alanine, valine, leucine, isoleucine and proline.
2. *Aromatic amino acids:* phenylalanine, tryptophan and tyrosine.
3. *Sulphur amino acids*: methionine and cystine.
4. *Hydroxyl amino acids*: serine and threonine.
5. *Basic amino acids:* arginine, lysine and histidine.
6. *Acidic amino acids:* aspartic acid and glutamic acid.

### 2.1.2.2 Protein synthesis

Protein synthesis consists of a collection of complex processes initiated by three forms of ribonucleic acid (RNA):

- messenger RNA (mRNA) which contains within its nucleotide sequence a code which determines the order in which amino acids are inserted into polypeptide chains.
- ribosomal RNA which combines with approximately 100 protein types in forming ribosomes (sub-cellular organelles) which organise the structural alignment of those different constituents involved in protein synthesis.
- transfer RNA (tRNA) of which there are specific examples for each amino acid. They are implicated in the activation and the bonding of successive amino acids within the ribosome in the sequence determined by mRNA which is itself situated within the ribosome.

Protein synthesis consists of three phases, within the overall process termed translation:

- initiation; a ribosome and a molecule of tRNA, being a specific initiator, present themselves at a particular site on a mRNA molecule at the beginning of the coding sequence. Another amino acyl tRNA may then attach itself to the complex already formed and this therefore effects the first peptide bond.
- elongation; the ribosome moves along the length of the mRNA and the polypeptide chain develops from amino acids in a sequence specifically determined by the order of nucleotides within the mRNA.

- termination; the ribosome reaches the site of coding for the end of the sequence and is released at the same time as mRNA once the protein chain has been completed.

In contrast to carbohydrates and lipids, which may be stored either as hepatic and muscular glycogen and body lipid reserves respectively when supplied in excess of requirements, amino acids cannot be accumulated within the bird. All those in excess of requirements for maintenance (tissue renewal) and production (growth in the young bird, egg protein synthesis) are catabolized. The amine group is removed and then excreted. The carbon skeleton may be used in metabolic pathways involving energy transfer by conversion into carbohydrates (glucogenesis) or into lipids (lipogenesis) or oxidised into $CO_2$ and expired.

In order for the bird to realize its genetic potential and to maximize productive performance through maximum rates of protein synthesis, amino acids must be provided in the necessary quantities, avoiding both excesses and deficiencies. A deficiency situation may be considered in terms of the concept of a limiting factor. When a productive bird (growing or egg laying) receives a certain quantity of each amino acid through its diet, the level of production achieved corresponds in general to the provision of that amino acid which is most limiting in relation to requirements. In the case of amino acids, those which are provided in excess in relation to that which is limiting are of no value and may even have a negative effect.

In the cases of excessive levels of individual amino acids, situations of antagonism may be observed. Antagonism may occur when the excess of an amino acid is associated with deficiencies of others whose requirements will therefore be increased. The best known situation is that of arginine and lysine. An excess of the latter (lysine:arginine ratio of 1.2) reduces growth rate of the young bird. An excess of lysine

induces an extremely high renal arginase activity which leads to an abnormally increased degradation of arginine.

### 2.1.2.3 Amino acids degradation

Excess amino acids are not stored in the body. They are either utilized immediately for their normal function or are oxidized as a source of energy although they are used inefficiently as a source of energy compared with carbohydrates and fat. Consequently it is desirable to feed the minimum protein level and supply the amino acids in the correct proportion.

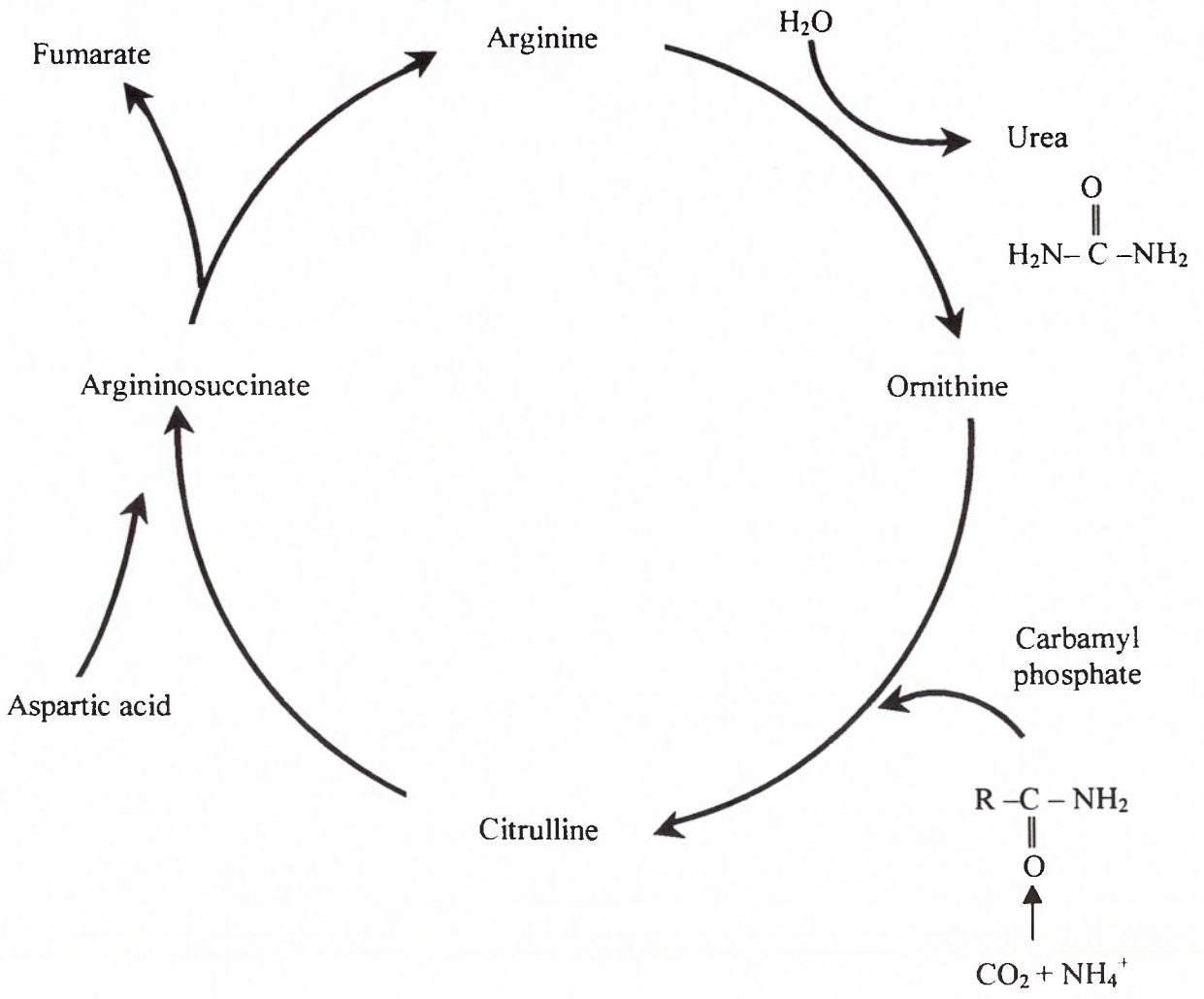
The degradation of amino acids, consists of two parts:

(1) The removal (deamination) of the amino group;

The ammonium ions produced through deamination are highly toxic, so must be removed quickly from the body. In mammals, ammonia is converted to urea which is very soluble and is excreted in urine (*Figure 2.1*). In birds the ammonia is converted to the less soluble uric acid. The major site of amino acid degradation in mammals is the liver. The α-amino group of many amino acids is transferred to α-keto-glutarate to form glutamic acid, which is oxidatively deaminated to yield $NH_4^+$;

E.g. Aspartic acid + α-keto-glutarate ⟷ oxaloacetate + glutamic acid

**Figure 2.1** Urea Cycle



Serine and threonine can be directly deaminated, because they have a hydroxyl group. Serine loses a hydrogen atom from its α-carbon and a hydroxyl group from its β-carbon atom to yield aminoacrylate. This unstable compound reacts with $H_2O$ to give pyruvate and $NH_4^+$. Some of the ammonium ions produced in the breakdown of amino acids are consumed in the biosynthesis of nitrogen compounds. The excess ammonium ions are converted into urea. $NH_4^+$ combines with $CO_2$, ATP and $H_2O$ to form carbamylphosphate. The carbamyl group is transferred to ornithine to form citrulline. Citrulline then condenses with aspartic acid, which provides the other nitrogen atom for urea, to form argininosuccinate. Argininosuccinase then cleaves argininosuccinate to form fumarate and arginine. Arginine is hydrolysed to urea and ornithine.

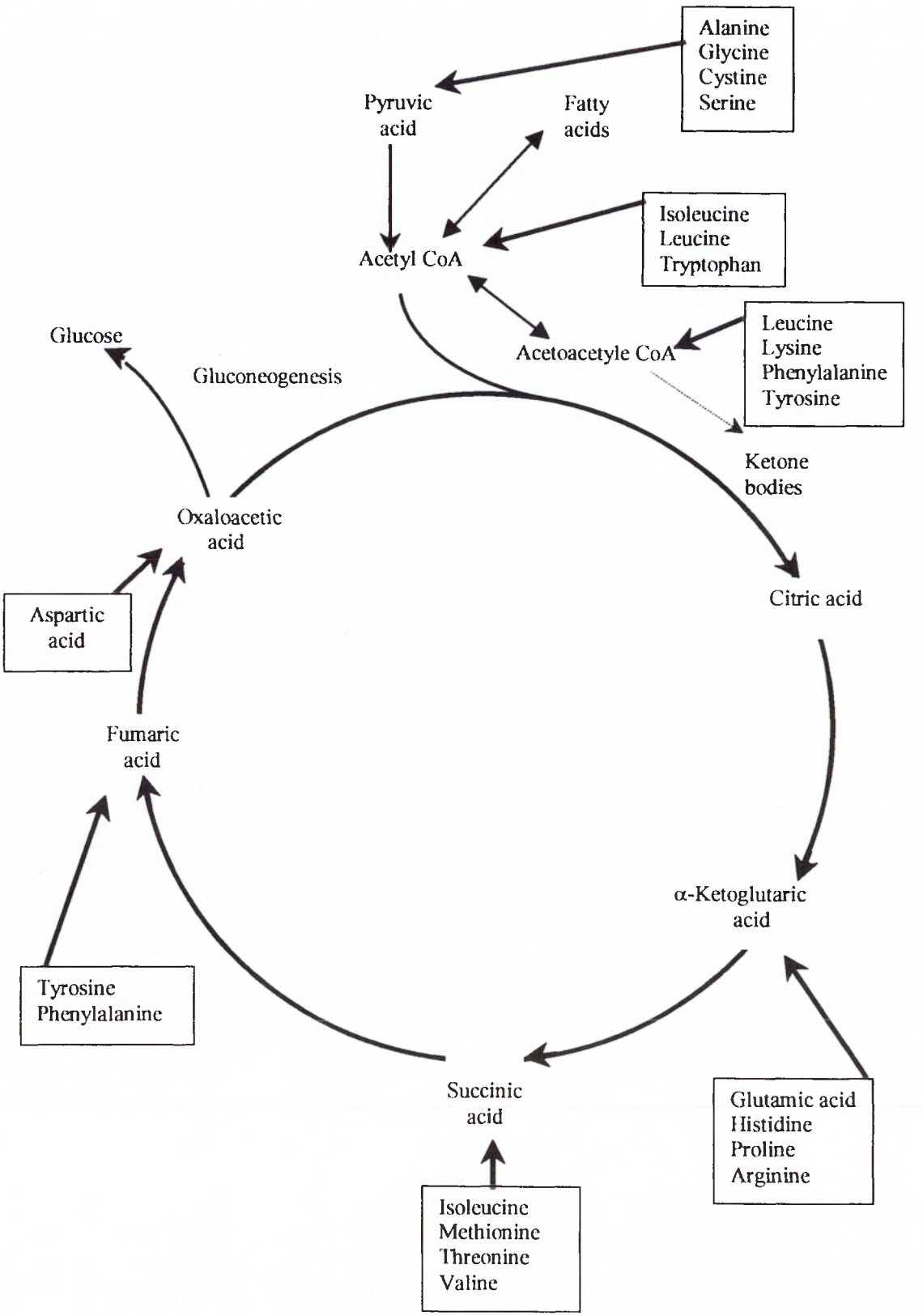
The formation of $NH_4^+$, and its incorporation into carbamylphosphate and the subsequent synthesize of citrulline occur in the mitochondrial matrix. In contrast, the next three reactions of the urea cycle, which lead to the formation of urea, take place in the cytosol.

The urea cycle is linked to the citric acid cycle by fumarate. Fumarate is hydrated to malate, which is in turn oxidized to oxaloacetate. Oxaloacetate can then be transaminated to aspartic acid or can be condensed with acetyl CoA to form citrate. Oxaloacetate can also be either converted into glucose by the gluconeogenic pathway or converted to pyruvate.

(2) The degradation of the carbon skeletons;

Amino acid degradation forms major intermediates that can be converted into glucose or be oxidized by the citric acid cycle (*Figure 2.2*). The carbon skeletons of the twenty

**Figure 2.2** Oxidation of carbon skeletons of amino acid in the citric acid cycle.



amino acids are degraded into only seven molecules: pyruvate, acetyl CoA, acetoacetyl CoA, α-keto-glutarate, succinyl CoA, fumarate and oxaloacetate.

Amino acids that are degraded to acetyl CoA or acetoacetyl CoA are called ketogenic, because they give rise to ketone bodies. Amino acids that are degraded to pyruvate, α-keto-glutarate, succinyl CoA, fumarate or oxaloacetate are called glucogenic. Net glucose synthesize is possible because these are citric acid intermediates and pyruvate can be converted into phosphoenol-pyruvate and then into glucose.

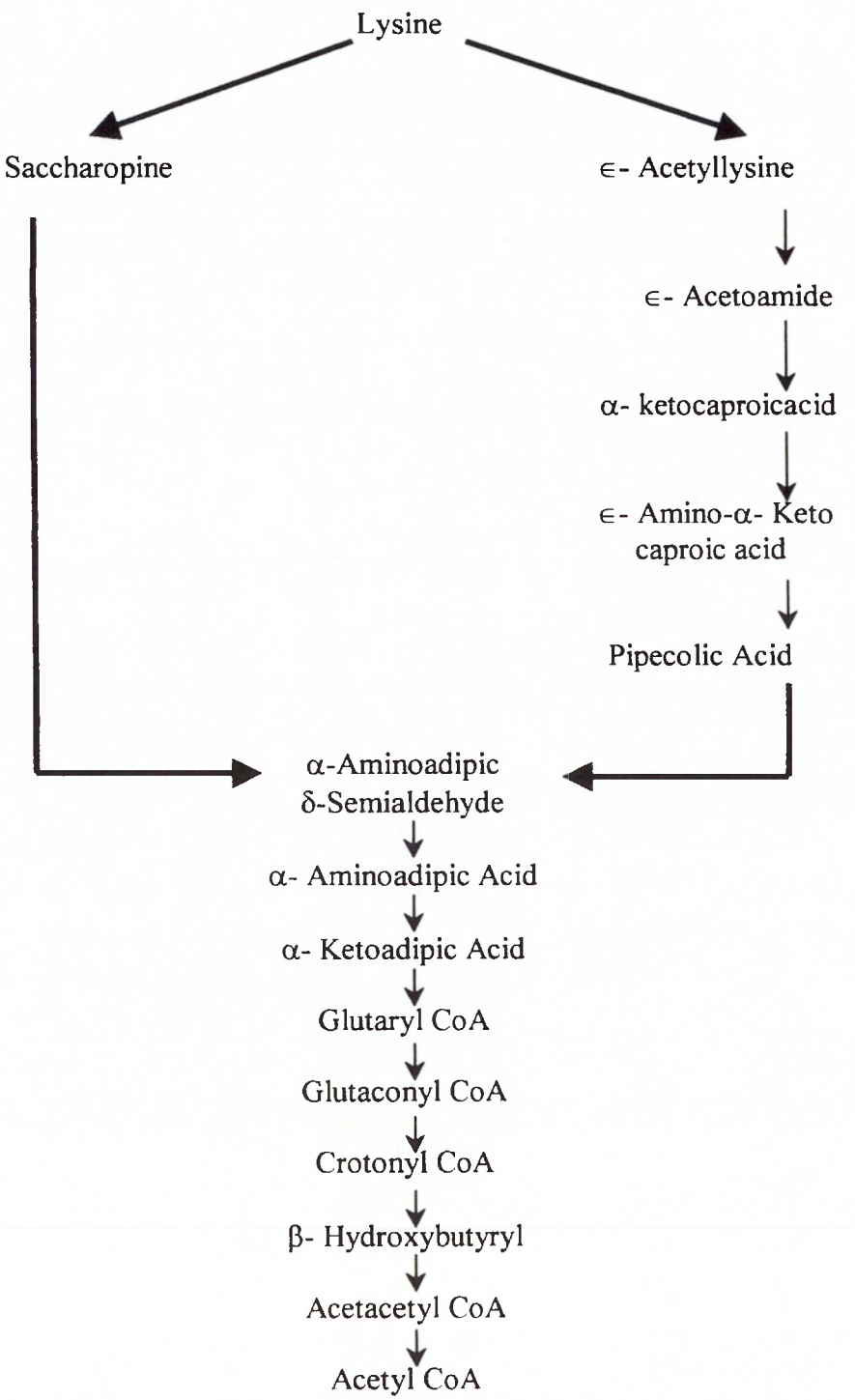
#### 2.1.2.4 Lysine degradation

The degradation of all amino acid groups will not be considered in detail in this document. However, some further information will be given on lysine oxidation because of its importance to the experimental work that is reported.

Lysine is degraded in chicken liver by an L-amino acid oxidase and lysine-ketoglutarate reductase, leading to the formation of pipecolic acid and saccharopine, respectively. Lysine is a ketogenic amino acid, which is ultimately converted to acetoacetyl CoA. This can occur via two distinct pathways (*Figure 2.3*).

Lysine condenses with α-keto-glutarate and is reduced to form saccharopine, which is cleaved to release glutamic acid and the α-semialdehyde of α-aminoadipate. The semialdehyde is oxidized to form α-aminoadipate, which can be transaminated to α-ketoadipate, which can be converted to crotonyl CoA. In birds, lysine is degraded in the liver but the pipecolic acid pathway may be the major route of lysine catabolism in the brain. It has also been suggested that lysine can be catabolised to form homocitrulline and homoarginine (Ryan and Wells, 1966).

**Figure 2.3** Lysine degradation



### 2.1.3 Responses to amino acids and described methods to express requirement

Amino acid levels in laying hen diets and the supply of an optimum dietary concentration are economically important to an egg laying enterprise. The level of a single essential amino acid that is either deficient or in excess of requirement may result in a diet that does not optimize the economic efficiency of an egg production system. There is a need to quantitatively describe the egg laying performance of hens to varying dietary levels of limiting amino acids.

Supplementation of a very deficient diet with the limiting amino acid will give a marked increase in egg mass output of the flock. Further supplementation of the diet with this limiting amino acid will give egg mass output responses that decrease in magnitude as the amino acid requirement for individual birds with the flock is reached (Morris, 1983). Limiting amino acids have the highest per unit weight economic value of all the major nutrients supplied in a poultry feed. Poultry nutritionists thus need the information to allow them to calculate the economic level of addition of the major limiting amino acids. Precise descriptions of the response curves that describe the increasing egg mass output, egg numbers and egg weights to increasing amino acid supply are essential information for this economic evaluation.

Standard texts of nutrient requirements for laying hens (e.g. NRC 1994) express requirement as a concentration of the diet for each essential amino acid (*Table 2.2*). However, Morris *et al.* (1999) have recently suggested that the best method of describing the amino acid requirements of growing chickens is to express as a proportion of the crude protein supply. There is a need to empirically assess which method of describing amino acid requirement gives the better explanation of reported experimental data in laying hens.

**Table 2.2** Methods of expressing amino acids requirements of laying hens. Adapted from NRC (1994)

| Amino acid | Methods of expressing requirement | | |
|---|---|---|---|
| | Proportion of diet (g/g) | Ideal balance as proportion of lysine supply (g/g) | Ideal balance as proportion of crude protein supply[†] (mg/g) |
| Lysine | 0.69 | — | 46.0 |
| Arginine | 0.70 | 1.01 | 46.7 |
| Histidine | 0.17 | 0.25 | 11.3 |
| Isoleucine | 0.65 | 0.94 | 43.3 |
| Leucine | 0.82 | 1.19 | 54.7 |
| Methionine | 0.30 | 0.43 | 20.0 |
| Methionine + Cystine | 0.58 | 0.84 | 39.0 |
| Phenylalanine | 0.47 | 0.68 | 31.3 |
| Phenylalanine + Tyrosine | 0.83 | 1.20 | 55.3 |
| Threonine | 0.47 | 0.68 | 31.3 |
| Tryptophan | 0.16 | 0.23 | 10.5 |
| Valine | 0.70 | 1.01 | 46.7 |

[†]Assuming a 150 g/kg Crude Protein diet (Specified by NRC (1994)).

A potential problem of describing a limiting amino acid response to the proportion of dietary crude protein is that the protein supply may also be limiting in another one or more amino acids. Therefore, a proportion of this supplied crude protein may not be utilizable by the hen for protein deposition because of the deficiency in the second limiting amino acid. ARC (1980) suggested describing the protein supply as the amount of ideally balanced protein. This is the amount of protein that has an ideal balance of amino acids relative to the requirements of the animal. It is possible that describing the amino acid response of laying hens as a proportion of the ideal crude protein may be a further refinement in describing their amino acid requirements.

The objectives of this study were, first, to quantitatively describe the relationship between increasing dietary levels of single limiting amino acids and the egg production characteristics of laying hens by a statistical analysis and assessment of the published literature. Second, to compare three methods of describing the dietary amino acid concentration; as a proportion of the diet (g/kg of feed), as a proportion of the crude protein (g/kg crude protein) or as a proportion of the ideal crude protein (g/kg ideal crude protein).

### 2.1.3.1 Methods of selection and analysis of data

A detailed literature search was conducted for all published laying hen experiments in which a single basal diet was supplemented with different levels of a single, first-limiting amino acid.

The selected experiments had to meet four main criteria:

1. The experiment must have examined the change of only a single dietary essential amino acid within an otherwise constant diet formulation.
2. The range of amino acid concentrations examined must have included the ideal balance for this limiting amino acid within the crude protein (as specified in *Table 2.4*).
3. One treatment group must have been at or within ±20% of this ideal balance for the limiting amino acid.
4. The crude protein and composition of all limiting amino acids must have been given in the published paper or, if not, the ingredient composition of the diet must have been given so that the amino acid concentrations could be calculated using feed composition data from NRC (1994).

Sufficient published experiments were available to give statistically valid comparisons of lysine (42 experiments), methionine (77 experiments), methionine *plus* cystine (77 experiments) and tryptophan (21 experiments) (*Table 2.3*). The selected experiments spanned 50 years and included a large number of production methods and different strains of laying hens kept under varied management conditions. There were, therefore, large variations between experiments in the egg production characteristics of the laying hens, so the amino acid treatment differences were described as a proportion of the treatment group within that experiment that were given a dietary treatment with the ideal balance of that amino acid. All the variables were expressed as a proportion (%) of the egg laying performance obtained in the experiment from the treatment group fed this ideal amino acid treatment. Linear and non-linear regression analyses were conducted on these data for each of the different single amino acids using their concentrations as the explanatory

**Table 2.3** Sources of data used to compare the egg production, egg weight and egg mass output of laying hens fed different amino acids levels

| Reference[1] | Experimental Basal diet | Crude Protein (CP) (g/kg) | Calculated Ideal CP (g/kg) | Amino Acid levels (g/kg CP) used within experiment |
|---|---|---|---|---|
| **Lysine** | | | | |
| Bray (1969)#5 | Maize + Soyabean meal | 119.7 | 119.7 | 26.23, 27.07, 28.74, 31.24, 34.59, 38.76, 43.78, 49.62, 56.31 |
| Chi *et al.* (1976) | Sesame meal + Maize starch | 140.5 | 140.5 | 25, 32.14, 39.29, 46.43, 53.57, 60.71 |
| Fontaine (1974) | Maize + Milo | 119.4 | 115.0 | 37.69, 45.23, 52.76, 60.3 |
| Fontaine and Reyntens (1968) | Maize + Barley | 132.5 | 132.5 | 45.28, 48.3, 51.32 |
| Gardiner and Dubetz (1978)#1a | Wheat + Soyabean meal | 160.0 | 160.0 | 39.38, 58.13 |
| Gardiner and Dubetz (1978)#1b | Wheat + Soyabean meal | 160.0 | 160.0 | 27.5, 46.25 |
| Gardiner and Dubetz (1978)#1c | Wheat + Soyabean meal | 174.0 | 174.0 | 30.46, 47.7 |
| Gruhn (1969) | Not specified | 125.0 | 110 | 28, 32, 36, 40 |
| Harms *et al.* (1995)§ #2 | Maize + Soyabean meal | 109.5 | 109.5 | 47.03, 49.77 |
| Harms *et al.* (1995)§ #3 | Maize + Soyabean meal | 104.38 | 104.38 | 45.88, 52.2 |
| Harms *et al.* (1995)§ #4 | Maize + Soyabean meal | 99.24 | 98.0 | 44.96, 54.94 |
| Harms *et al.* (1995)§ #5 | Maize + Soyabean meal | 94.10 | 90.0 | 43.89, 57.92 |
| Harms *et al.* (1995)§ #6 | Maize + Soyabean meal | 89.0 | 84.0 | 42.7, 61.24 |
| Hijikuro and Horiguchi (1974) | Maize + Maize gluten meal | 160.0 | 95.0 | 21.25, 36.25 |
| Ingram *et al.* (1951)#2a | Maize + Maize gluten meal | 190.0 | 190.0 | 21.05, 35.95, 65.26 |
| Ingram *et al.* (1951)#2b | Maize + Maize gluten meal | 190.0 | 190.0 | 21.05, 31.58, 38.95, 47.37, 65.26 |
| Jensen *et al.* (1974a) | Wheat + Soyabean meal | 160.6 | 143.0 | 35.62, 43.71, 48.07 |
| Jensen *et al.* (1974b) | Maize + Soyabean meal | 155.1 | 133.0 | 32.24, 38.68, 45.13, 51.58 |
| Karunajeewa (1974) § | Wheat + Barley + Peanut meal | 158.0 | 95.0 | 32.28, 34.81, 50.63 |
| Karunajeewa and Tham (1987)#2a | Wheat + Barley | 137.5 | 137.5 | 48.23, 51.24, 54.26 |
| Karunajeewa and Tham (1987)#2b | Oats groats + Barley | 132.7 | 132.7 | 46.51, 49.42, 52.33 |
| Koelkebeck *et al.* (1991) | Maize + Soyabean meal | 160.0 | 130.0 | 49.38, 55.63 |
| Latshaw (1976)#1a | Maize + Maize gluten meal | 140.0 | 140.0 | 32.14, 36.43, 40, 43.57 |
| Latshaw (1976)#1b | Maize + Maize gluten meal | 140.0 | 140.0 | 40.71, 44.29, 47.86, 51.43 |
| Latshaw (1976)#2a | Maize + Maize gluten meal | 140.0 | 140.0 | 32.14, 35, 37.86, 40.71 |
| Latshaw (1976)#2b | Maize + Maize gluten meal | 140.0 | 140.0 | 35, 37.86, 40.71, 43.57 |
| McDonald (1979)#1 | Wheat + Sorghum | 166.0 | 115.0 | 32.53, 37.35 |
| Nathanael *et al.* (1980)#1 | Maize + Maize gluten meal | 152.0 | 125.0 | 30.07, 36.64, 43.22, 49.8 |
| Nathanael *et al.* (1980)#2 | Maize + Maize gluten meal | 133.0 | 133.0 | 42.86, 45.11, 47.37, 49.62, 51.88, 54.14, 56.39, 58.65 |

[1]Number and letter represent the experiment number within the published paper (number) and the diet series within a specific experiment (letter).
⇧Brown feathered-laying hens. § Broiler Breeders. NA= Sufficient data not available to calculate ideal CP.

**Table 2.3** *(Continued)*

| Reference | Experimental basal diet | Crude Protein (CP) (g/kg) | Calculated Ideal CP (g/kg) | Amino acid levels (g/kg CP) used within experiment |
|---|---|---|---|---|
| Pepper *et al.* (1962)#1a | Maize + Wheat + Soyabean meal | 158.0 | 158.0 | 56.96, 60.13 |
| Pepper *et al.* (1962)#1b | Maize + Wheat + Soyabean meal | 142.0 | 142.0 | 51.41, 60.21 |
| Pepper *et al.* (1962)#1c | Maize + Wheat + Soyabean meal | 124.0 | 124.0 | 45.16, 65.32 |
| Prochaska *et al.* (1996)#1 | Milo + Soyabean meal | 136.0 | 136.0 | 51.47, 83.09, 116.18 |
| Prochaska *et al.* (1996)#2 | Milo + Soyabean meal | 151.3 | 151.3 | 47.59, 58.82, 76.01, 90.55 |
| Schutte and Smink (1998) | Maize + Soyabean meal | 164.0 | 143.0 | 39.63, 42.07, 44.51, 46.95, 49.39, 51.83, 54.27, 56.71 |
| Summers *et al.* (1991) | Maize + Soyabean meal | 100.0 | 85.0 | 38, 64 |
| Uzu and Larbier (1985)† 1a | Maize + Soyabean meal | 130.0 | 130.0 | 44.62, 50.0, 57.69 |
| Uzu and Larbier (1985)† 1b | Maize + Soyabean meal | 145.0 | 145.0 | 44.83, 51.72 |
| Uzu and Larbier (1985)† 2a | Maize + Soyabean meal | 130.0 | 130.0 | 44.62, 49.23, 52.31 |
| Uzu and Larbier (1985)† 2b | Maize + Soyabean meal | 145.0 | 145.0 | 44.83, 47.24, 49.66 |
| Uzu and Larbier (1985)† 2c | Maize + Soyabean meal | 160 | 160 | 44.38, 46.88 |
| Van Weerden and Schutte (1980) | Maize + Soyabean meal | 135.0 | 135.0 | 47.41, 51.11, 54.81, 62.22 |
| **Methionine** | | | | |
| Bertram *et al.*(1995a)#1 | Maize + Tapioca + Soyabean meal | 148.0 | 131.0 | 16.89, 20.27, 23.65, 27.03, 30.41 |
| Bertram *et al.*(1995a)#2 | Maize + Tapioca + Soyabean meal | 151.0 | 128.0 | 16.56, 19.87, 23.18, 26.49, 29.80 |
| Bertram *et al.*(1995b)† | Maize + Tapioca + Soyabean meal | 156.0 | 126.0 | 14.74, 17.31, 19.87, 22.44, 25.0, 27.56 |
| Bray (1965)#1a | Maize Starch + Soyabean meal | 70.0 | 70.0 | 11, 16, 21, 26, 31, 36 |
| Bray (1965)#1b | Maize Starch + Soyabean meal | 100.0 | 100.0 | 11, 16, 21, 26, 31, 36 |
| Bray (1965)#3 | Maize Starch + Soyabean meal | 120.0 | 120.0 | 11.83, 14.75, 17.67, 20.58, 23.5, 26.42, 29.33 |
| Bray (1965)#4 | Maize Starch + Soyabean meal | 120.0 | 120.0 | 13.08, 13.92, 14.75, 15.58, 17.25, 19.75, 23.08, 27.25 |
| Calderon and Jensen (1990)#1a | Maize + Soyabean meal | 130.0 | 130.0 | 19.62, 20.0, 20.38, 20.77 |
| Calderon and Jensen (1990)#1b | Maize + Soyabean meal | 160.0 | 145.0 | 15.94, 16.25, 16.56, 16.88 |
| Calderon and Jensen (1990)#2a | Maize + Soyabean meal | 130.0 | 130.0 | 19.62, 21.54, 23.46, 25.38, 27.31, 29.23 |
| Calderon and Jensen (1990)#2b | Maize + Soyabean meal | 160.0 | 149.0 | 15.94, 17.50, 19.06, 20.63, 22.19, 23.75 |
| Calderon and Jensen (1990)#2c | Maize + Soyabean meal | 190.0 | 167.0 | 13.42, 14.74, 16.05, 17.37, 18.68, 20.0 |
| Campbell *et al.*(1980)#1a | Barley + Fababean + Soyabean meal | 160.0 | 118.0 | 17.05, 20.0, 22.5 |
| Campbell *et al.*(1980)#1b | Barley + Fababean + Soyabean meal | 160.0 | 118.0 | 19.38, 30.0, 36.25 |
| Cave and De Grote (1990)#1a | Maize + Soyabean meal | 135.0 | 135.0 | 18.44, 20.67, 22.89, 25.11, 27.33, 29.56 |
| Cave and De Grote (1990)#1b | Maize + Soyabean meal | 155.0 | 155.0 | 16.06, 18.0, 19.94, 21.87, 23.81, 25.74 |

**Table 2.3** *(Continued)*

| Reference | Experimental basal diet | Crude Protein (CP) (g/kg) | Calculated Ideal CP (g/kg) | Amino acid levels (g/kg CP) used within experiment |
|---|---|---|---|---|
| Daenner and Bessei (2000) | Maize + Barley + Soyabean meal | 150.0 | 126.0 | 14.67, 17.33, 20.67, 24.0 |
| Daghir *et al.*(1964)§ | Maize + Soyabean meal | 155.4 | 136.0 | 18.02, 21.24, 24.45, 27.67 |
| Elwinger and Wahlstrom (2000) | Maize + Barley + Soyabean meal | 159.0 | 159.0 | 19.50, 27.04 |
| Fontaine (1974) | Maize + Milo | 119.4 | 98.0 | 20.94, 25.13, 29.31, 33.5 |
| Harms *et al.* (1998)#1a | Maize + Soyabean meal | 127.0 | 127.0 | 19.69, 21.65, 23.62 |
| Harms *et al.* (1998)#1b | Maize + Soyabean meal | 150.0 | 133.0 | 16.67, 18.33, 20.0 |
| Harms *et al.* (1999) | Maize + Soyabean meal | 170.0 | 154.0 | 17, 18.59, 20.18, 21.76, 23.29, 24.82 |
| Heywang (1956)#1 | Maize + Soyabean meal | 169.0 | 169.0 | 15.98, 18.32, 21.01 |
| Heywang (1956)#2a | Maize + Soyabean meal | 155.0 | 155.0 | 17.42, 20.65, 22.26 |
| Heywang (1956)#2b | Maize + Soyabean meal + Fish meal | 155.0 | 155.0 | 18.71, 20.32, 21.94 |
| Hsu *et al.* (1998) | Maize + Soyabean meal | 140.3 | 135.0 | 16.89, 26.73 |
| Jackson *et al.* (1987) | Maize + Soyabean meal | 122.0 | 122.0 | 19.10, 21.56, 24.02, 26.48, 28.93, 31.39 |
| Jensen *et al.* (1974b)#1a | Maize + Soyabean meal | 160.0 | 160.0 | 17.5, 20.0, 22.5, 25.0 |
| Jensen *et al.* (1974b)#1b | Maize + Peas | 160.0 | 129.0 | 15.5, 18.0, 20.5, 23.0, 25.5, 28.0 |
| Jensen *et al.* (1974b)#2 | Maize + Soyabean meal | 140.0 | 140.0 | 17.79, 20.64 |
| Karunajeewa (1974) § | Wheat + Barley + Peanut meal | 158.0 | 100.0 | 12.03, 15.19, 24.05 |
| Kim and McGinnis (1972) | Maize + Wheat + Soyabean meal | 135.0 | 90.0 | 12.59, 16.30, 20.0, 23.7, 27.41 |
| Koelkebeck *et al.* (1991) | Maize + Soyabean meal | 160.0 | 141.0 | 16.25, 22.5 |
| Latshaw (1974) | Maize + Soyabean meal | 148.0 | 131.0 | 16.89, 30.41 |
| Leong and McGinnis (1952) | Maize + Peas | 152.0 | 136.0 | 11.84, 14.47, 15.79, 18.42 |
| McDonald (1979)#1 | Wheat + Sorghum | 166.0 | 117.0 | 13.86, 19.88 |
| Mueller (1967) | Maize + Soyabean meal | 164.0 | 144.0 | 17.68, 35.98 |
| Muller and Balloun (1974)#1a | Maize + Soyabean meal | 120.0 | 95.0 | 17.50, 25.83 |
| Muller and Balloun (1974)#1b | Maize + Soyabean meal | 140.0 | 110.0 | 16.43, 20.0 |
| Muller and Balloun (1974)#1c | Maize + Soyabean meal | 160.0 | 123.0 | 16.25, 19.38 |
| Muller and Balloun (1974)#2 | Maize + Soyabean meal | 160.0 | 123.0 | 16.25, 19.38 |
| Muller and Balloun (1974)#3a | Maize + Soyabean meal | 120.0 | 77.0 | 16.67, 20.83, 25.0 |
| Muller and Balloun (1974)#3b | Maize + Soyabean meal | 160.0 | 108.0 | 15.63, 18.75, 21.88 |
| Muller and Balloun (1974)#4a | Maize + Soyabean meal | 135.0 | 92.0 | 14.81, 18.52, 22.22 |
| Muller and Balloun (1974)#4b | Maize + Soyabean meal | 155.0 | 100.0 | 14.84, 18.06, 21.29 |
| Parsons and Leeper (1984)#1a | Maize + Soyabean meal | 140.0 | 140.0 | 18.57, 25.71 |
| Parsons and Leeper (1984)#1b | Maize + Soyabean meal | 160.0 | 136.0 | 16.25, 22.50 |
| Pepper *et al.* (1962)#1a | Maize + Wheat + Soyabean meal | 158.0 | 158.0 | 22.78, 25.95 |
| Pepper *et al.* (1962)#1b | Maize + Wheat + Soyabean meal | 142.0 | 142.0 | 22.54, 24.65 |

**Table 2.3** *(Continued)*

| Reference | Experimental basal diet | Crude Protein (CP) (g/kg) | Calculated Ideal CP (g/kg) | Amino acid levels (g/kg CP) used within experiment |
|---|---|---|---|---|
| Pepper *et al.* (1962)#1c | Maize + Wheat + Soyabean meal | 124.0 | 124.0 | 22.58, 27.42 |
| Petersen *et al.* (1983) | Maize + Milo + Soyabean meal | 170.0 | 170.0 | 15.0, 15.88, 16.76, 17.65 |
| Pourreza and Smith (1988)☆ | Wheat + Soyabean meal | 165.0 | 138.0 | 15.76, 17.58, 19.39, 21.21, 23.03 |
| Reid and Weber (1974)#1a | Milo + Soyabean meal | 140.0 | 116.0 | 14.29, 17.86, 19.29, 21.43 |
| Reid and Weber (1974)#1b | Milo + Soyabean meal | 162.0 | 128.0 | 14.81, 18.52 |
| Roberson and Trujillo (1975) | Maize + Milo + Soyabean meal | 160.0 | 126.0 | 15.63, 20.0 |
| Salman and McGinnis (1968) | Maize + Soyabean meal | 170.0 | 126.0 | 14.12, 20.0, 25.88, 31.18, 37.65, 43.53 |
| Schutte and Van Weerden (1978)#1 | Maize + Soyabean meal | 135.0 | 128.0 | 16.67, 20.45, 24.24, 28.03 |
| Schutte *et al.* (1983)#1a | Maize + Soyabean meal | 141.0 | 141.0 | 23.4, 26.95, 39.72 |
| Schutte *et al.* (1983)#1b | Maize + Soyabean meal | 142.0 | 128.0 | 19.72, 23.24, 24.65 |
| Schutte *et al.* (1984) | Maize + Soyabean meal | 138.0 | 128.0 | 16.67, 20.29, 23.91, 27.54, 31.16 |
| Schutte *et al.* (1994)#1 | Maize + Soyabean meal | 145.0 | 123.0 | 15.86, 19.31, 20.69, 22.41, 24.48, 27.24 |
| Schutte *et al.* (1994)#2 | Maize + Soyabean meal | 154.0 | 131.0 | 22.08, 25.32, 28.57, 31.82 |
| Scott *et al.*(1975) | Milo + Soyabean meal | 120.0 | 103.0 | 16.67, 23.33 |
| Shafer *et al.*(1996)#1 | Maize + Soyabean meal | 147.5 | 138.0 | 18.98, 29.15 |
| Shafer *et al.*(1996)#2 | Maize + Soyabean meal | 147.0 | 147.0 | 21.09, 23.13, 24.49, 26.53 |
| Shafer *et al.*(1998) | Maize + Soyabean meal | 165.0 | 144.0 | 23.03, 27.88, 32.12 |
| Slinger *et al.*(1972)#1a | Wheat + Soyabean meal | 122.0 | 97.0 | 15.57, 20.74 |
| Slinger *et al.*(1972)#1b | Wheat + Soyabean meal | 138.0 | 108.0 | 15.22, 19.78 |
| Slinger *et al.*(1972)#1c | Wheat + Soyabean meal | 153.0 | 118.0 | 15.03, 19.15 |
| Slinger *et al.*(1972)#1d | Wheat + Soyabean meal | 168.0 | 128.0 | 14.88, 18.63 |
| Slinger *et al.*(1972)#1e | Wheat + Maize + Soyabean meal | 135.0 | 108.0 | 17.04, 21.70 |
| Slinger *et al.*(1972)#1f | Wheat + Blood meal | 135.0 | 105.0 | 14.81, 24.07 |
| Speers and Chi (1974) | Maize + Soyabean meal | 130.0 | NA | 17.69, 21.54, 25.38 |
| Summers *et al.*(1991) | Maize + Soyabean meal | 100.0 | 83.0 | 20.0, 52.0 |
| Waldroup and Hellwing (1995) | Maize + Soyabean meal | 122.0 | 110.0 | 19.1, 21.56, 24.02, 26.48, 28.93, 31.39 |
| Yamazaki and Takemasa (1998) | Maize + Milo | 155.0 | 155.0 | 19.35, 25.81 |
| **Methionine + Cystine** | | | | |
| Bertram *et al.*(1995a)#1 | Maize + Tapioca + Soyabean meal | 156.0 | 115.0 | 32.05, 34.62, 37.18, 39.74, 42.31 |
| Bertram *et al.*(1995a)#2 | Maize + Tapioca + Soyabean meal | 148.0 | 125.0 | 33.78, 37.16, 40.54, 37.16, 40.54, 43.92 |
| Bertram *et al.*(1995b)☆ | Maize + Wheat + Soyabean meal | 151.0 | 125.0 | 33.11, 36.42, 39.74, 43.05, 46.36 |

**Table 2.3** *(Continued)*

| Reference | Experimental basal diet | Crude Protein (CP) (g/kg) | Calculated Ideal CP (g/kg) | Amino acid levels (g/kg CP) used within experiment |
|---|---|---|---|---|
| Bray (1965)#1a | Maize Starch + Soyabean meal | 70.0 | 70.0 | 19.0, 26.14, 33.14, 35.43, 41.5 |
| Bray (1965)#1b | Maize Starch + Soyabean meal | 100.0 | 100.0 | 18.5, 28.7, 34.3, 41.6, 46.4, 51.8 |
| Bray (1965)#3 | Maize Starch + Soyabean meal | 120.0 | 120.0 | 25.0, 33.5, 36.17, 38.42, 41.58, 46.33, 47.17 |
| Bray (1965)#4 | Maize Starch + Soyabean meal | 120.0 | 120.0 | 23.5, 25.0, 26.08, 26.83, 30.33, 34.17, 37.58, 43.0 |
| Calderon and Jensen (1990)#1a | Maize + Soyabean meal | 130.0 | 130.0 | 39.23, 43.08, 46.92, 50.77 |
| Calderon and Jensen (1990)#1b | Maize + Soyabean meal | 160.0 | 145.0 | 31.88, 35.0, 38.13, 41.25, 41.25 |
| Calderon and Jensen (1990)#2a | Maize + Soyabean meal | 190.0 | 163.0 | 26.84, 29.47, 32.11, 34.47 |
| Calderon and Jensen (1990)#2b | Maize + Soyabean meal | 130.0 | 130.0 | 39.23, 41.15, 43.08, 45.0 46.92, 48.85 |
| Calderon and Jensen (1990)#2c | Maize + Soyabean meal | 160.0 | 145.0 | 31.88, 33.44, 35.0, 36.56, 38.13, 39.69 |
| Calderon and Jensen (1990)#2c | Maize + Soyabean meal | 190.0 | 163.0 | 26.84, 28.16, 29.47, 30.79, 32.11, 33.42 |
| Campbell *et al.*(1980)#1a | Barley + Fababean + Soyabean meal | 160.0 | 140.0 | 28.75, 31.25, 33.75 |
| Campbell *et al.*(1980)#1b | Barley + Fababean + Soyabean meal | 160.0 | 140.0 | 28.75, 39.38, 45.63 |
| Cave and De Grote (1990)#1a | Maize + Soyabean meal | 135.0 | 125.0 | 37.78, 40.0, 42.22, 44.44, 46.67, 48.89 |
| Cave and De Grote (1990)#1b | Maize + Soyabean meal | 155.0 | 125.0 | 32.90, 34.84, 36.77, 38.71, 40.65, 42.58 |
| Daenner and Bessei (2000) | Maize + Barley + Soybean meal | 150.0 | 126.0 | 32.67, 35.33, 38.67, 42.0 |
| Daghir *et al.*(1964)§ | Maize + Soyabean meal | 155.4 | 140.0 | 34.11, 37.42, 40.54, 43.76 |
| Elwinger and Wahlstrom (2000) | Wheat + Barley + Soyabean meal | 159.0 | 159.0 | 37.74, 45.9 |
| Fontaine (1974) | Maize + Milo | 119.4 | 98.0 | 37.69, 41.88, 46.06, 50.25 |
| Harms *et al.* (1998)#1a | Maize + Soyabean meal | 127.0 | 127.0 | 39.37, 41.34, 43.31 |
| Harms *et al.* (1998)#1b | Maize + Soyabean meal | 150.0 | 150.0 | 33.33, 35.0, 36.67 |
| Harms *et al.* (1999) | Maize + Soyabean meal | 170.0 | 170.0 | 34.71, 36.29, 39.47, 41.06, 42.65 |
| Heywang (1956)#1 | Maize + Soyabean meal | 169.0 | 169.0 | 33.14, 35.5, 38.17 |
| Hsu *et al.* (1998) | Maize + Soyabean meal | 140.3 | 135.0 | 34.14, 43.98 |
| Jackson *et al.*(1987) | Maize + Soyabean meal | 122.0 | 122.0 | 35.49, 37.95, 40.41, 42.87, 45.33, 47.79 |
| Jensen *et al.* (1974b)#1a | Maize + Soyabean meal | 160.0 | 140.0 | 38.13, 40.63, 43.13, 45.63 |
| Jensen *et al.* (1974b)#1b | Maize + Peas | 160.0 | 124.0 | 31.38, 33.88, 36.38, 38.88, 40.13, 42.63 |
| Jensen *et al.* (1974b)#2 | Maize + Soyabean meal | 140.0 | 123.0 | 37.64, 40.5 |
| Karunajeewa (1974) § | Wheat + Barley + Peanut meal | 158.0 | 95.0 | 24.68, 27.85, 36.71 |
| Kim and McGinnis (1972) | Maize + Wheat + Soyabean meal | 138.0 | 85.0 | 25.93, 29.63, 33.33, 37.04, 40.74 |
| Koelkebeck *et al.* (1991) | Maize + Soyabean meal | 160.0 | 130.0 | 34.38, 40.63 |
| Latshaw (1974) | Maize + Soyabean meal | 148.0 | 125.0 | 34.46, 47.97 |

**Table 2.3** *(Continued)*

| Reference | Experimental basal diet | Crude Protein (CP) (g/kg) | Calculated Ideal CP (g/kg) | Amino acid levels (g/kg CP) used within experiment |
|---|---|---|---|---|
| Leong and McGinnis (1952) | Maize + Peas | 152.0 | 140.0 | 34.87, 36.18, 37.5, 38.82 |
| McDonald (1979)#1 | Wheat + Sorghum | 166.0 | 115.0 | 30.12, 36.14 |
| Mueller (1967) | Maize + Soyabean meal | 165.0 | 145.0 | 34.15, 58.54 |
| Muller and Balloun (1974)#1a | Maize + Soyabean meal | 120.0 | 105.0 | 30.83, 35.0 |
| Muller and Balloun (1974)#1b | Maize + Soyabean meal | 140.0 | 115.0 | 30.71, 34.29 |
| Muller and Balloun (1974)#1c | Maize + Soyabean meal | 160.0 | 130.0 | 30.0, 33.13 |
| Muller and Balloun (1974)#3a | Maize + Soyabean meal | 120.0 | 100.0 | 25.0, 29.17, 33.33 |
| Muller and Balloun (1974)#3b | Maize + Soyabean meal | 160.0 | 125.0 | 26.25, 29.38, 32.5 |
| Muller and Balloun (1974)#4a | Maize + Soyabean meal | 135.0 | 100.0 | 34.07, 37.78, 41.48 |
| Muller and Balloun (1974)#4b | Maize + Soyabean meal | 155.0 | 115.0 | 33.55, 36.77, 40.0 |
| Parsons and Leeper (1984)#1a | Maize + Soyabean meal | 140.0 | 130.0 | 37.86, 45.0 |
| Parsons and Leeper (1984)#1b | Maize + Soyabean meal | 160.0 | 130.0 | 33.13, 39.38 |
| Pepper *et al.* (1962)#1a | Maize + Wheat + Soyabean meal | 158.0 | 158.0 | 39.24, 42.41 |
| Pepper *et al.* (1962)#1b | Maize + Wheat + Soyabean meal | 142.0 | 142.0 | 43.66, 47.18 |
| Pepper *et al.* (1962)#1c | Maize + Wheat + Soyabean meal | 124.0 | 124.0 | 52.7, 49.4 |
| Petersen *et al.* (1983) | Maize + Milo + Soyabean meal | 170.0 | 170.0 | 30.59, 31.47, 32.35, 33.24 |
| Pourreza and Smith (1988)♆ | Wheat + Soyabean meal | 165.0 | 123.0 | 33.33, 35.76, 37.58, 39.39, 41.21 |
| Reid and Weber (1974)#1a | Milo + Soyabean meal | 140.0 | 100.0 | 32.14, 35.71, 39.29, 42.86 |
| Reid and Weber (1974)#1b | Milo + Soyabean meal | 162.0 | 120.0 | 27.78, 30.86, |
| Roberson and Trujillo (1975) | Maize + Milo + Soyabean meal | 160.0 | 125.0 | 32.14, 35.71, 39.29, 42.86 |
| Salman and McGinnis (1968) | Maize + Soyabean meal | 170.0 | 120.0 | 29.41, 35.29, 41.18, 47.06, 52.94, 58.82 |
| Schutte and VanWeerden (1978)#1 | Maize + Soyabean meal | 165.0 | 135.0 | 36.36, 39.39 |
| Schutte *et al.* (1983)#1a | Maize + Soyabean meal | 141.0 | 115.0 | 35.46, 39.0, 51.77 |
| Schutte *et al.* (1983)#1b | Maize + Soyabean meal | 142.0 | 115.0 | 33.21, 38.73, 40.14 |
| Schutte *et al.* (1984) | Maize + Soyabean meal | 138.0 | 115.0 | 36.23, 39.86, 43.48, 47.1, 50.72 |
| Schutte *et al.* (1994)#1 | Maize + Soyabean meal | 145.0 | 115.0 | 33.1, 36.55, 37.93, 39.66, 41.38, 68.97 |
| Schutte *et al.* (1994)#2 | Maize + Soyabean meal | 154.0 | 120.0 | 32.9, 36.13, 39.35, 42.58 |
| Scott *et al.*(1975) | Milo + Soyabean meal | 120.0 | 100.0 | 33.33, 40.0 |
| Shafer *et al.*(1996)#1 | Maize + Soyabean meal | 147.5 | 147.5 | 36.61, 46.78 |
| Shafer *et al.*(1996)#2 | Maize + Soyabean meal | 147.0 | 147.0 | 40.82, 42.86 |
| Shafer *et al.*(1998) | Maize + Soyabean meal | 165.0 | 135.0 | 43.52, 49.39, 51.45 |
| Slinger *et al.*(1972)#1a | Wheat + Soyabean meal | 122.0 | 95.0 | 31.15, 36.31 |
| Slinger *et al.*(1972)#1b | Wheat + Soyabean meal | 138.0 | 105.0 | 30.43, 35.0 |
| Slinger *et al.*(1972)#1c | Wheat + Soyabean meal | 153.0 | 115.0 | 30.07, 34.18 |

**Table 2.3** *(Continued)*

| Reference | Experimental basal diet | Crude Protein (CP) (g/kg) | Calculated Ideal CP (g/kg) | Amino acid levels (g/kg CP) used within experiment |
|---|---|---|---|---|
| Slinger *et al.*(1972)#1d | Wheat + Soyabean meal | 168.0 | 125.0 | 29.76, 33.51 |
| Slinger *et al.*(1972)#1e | Wheat + Maize + Soyabean meal | 135.0 | 115.0 | 31.11, 35.78 |
| Slinger *et al.*(1972)#1f | Wheat + Blood meal | 135.0 | 100.0 | 30.37, 39.63 |
| Speers and Chi (1974) | Maize + Soyabean meal | 130.0 | NA | 43.08, 46.92, 50.77 |
| Summers *et al.*(1991) | Maize + Soyabean meal | 100.0 | 83.0 | 33.0, 65.0 |
| Vogt and Krieg (1983) | Maize + Soyabean meal | 145.0 | 145.0 | 41.38, 44.14, 46.9, 49.66, 52.41, 55.17, 47.93 |
| Waldroup and Hellwing (1995) | Maize + Soyabean meal | 122.0 | 115.0 | 35.25, 37.7, 40.16, 42.62, 45.08 |
| Yamazaki and Takemasa (1998) | Maize + Milo | 155.0 | 155.0 | 38.71, 45.16 |
| **Tryptophan** | | | | |
| Al-saffar and Rose (2000a)♆ | Maize + Maize gluten meal | 170.0 | 161.0 | 6.3, 10.5, 15, 20.0 |
| Bray (1969)#6 | Maize + Soyabean meal | 119.7 | 119.7 | 6.27, 6.52, 7.02, 7.94, 9.27, 11.1 11.2, 13.7, 16.96, 20.97, 25.7 |
| Ingram *et al.* (1951)#2a | Maize + Maize gluten meal | 190.0 | 190.0 | 6.32, 14.74, 22.11, 30 |
| Ingram *et al.* (1951)#2b | Maize + Maize gluten meal | 190.0 | 190.0 | 6.32, 8.95, 11.58, 16.84, 22.11 |
| Ishibashi (1985)#1 | Maize + Fish meal | 151.0 | 151.0 | 6.16, 8.28, 11.13, 16.16, 21.19 |
| Ishibashi (1985)#2 | Maize + Fish meal | 154.0 | 154.0 | 7.27, 8.18, 9.16, 10.13, 11.10 |
| Ishibashi (1985)#3 | Maize + Fish meal | 154.0 | 154.0 | 5.66, 6.58, 7.5, 8.42, 10.26 |
| Ishibashi (1985)#4 | Maize + Fish meal | 152.0 | 152.0 | 7.37, 9.28, 11.25, 13.16, 15.13 |
| Jensen *et al.* (1990)#1 | Maize + Soyabean meal | 145.0 | 145.0 | 9.31, 11.03, 12.76, 14.48 |
| Jensen *et al.* (1990)#3a | Maize + Soyabean meal | 140.0 | 140.0 | 9.29, 10.71, 12.14, 13.57, 15 |
| Jensen *et al.* (1990)#3b | Maize + Soyabean meal | 160.0 | 160.0 | 10, 11.25, 12.5, 13.75, 15 |
| Jensen *et al.* (1990)#3c | Maize + Soyabean meal | 180.0 | 162.0 | 10.56, 11.67, 12.78, 13.89, 15 |
| Jensen *et al.* (1990)#4a | Maize + Soyabean meal | 140.0 | 140.0 | 7.86, 9.29, 10.71, 12.14, 13.57 |
| Jensen *et al.* (1990)#4b | Maize + Soyabean meal | 160.0 | 160.0 | 8.13, 9.38, 10.63, 11.88, 13.13 |
| Jensen *et al.* (1990)#4c | Maize + Soyabean meal | 180.0 | 180.0 | 8.33, 9.44, 10.56, 11.67, 12.78 |
| Koelkebeck *et al.* (1991)#3 | Maize + Soyabean meal | 160.0 | 130.0 | 11.25, 17.5 |
| Morris and Wethli (1978) | Maize + Maize gluten meal | 279.0 | 241.0 | 8.4, 9.6, 10.8, 12, 14.4, 16.8 |
| Ohtani *et al.* (1989) | Maize + Soyabean meal | 152.4 | 139.0 | 9.84, 11.48, 13.12 |
| Russell and Harms (1999) | Maize + Soyabean meal | 131.9 | 131.9 | 8.34, 9.86, 11.37, 12.89, 14.4, 15.92, 17.44 |
| Tasaki (1983) | Maize + Soyabean meal | 160.0 | 128.0 | 4.13, 6.06, 8.0, 13.81, 45.06 |
| Wethli and Morris (1978) | Maize + Maize gluten meal | 155.0 | NA | 8.4, 10.2, 12, 13.8, 15.6, 17.4, 19.2 |

[1]Number and letter represent the experiment number within the published paper (number) and the diet series within a specific experiment (letter).
♆Brown feathered-laying hens. § Broiler Breeders. NA= Sufficient data not available to calculate ideal CP.

variable and with egg numbers, egg weights and egg mass outputs as the dependent variables.

Amino acid concentration was described by three different methods:

1. Concentration in diet (g/kg of feed).
2. Concentration in crude protein (g/kg Crude Protein).
3. Concentration in ideal crude protein (g/kg Ideal Crude Protein). Ideal crude protein was calculated by examining the total essential amino acid composition of the basal diet. The concentration of each of the amino acids was examined. If any amino acid, apart from the single amino acid that was being tested, was deficient then the ideal crude protein concentration was changed to become less than the crude protein concentration. The percentage deficiency of this amino acid was determined and the crude protein concentration of the diet was multiplied by this factor to derive the ideal crude protein concentration. An example of this calculation is as follows: An experiment that examined different levels of tryptophan had a basal diet that contained 150 g/kg crude protein. Examination of the amino acid composition of the diet indicated (apart from tryptophan) that lysine (5.8 g/kg) was the next limiting amino acid in the protein. The diet supplied 5.8 g/kg of lysine (equivalent to (5.8x1000÷150= 38.7g/kg of the crude protein)). The ideal balance of lysine within protein is 46 (*Table 2.2*), therefore the deficiency of lysine was (38.7÷46=0.826). Therefore, the amount of ideal crude protein supplied was (0.826x150=123.9 g/kg).

### 2.1.3.2 Results

The relationships between dietary amino acid concentration expressed by the three methods, with three variables of egg laying performance are shown in *Figures 2.4 to 2.15*. The results show that increases in dietary amino acid concentration gave small increases in egg numbers, egg weights and egg mass output of the laying hens until a critical concentration was reached. A curvilinear exponential model (equation 1) gave the best fit to these data sets for all variables except egg weights with tryptophan (no significant relationship ($p>0.05$)). Positive linear responses gave the best fit to the data sets for egg weights and egg mass output with methionine *plus* cystine.

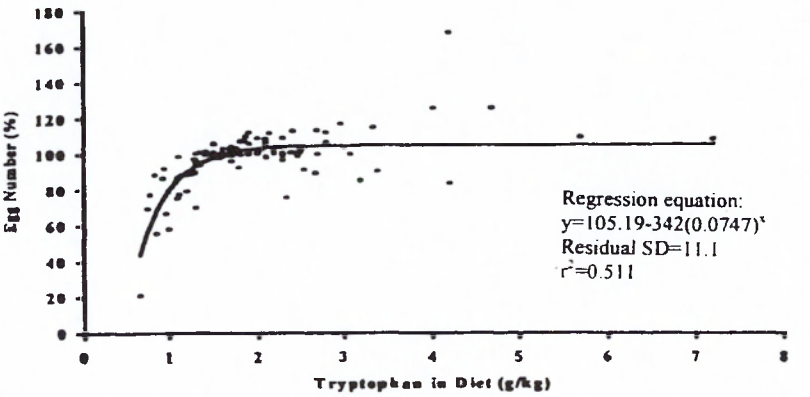
Equation 1: $y = a + b\ (r^{x})$

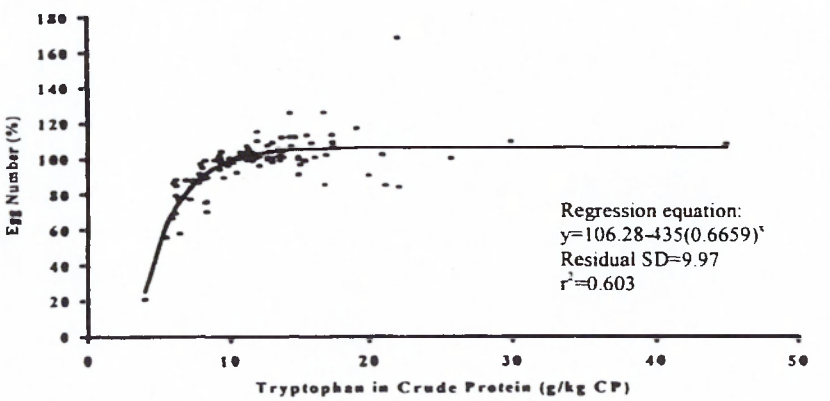
Where y = egg laying response (expressed as a percent of the laying response of birds given a diet with an ideal balance of that amino acid), x = proportion of crude limiting amino acid concentration and b and r are constants. The ideal balances used for the individual amino acids are given in *Table 2.2*.

### 2.1.3.3 Interpretation of results

The balance of amino acids in laying hen diets is an important nutritional variable that affects the economic efficiency of an egg laying enterprise. Lysine, methionine, methionine *plus* cystine and tryptophan are the major amino acids that can be limiting in practical laying hen feeds. Knowledge of the exact shape of the response curves to changes of each of these amino acids is important in formulating practical diets that

**Figure 2.4** Relationship between dietary variation in tryptophan concentration and the numbers of eggs laid by hens. Eligible data for all published experiments are included and egg numbers are expressed as a percentage of the treatment group within the experiment that was given 10.5 g/kg of tryptophan within the crude or ideal crude protein. Three methods of describing the tryptophan supply are compared.



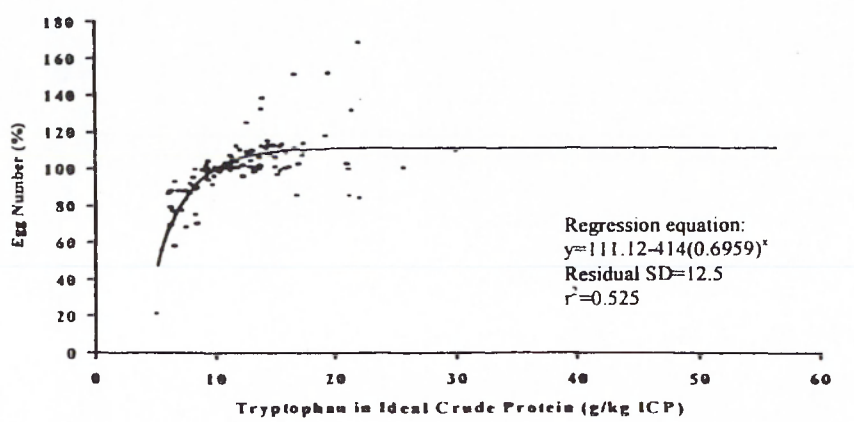
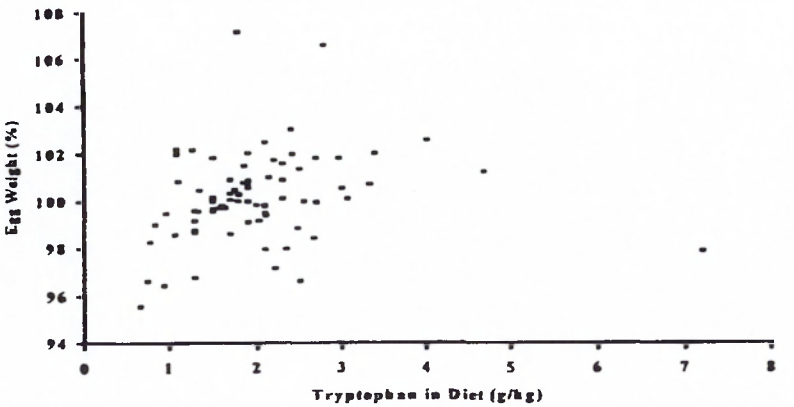
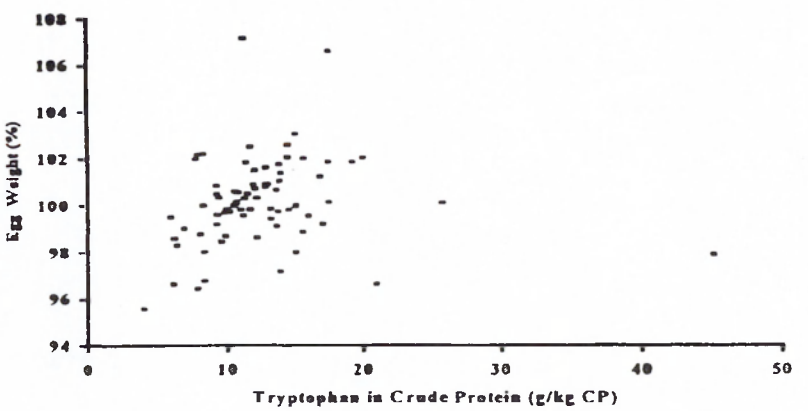



**Figure 2.5** Relationship between dietary variation in tryptophan concentration and the mean weights of eggs laid by hens. Eligible data for all published experiments are included and mean egg weights are expressed as a percentage of the treatment group within the experiment that was given 10.5 g/kg of tryptophan within the crude or ideal crude protein. Three methods of describing the tryptophan supply are compared.



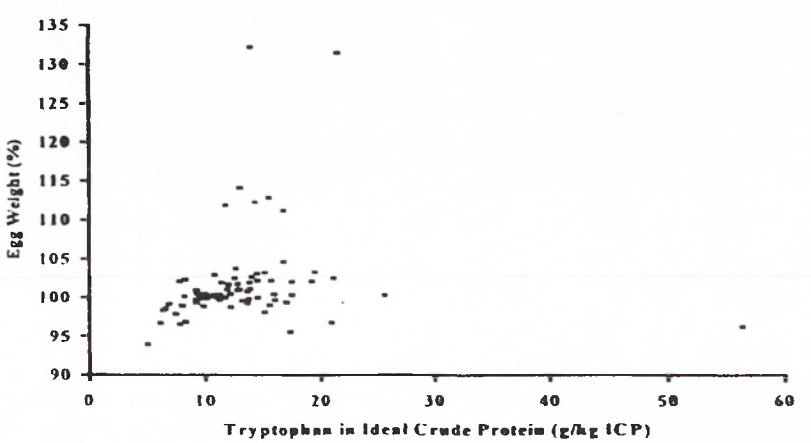



**Figure 2.6** Relationship between dietary variation in tryptophan concentration and the mass outputs of eggs laid by hens. Eligible data for all published experiments are included and egg mass outputs are expressed as a percentage of the treatment group within the experiment that was given 10.5 g/kg of tryptophan within the crude or ideal crude protein. Three methods of describing the tryptophan supply are compared.

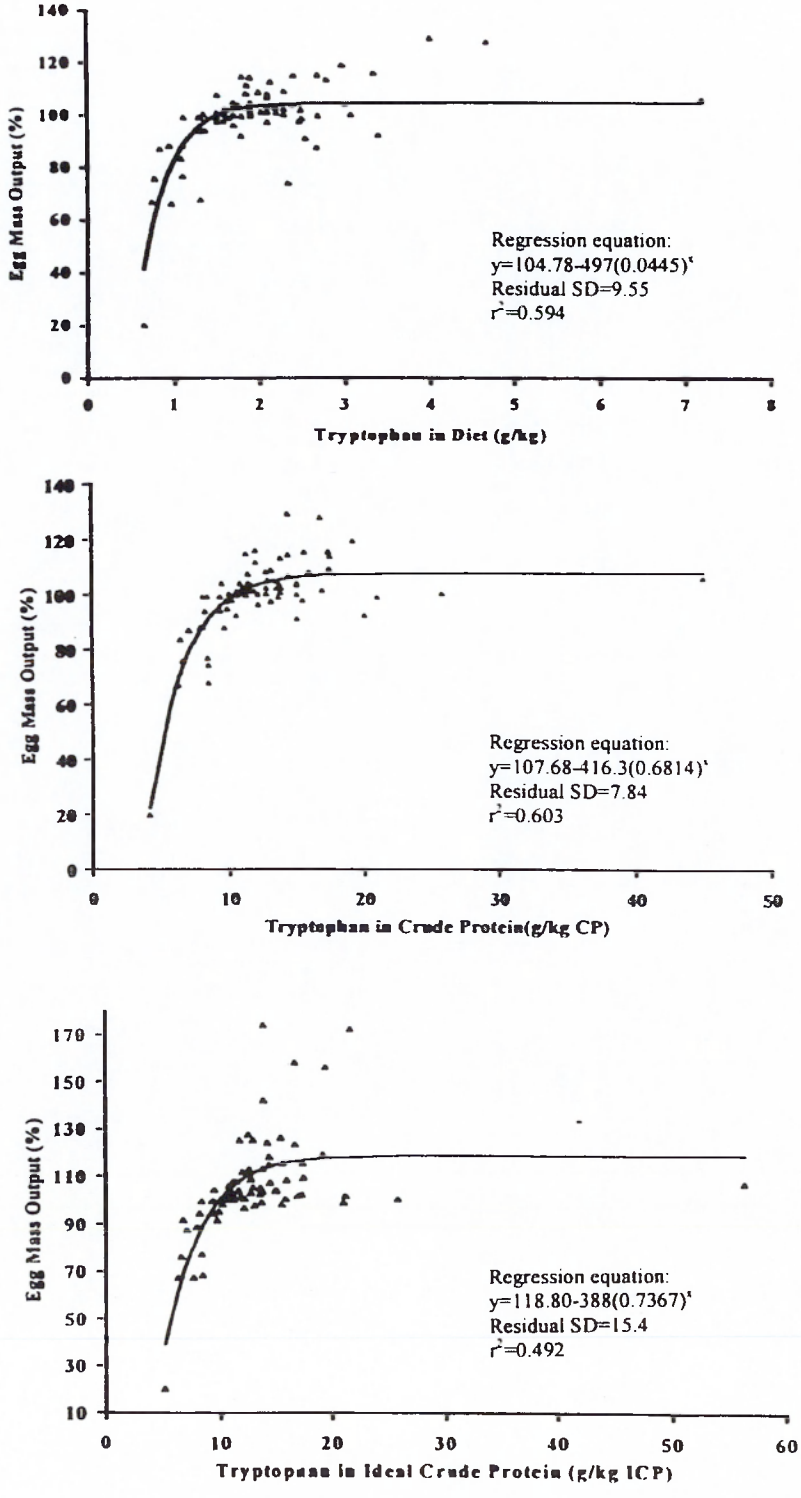


**Figure 2.7** Relationship between dietary variation in lysine concentration and the numbers of eggs laid by hens. Eligible data for all published experiments are included and egg numbers are expressed as a percentage of the treatment group within the experiment that was given 46 g/kg of lysine within the crude or ideal crude protein. Three methods of describing the lysine supply are compared.

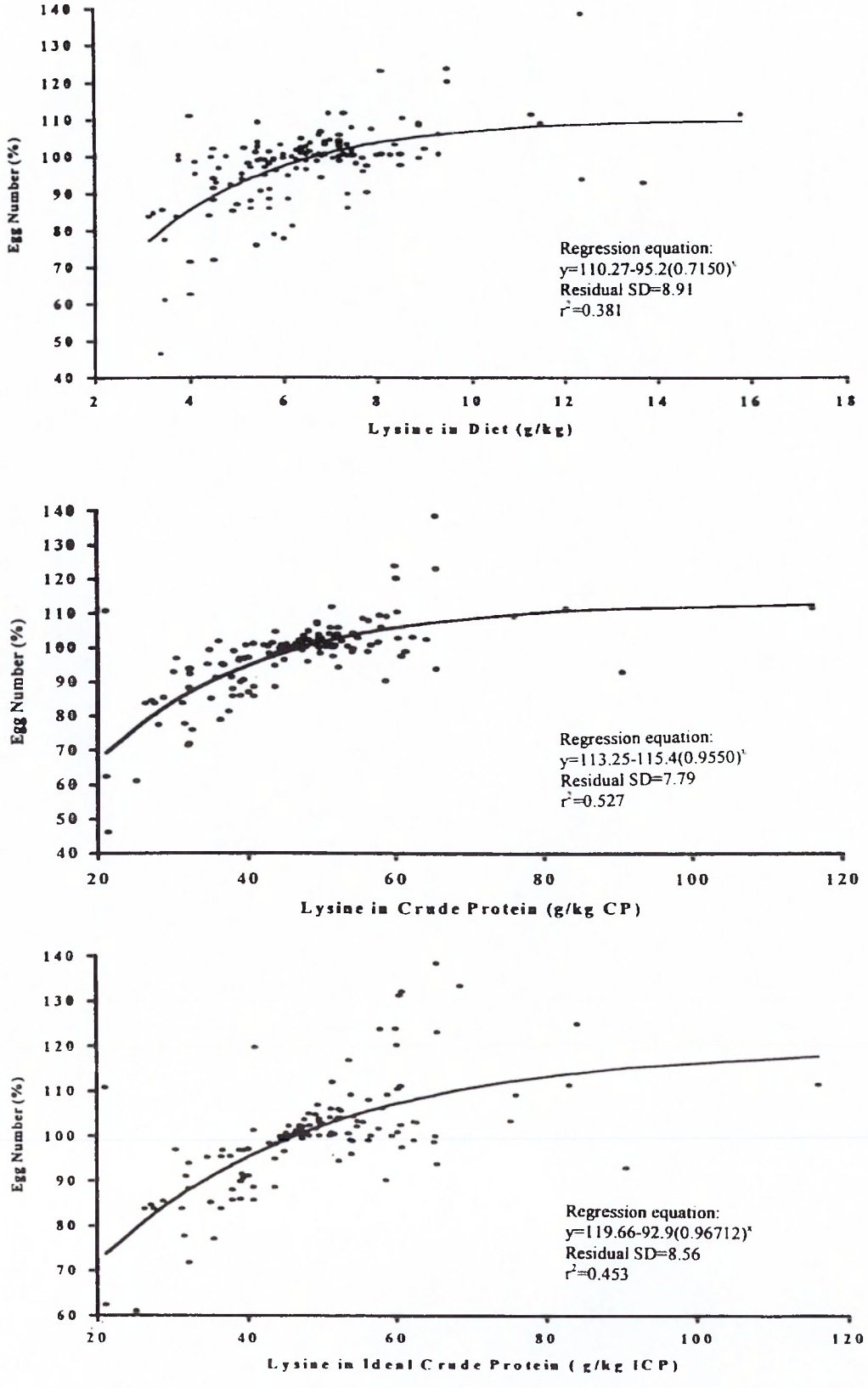
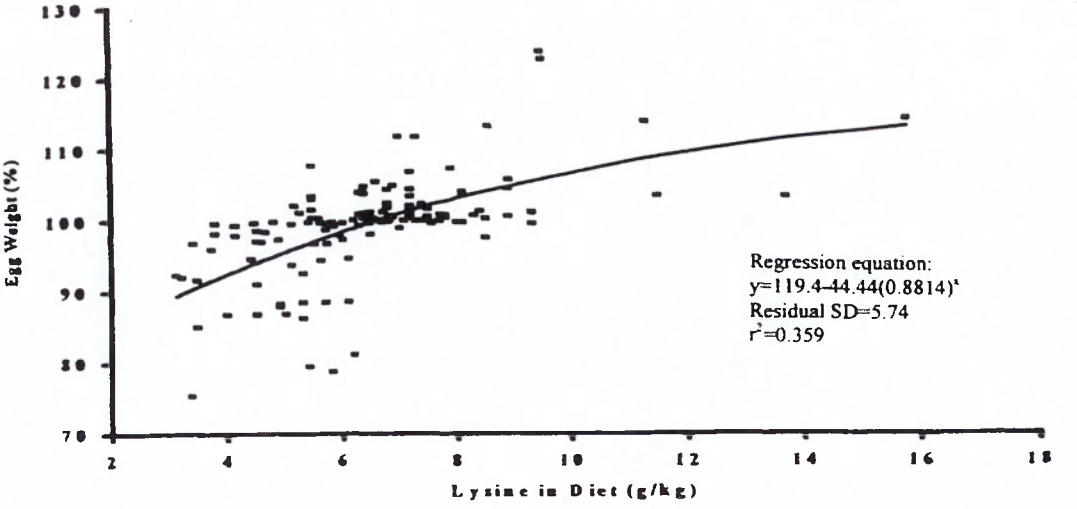
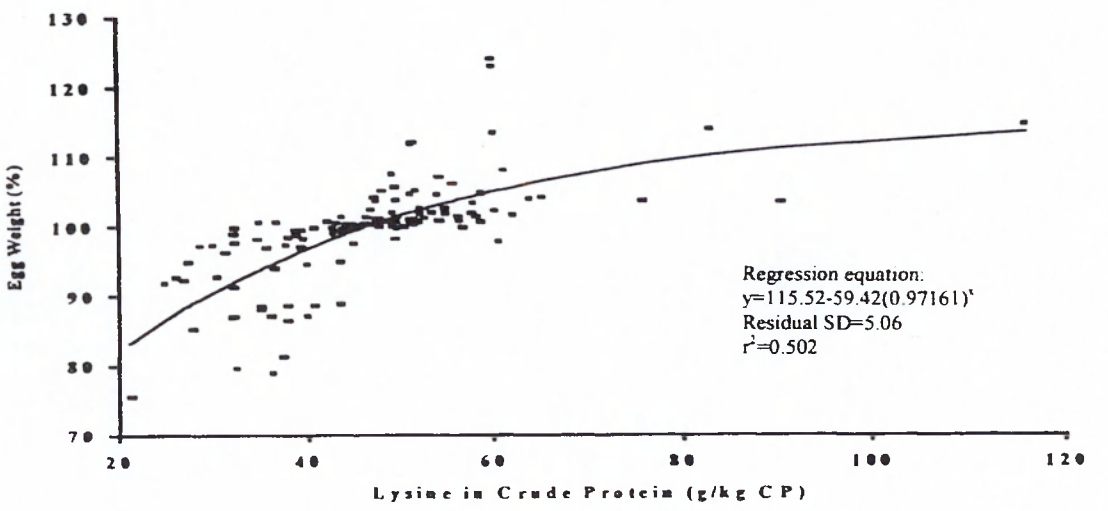


**Figure 2.8** Relationship between dietary variation in lysine concentration and the mean weights of eggs laid by hens. Eligible data for all published experiments are included and mean egg weights are expressed as a percentage of the treatment group within the experiment that was given 46 g/kg of lysine within the crude or ideal crude protein. Three methods of describing the lysine supply are compared.



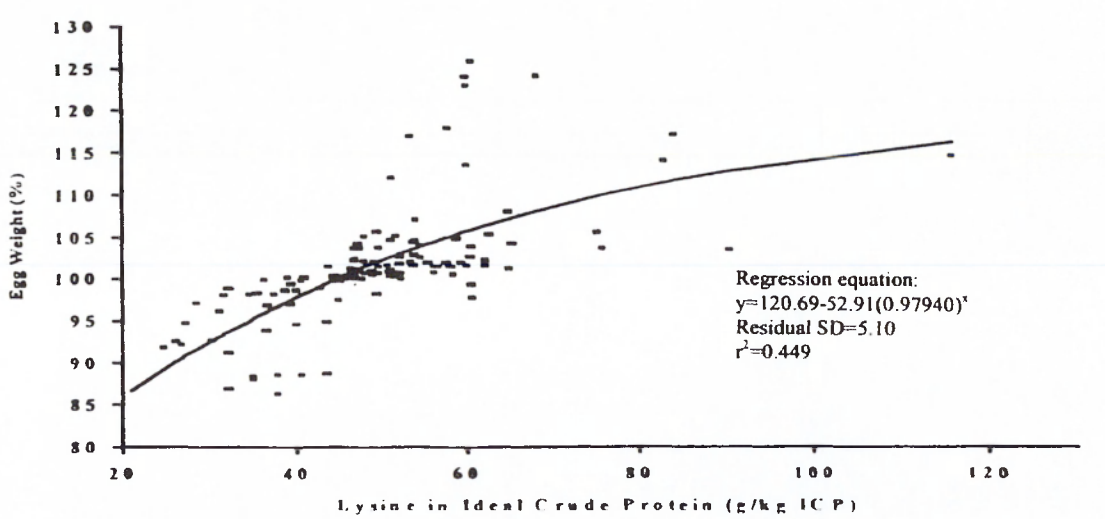
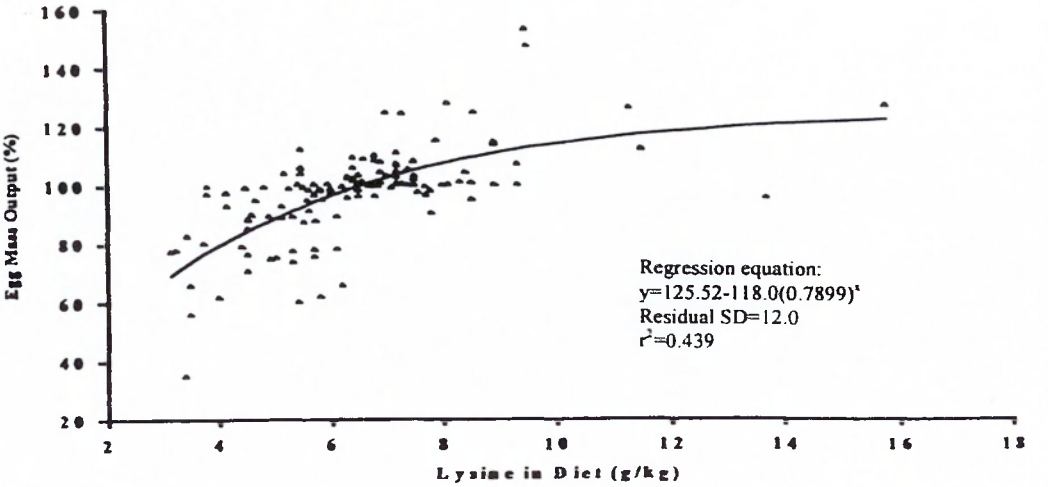
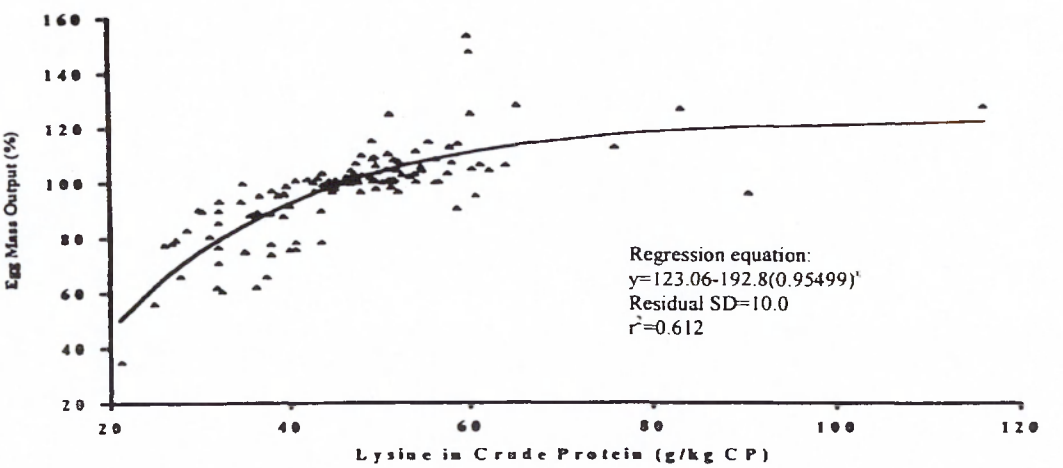



**Figure 2.9** Relationship between dietary variation in lysine concentration and the mass outputs of eggs laid by hens. Eligible data for all published experiments are included and egg mass outputs are expressed as a percentage of the treatment group within the experiment that was given 46 g/kg of lysine within the crude or ideal crude protein. Three methods of describing the lysine supply are compared.



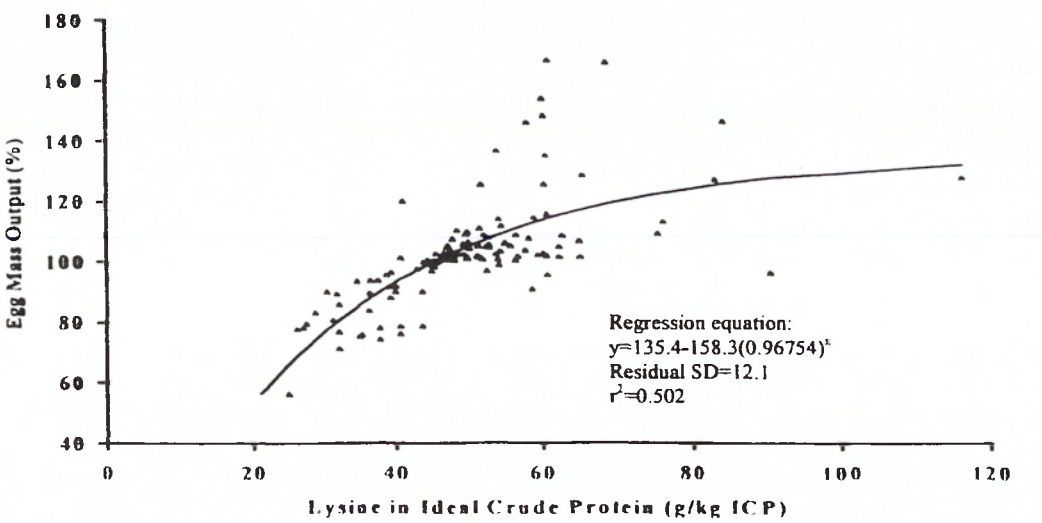
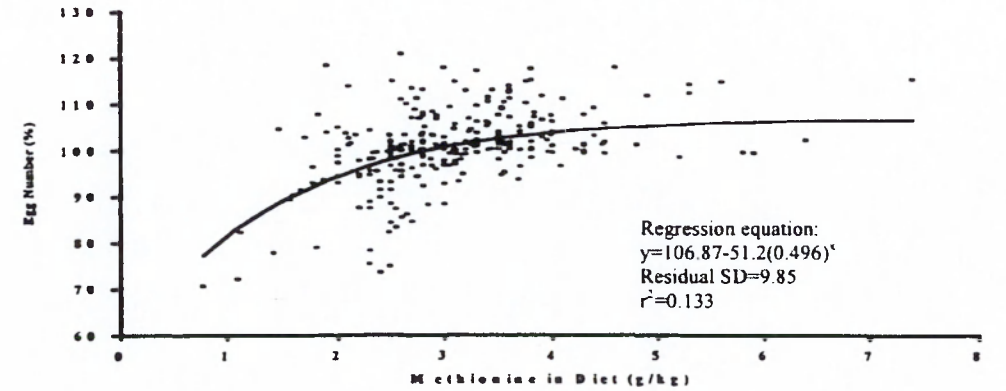
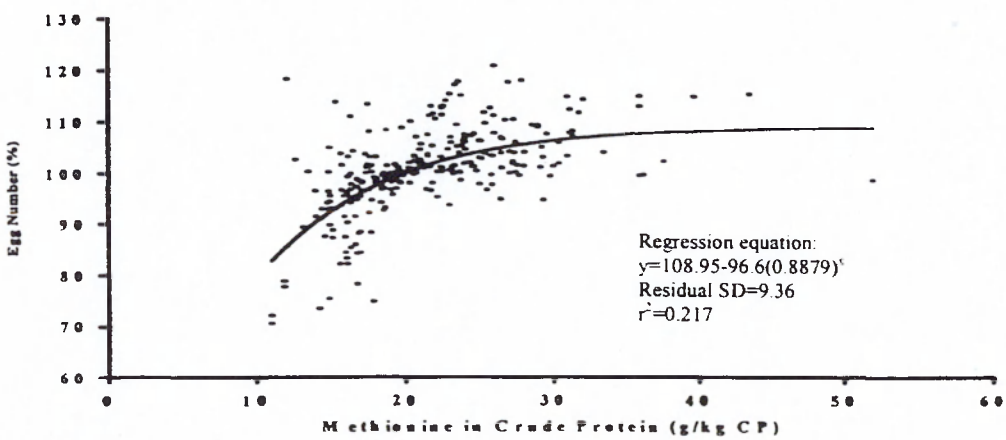



**Figure 2.10** Relationship between dietary variation in methionine concentration and the numbers of eggs laid by hens. Eligible data for all published experiments are included and egg numbers are expressed as a percentage of the treatment group within the experiment that was given 20 g/kg of methionine within the crude or ideal crude protein. Three methods of describing the methionine supply are compared.



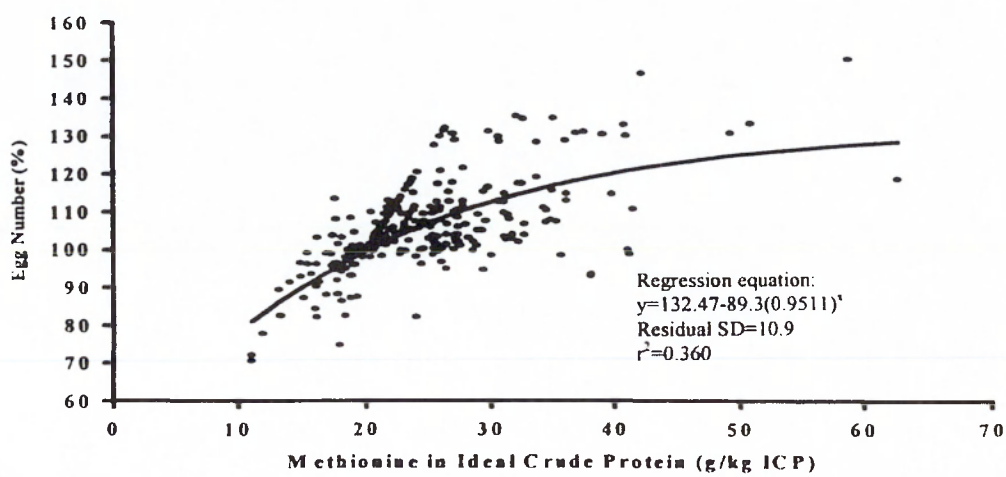
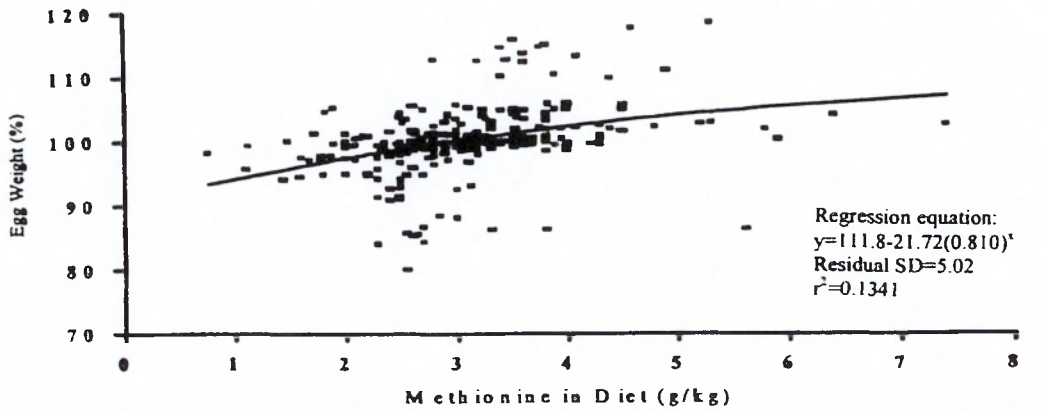
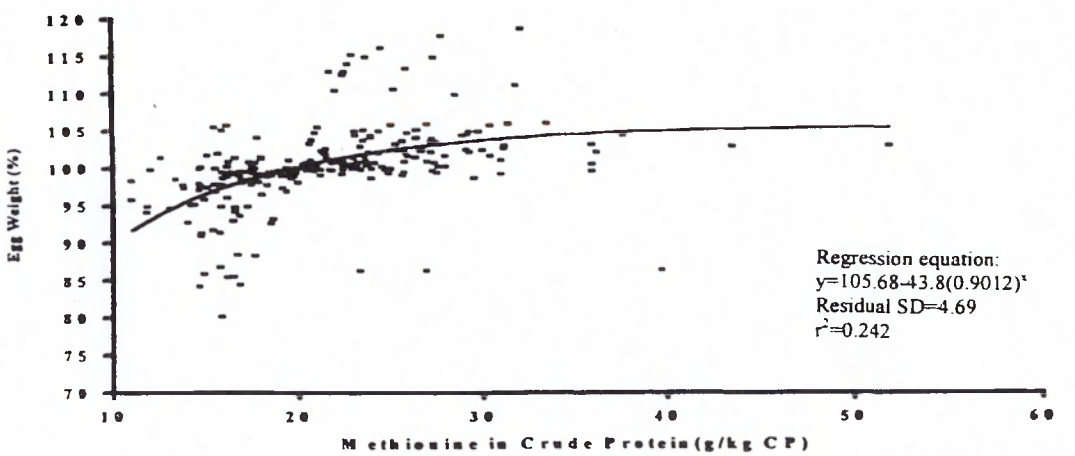



**Figure 2.11** Relationship between dietary variation in methionine concentration and the mean weights of eggs laid by hens. Eligible data for all published experiments are included and mean egg weights are expressed as a percentage of the treatment group within the experiment that was given 20 g/kg of methionine within the crude or ideal crude protein. Three methods of describing the methionine supply are compared.



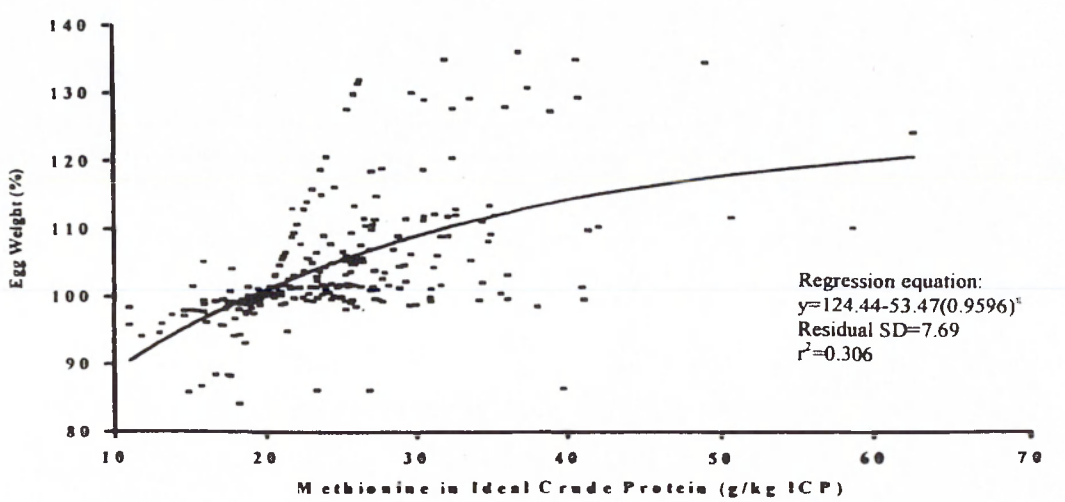
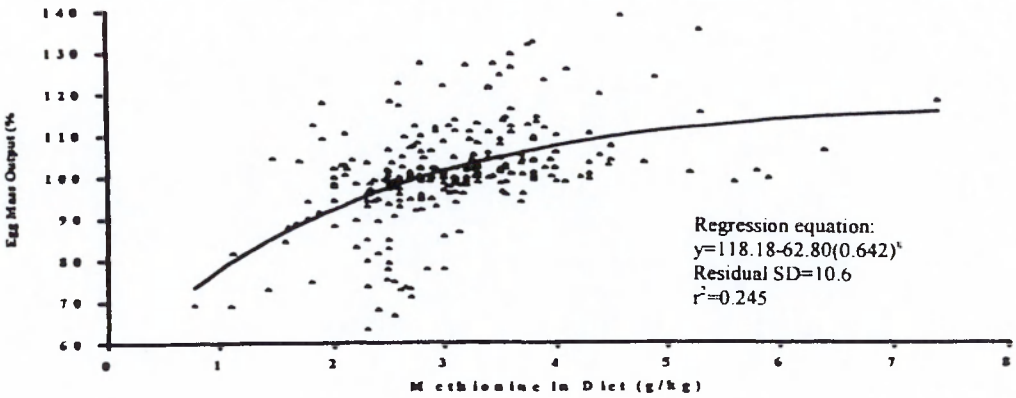
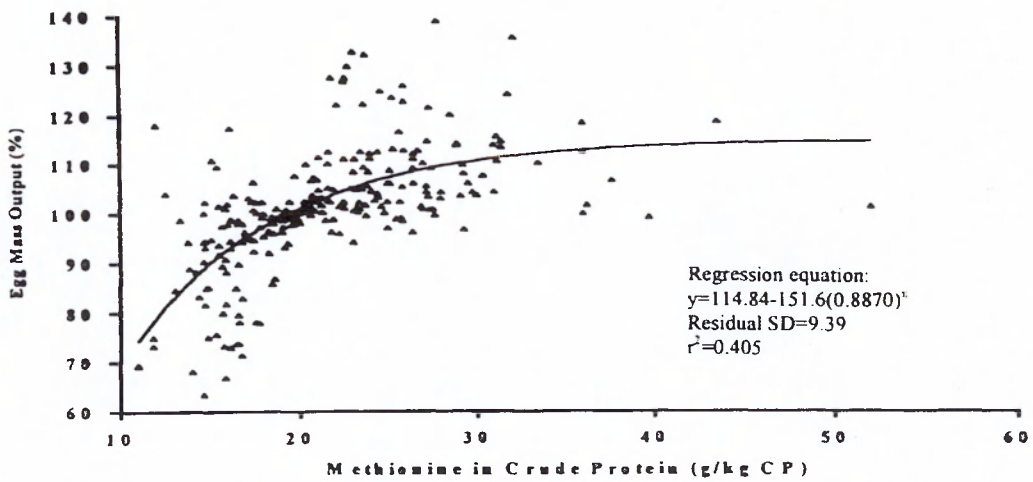



**Figure 2.12** Relationship between dietary variation in methionine concentration and the mass outputs of eggs laid by hens. Eligible data for all published experiments are included and egg mass outputs are expressed as a percentage of the treatment group within the experiment that was given 20 g/kg of methionine within the crude or ideal crude protein. Three methods of describing the methionine supply are compared.



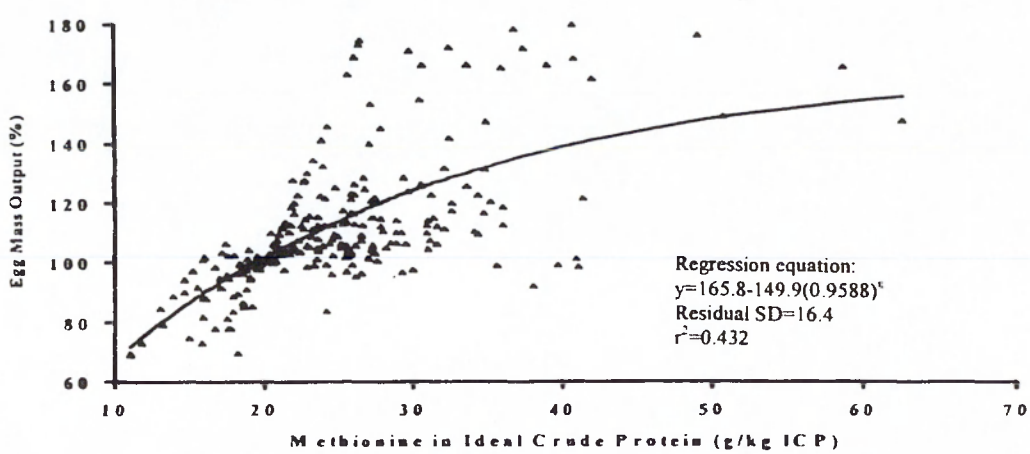



**Figure 2.13** Relationship between dietary variation in methionine + cystine concentration and the numbers of eggs laid by hens. Eligible data for all published experiments are included and egg numbers are expressed as a percentage of the treatment group within the experiment that was given 39 g/kg of methionine + cystine within the crude or ideal crude protein. Three methods of describing the methionine + cystine supply are compared.

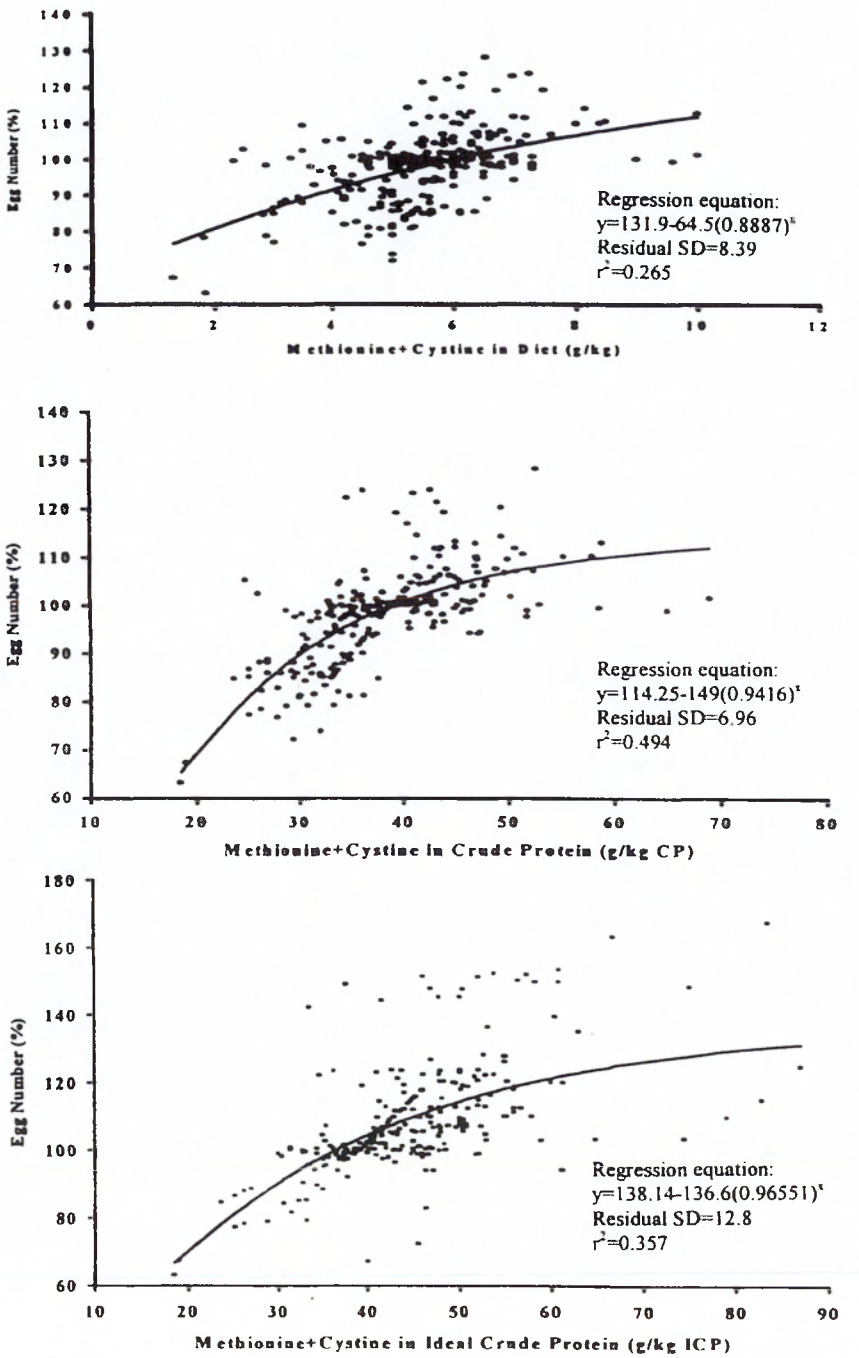


**Figure 2.14** Relationship between dietary variation in methionine + cystine concentration and the mean weights of eggs laid by hens. Eligible data for all published experiments are included and mean egg weights are expressed as a percentage of the treatment group within the experiment that was given 39 g/kg of methionine + cystine within the crude or ideal crude protein. Three methods of describing the methionine + cystine supply are compared.

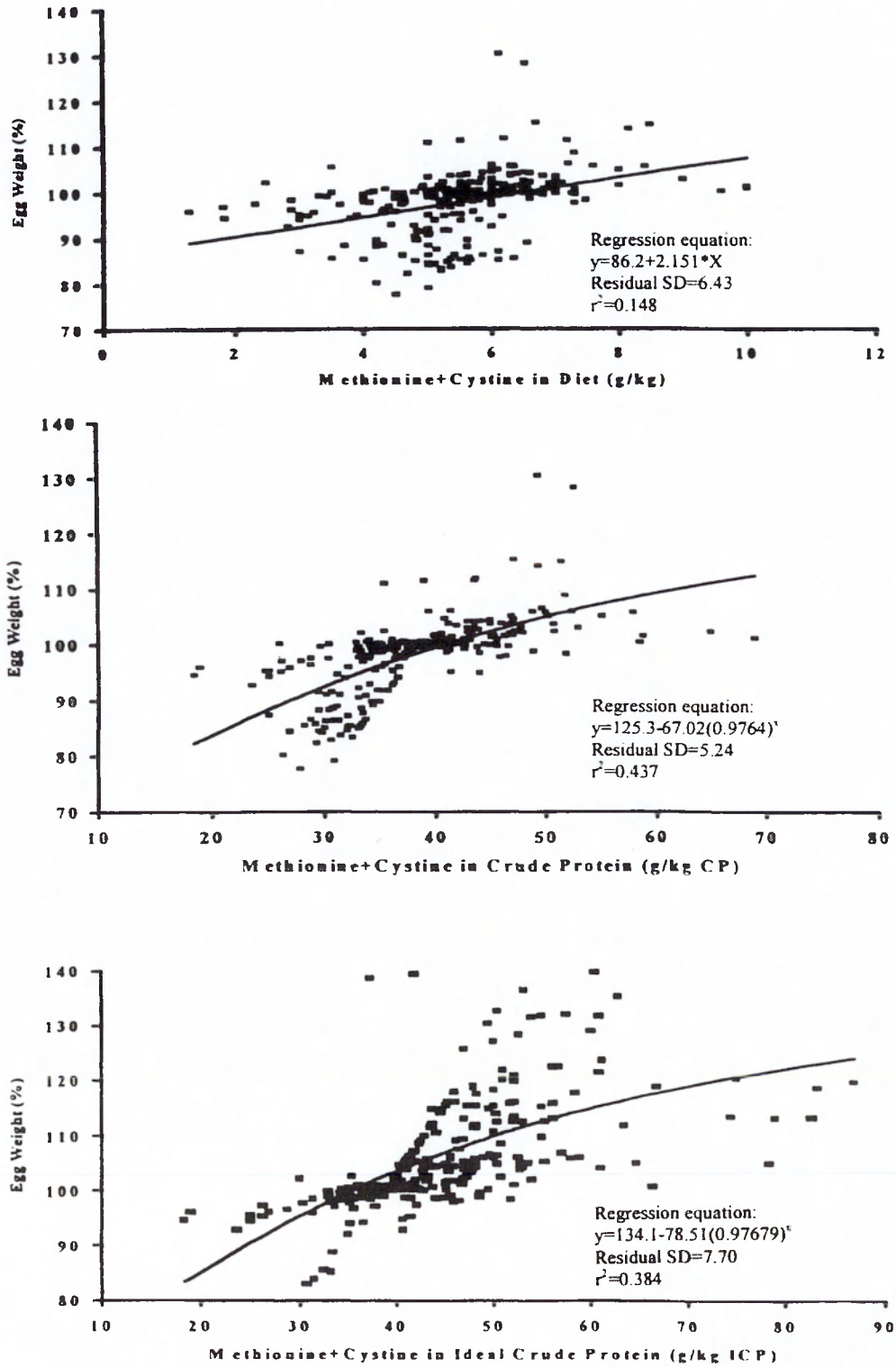
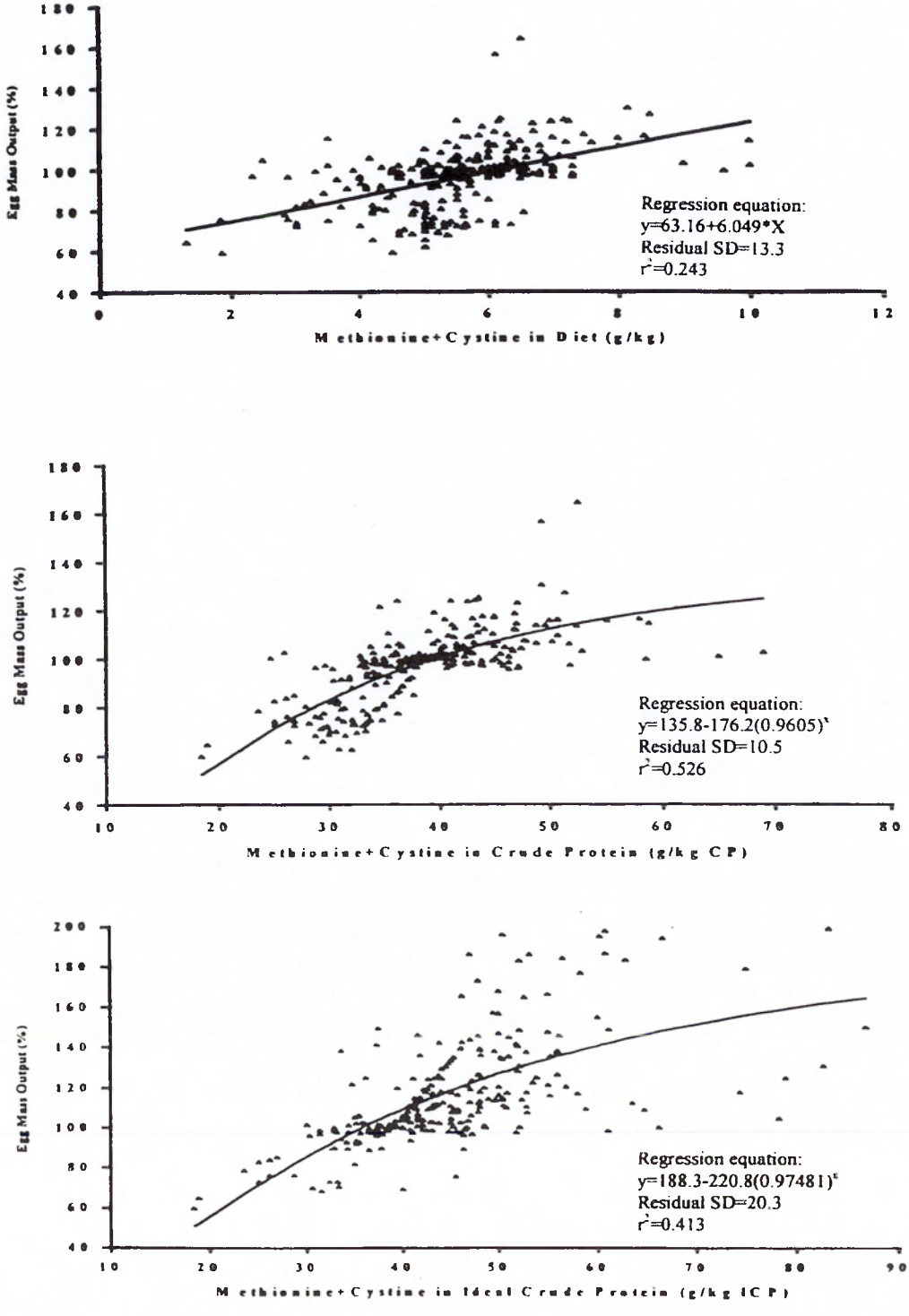


**Figure 2.15** Relationship between dietary variation in methionine + cystine concentration and the mass outputs of eggs laid by hens. Eligible data for all published experiments are included and egg mass outputs are expressed as a percentage of the treatment group within the experiment that was given 39 g/kg of methionine + cystine within the crude or ideal crude protein. Three methods of describing the methionine + cystine supply are compared.



optimize the economic efficiency of a laying hen production system, especially in countries that need to import large quantities of protein concentrates.

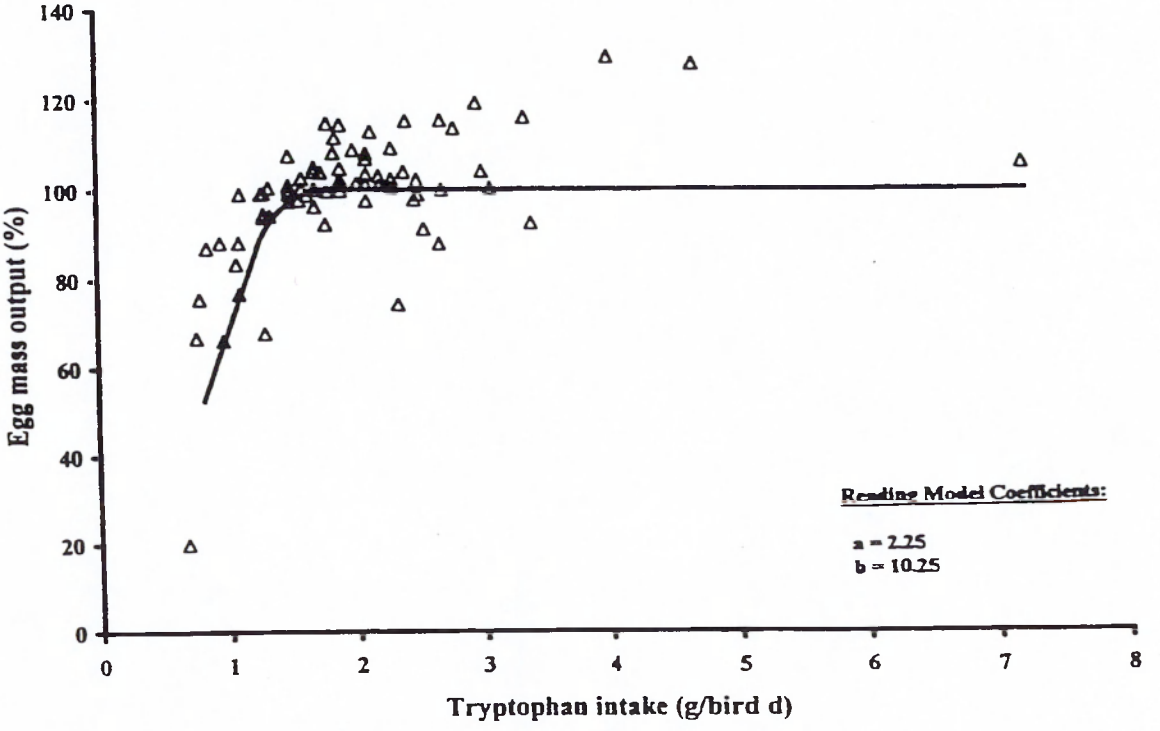
The statistical analysis used in this study indicated that a curvilinear exponential model gave the best fit to almost all egg production variables with all four sets of limiting amino acid data (Egg weight and egg mass output responses with methionine *plus* cystine were exceptions). There was no evidence of a reduction in productive output with an increase in the limiting amino acid concentration above an optimum. This contrasts with the response of broiler chickens to dietary amino acids in which there is a reduction of growth performance above an optimum and the response curve is best described by a quadratic equation (Abebe and Morris, 1990a). Broiler chickens given a very high dietary concentration of a single amino reduce their voluntary feed intakes consequently reducing growth and feed conversion efficiency, whereas laying hen do not appear to reduce feed intakes with an excess of a single dietary amino acid.

Morris (1983) compared the mathematical models that can be used to describe the response of laying hens to dietary amino acid supply. He concluded that there were deficiencies in all of these models but that an asymptotic curve gave a good fit to the experimental data. Morris (1983) also suggested that the Reading Model gave an equally as good fit. *Figure 2.16* shows that applying the Reading Model to the present data set also gave a good fit to the data for tryptophan, lysine, methionine and methionine plus cystine. This indicated that this factorial method of estimating the responses of layers to increasing amounts of a limiting dietary amino acid gave a good representation of the published experimental data. The coefficients that gave the best fit to the data for each of the limiting amino acids that were studied are given in *Figure 2.16*. However, this approach of describing the data was not pursued further in this evaluation because it

**Figure 2.16** The response in egg mass output of laying hens to increasing intakes of dietary amino acids fitted using the Reading Model. The equation to predict egg mass output of individual birds within flock was:

Egg mass output = (amino acid intake – (b x W)) / a

Where W = body weight of birds (kg). A mean body weight of (1.7 kg) was used and a constant feed intake was assumed (112.8 g/ bird d). A normal distribution of the maximum egg outputs of individual birds within a flock was assumed with a standard deviation of 10% of the mean of 56.9 g/bird d. *a* and *b* are coefficients that represent the requirement for production ( mg/kg egg mass output) and maintenance ( mg/kg body weight) respectively.



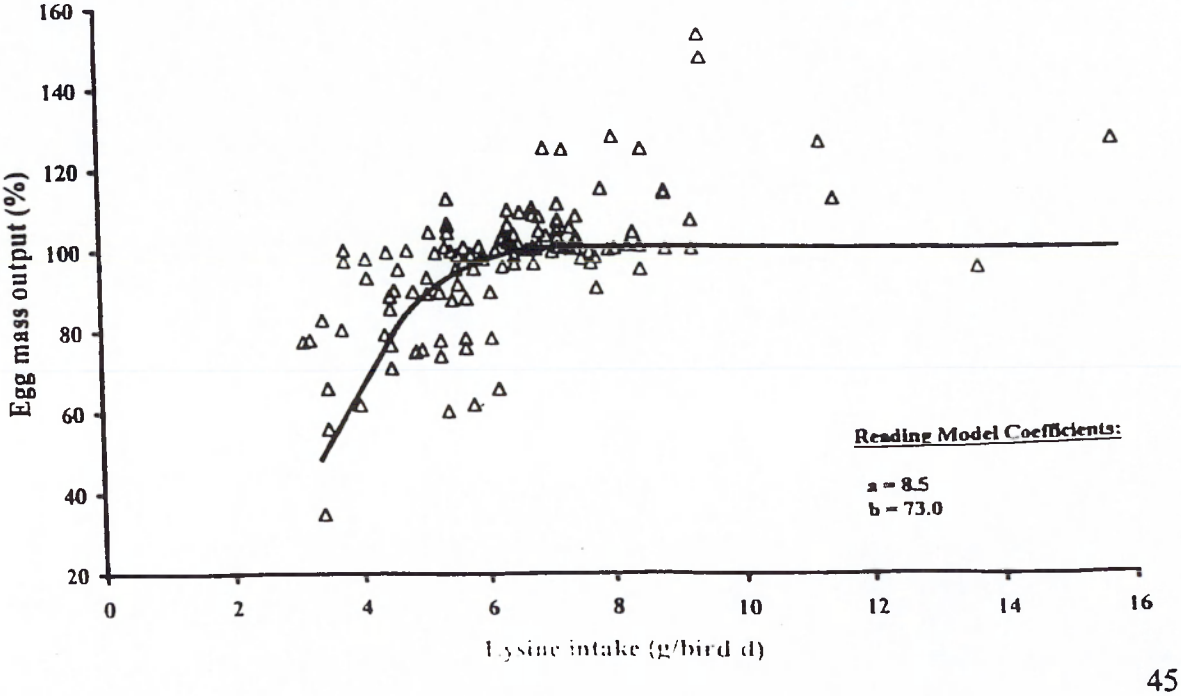
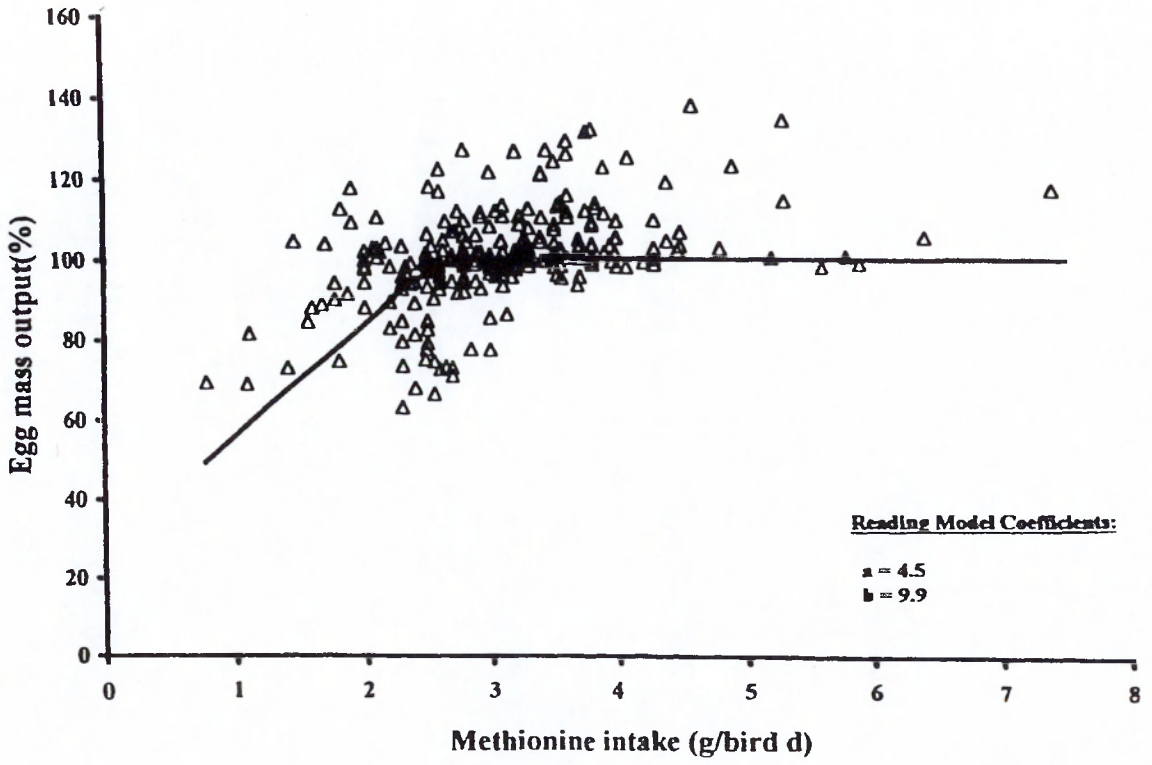
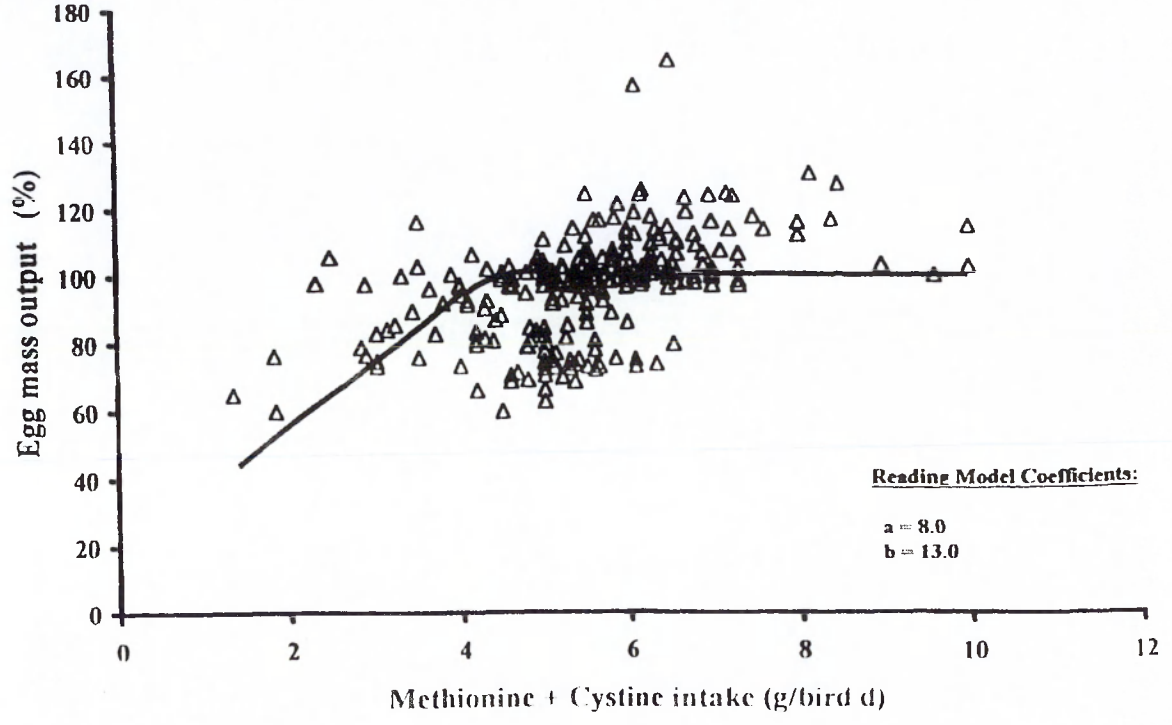



**Figure 2.16** ***(Continued)***



would not allow for a comparison of the different methods of expressing dietary amino acid concentrations.

Work from the 1960s onward has demonstrated that the limiting amino acid requirements of growing chickens are directly related to the concentration of the total dietary protein. Bornstein (1970) observed a constant lysine: crude protein requirement in chickens and Boomgaardt and Baker (1971) observed a similar response with dietary tryptophan. Nelson *et al.* (1960) observed that the maximum growth rates of chickens were obtained at a constant sulphur amino acid: total crude protein rates although this has not been supported in some other studies (Boomgaardt and Baker, 1973). The constant amino acid: crude protein relationship with growing chicken has also been confirmed in recent studies (Abebe and Morris, 1990a, b; Morris *et al.*, 1992). Morris *et al.* (1987) concluded that expressing dietary limiting amino acid supply as a proportion of the crude protein was the best practical method of describing the requirements of flocks of growing broiler chicks. Morris *et al.* (1999) re-examined these experiments with growing chicks and concluded that practical diet formulation programs should be modified to maintain an optimum proportion of essential amino acids to the total crude protein supply.

Laying hens are expected to respond in a similar manner to growing chickens (Almquist, 1952). However, there is a lack of experimental evidence that directly examines whether expressing amino acid supply as a proportion of crude protein supply is preferable for laying hens. A major objective of the present study was to compare this method of description of amino acid supply in layers. This comparison has shown that, in all cases, expressing the egg production responses as a proportion of the crude protein gave a reduction in the residual standard deviation and increased the $r^2$ value. The present statistical analysis has thus clearly demonstrated that describing amino acid

supply as a proportion of the diet protein supply (lysine, methionine, methionine *plus* cystine and tryptophan) was the preferable method of describing their dietary concentrations.

Cole (1979) and ARC (1981) proposed that, to quantify the amount of dietary protein that was available to an animal, the amount of ideal protein should be described in a feed. The ideal protein consisted of the amount of protein in which there was an ideal balance of all essential amino acids and an ideal balance of essential to non-essential amino acids. ARC (1981) discussed and proposed an ideal amino acid balance for pigs but not for poultry. No expert committee has specifically considered the ideal amino acid balance for poultry, so we used the crude protein and amino acid specifications of NRC (1994) for laying hens to derive an ideal balance.

All the experiments considered in this study examined a single basal diet formulation that was deficient in the test amino acid, and supplemented it with different levels of the test amino acid. However, the basal feed protein could also have been deficient in one or more other essential amino acids. Supplementation of the test amino acid in this type of basal feed would not be expected to give the same egg production response compared to supplementation of a basal diet that otherwise had an ideal balance of all other amino acids.

However, the statistical comparisons in this present study indicated that there was no ($p>0.05$) improvement in precision by describing the limiting amino acid supply as a proportion of the ideal crude protein concentration. It is possible that laying hen responses to an amino acid supply do not depend upon the balance of other amino acids. Alternatively, in the majority of the data sets used, the total essential amino acid balance was not determined, and so was predicted from published values (NRC 1994) for each of the feed ingredients in the basal feed. It may be that this calculation method was not

precise enough to give any measurable improvement in the precision in describing the experimental data.

In conclusion, this study has shown that the egg laying responses of hens to a limiting dietary amino acid are curvilinear. The Reading Model can be used to give a good fit to these data but an asymptotic equation also gave a good fit and allowed for statistical comparisons of the methods of describing amino acid concentrations. Expressing dietary amino acid concentrations as a proportion of the crude protein supply reduced the unexplained variation in the data compared to expressing as a proportion of the diet. The study therefore supports the proposal by Morris *et al.* (1999) that in practical feed formulation, amino acid concentration is best described as a proportion of the crude protein and also gives evidence that this approach is valuable not only for growing chicken but also for laying hen diets. No improvement in precision could be demonstrated by describing the amino acid concentration as a proportion of the ideal crude protein.

## 2.2 Temperature

### 2.2.1 Physiological response to different temperatures

Temperature is the most pervasive environmental factor that influences livestock and poultry. There is always an environmental temperature, whereas other factors may or may not be present. Domestic fowl are homeotherms and they must maintain a constant body temperature by biochemical, physiological and behavioural responses.

Birds moved to cold environments respond by increasing metabolic thermogenesis (Freeman, 1965; McDonald *et al*, 1966; Smith and Oliver, 1971), reducing blood flow to body extremities (Sturkie, 1970), and huddling to other individuals in the flock (Baldwin, 1974; Esmay, 1978). Birds moved to hot environments respond by panting to increase evaporate heat loss (Freeman, 1965; Sturkie, 1965; Romijan and Lokhorst, 1966; Hafez, 1968), they will increase blood flow to body extremities (Sturkie, 1970) and have behavioural and postural changes that increase body heat loss (Freeman, 1969; Wathes, 1978).

### 2.2.2 Egg laying response to different temperatures

The general model of thermogenesis of farm animals' responses to temperatures is that there is a thermoneutral zone where changing ambient temperatures do not change the metabolic rate of the animal and that all body temperature regulation is achieved by physiological and behavioural means (van Kampen, 1981). However, there is evidence that, in poultry, throughout the whole range of environmental temperatures there are

physiological responses that affect their productive performance and efficiency of feed utilisation (Howlider and Rose, 1987; Marsden and Morris, 1987). Emmans and Charles (1977) and Charles (1985) stated that 21°C is the optimum house temperature for laying hens. However, because laying performance changes continuously with ambient temperature, the optimum economic temperature may change depending on the economic conditions that prevail for a specific egg unit. There is a need for a precise description of the relative changes in the egg laying characteristics of laying hens with changing ambient temperatures to enable individual egg producers to decide upon their ideal house temperature. One previous study has examined this subject (Marsden and Morris, 1987). However it used a limited data set that examined experiments mostly published prior to 1980. There is now a need to re-evaluate and include the large amount of recent published information that has been generated on the production responses of hens kept under different environmental temperatures.

Birds kept in cold climates are predominately kept in controlled environmental housing where there will be only a relatively small diurnal variation in ambient temperature. However the growing importance of free-range egg production in parts of Europe results in large diurnal temperature variations for this class of poultry. Birds kept in hot climates frequently will experience large diurnal variations in their ambient temperature. Open-sided housing may be used in hot climates and this gives little environmental control. Controlled environment housing may still not be able to reduce the high amplitude of the diurnal variation in ambient temperature in these climates.

There is a practical need to understand and quantify the egg laying responses of hens to these diurnal temperature effects. De Andrade *et al.* (1977) suggested that the responses of birds could be calculated as the result of the mean daily temperature. However, no quantitative data have yet been examined to statistically test this

**Table 2.4** Sources of data used in computation of equations of egg production, egg composition and shell strength of laying hens kept under different environmental temperatures

| Reference | Number of Birds in experiment | Experiment duration (weeks) | Experimental temperatures (°C) | Bird details | | Data availability | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Single (S) or Group (G) housed birds | White (W)or Brown (B) feathered | Laying egg performance | Egg composition | Shell strength |
| Ahmad *et al.* (1974) | 64 | 22 | 21, 30 | S | W | ✓ | ✓ | ✓ |
| Ahvar *et al.* (1982) | 837 | 50 | 21, 32 | G | W | | ✓ | ✓ |
| Blake *et al.* (1984) | 16 | 4 | 21, 30 | S | W | ✓ | | |
| Carmon *et al.* (1965 )#1 | 40 | 8 | 19, 30 | S | W | | ✓ | |
| Carmon *et al.* (1965 )#2 | 75 | 3 | 8, 19, 30 | S | W | | ✓ | |
| Cheng *et al.* (1990) | 248 | 12 | 23.9, 31.1 | G | W | ✓ | ✓ | ✓ |
| Cowan and Michie (1980) | 480 | 24 | 21, 27 | G | B | ✓ | | |
| DE Andrade *et al.* (1976) | 216 | 18 | 21, 32 | S | W | ✓ | ✓ | ✓ |
| DE Andrade *et al.* (1977) | 216 | 12 | 21, 31 | S | W | ✓ | ✓ | ✓ |
| Emery *et al.* (1984)#1 | 54 | 15 | 23.9, 26.7, 29.4 | S | W | ✓ | | ✓ |
| Emery *et al.* (1984)#2 | 36 | 14 | 23.9, 26.4 | S | W | ✓ | | ✓ |
| Emmans *et al.* (1977)#1 | 4560 | 56 | 19, 24 | G | W | ✓ | | |
| Emmans *et al.* (1977)#2 | 3648 | 56 | 19, 24 | G | B | ✓ | | |
| Emmans *et al.* (1977)#3 | 4560 | 52 | 18, 20, 22, 24 | G | W | ✓ | | |
| Emmans *et al.* (1977)#4 | 3648 | 52 | 18, 20, 22, 24 | G | B | ✓ | | |
| Emmans *et al.* (1977)#5 | 4560 | 52 | 18, 20, 22, 24 | G | W | ✓ | | |
| Emmans *et al.* (1977)#6 | 3648 | 52 | 18, 20, 22, 24 | G | B | ✓ | | |
| Goto *et al.* (1982) | 50 | 3 | 21, 32 | S | W | | | ✓ |
| Hill *et al.* (1988) | 7680 | 40 | 18, 21 | G | B | ✓ | | |
| Huston *et al.* (1972)#1 | 90 | 24 | 8, 19, 30 | S | W | ✓ | | |
| Huston *et al.* (1972)#2 | 90 | 24 | 8, 19, 30 | S | B | ✓ | | |
| Hvidsten *et al.* (1976)#1 | 1260 | 56 | 10, 16, 22, 28 | G | W | ✓ | | ✓ |
| Hvidsten *et al.* (1976)#2 | 1260 | 60 | 10, 16, 22, 28 | G | W | ✓ | | ✓ |
| Jones *et al.* (1976) | 45 | 3 | 4.5, 21, 35 | S | W | ✓ | | |
| Lillie *et al.* (1976) | 1620 | 24 | 13, 21.5, 29.5 | G | W | ✓ | | ✓ |
| Marsden *et al.* (1981)#1 | 2160 | 23 | 15, 18, 21, 24, 27, 30 | G | W | ✓ | ✓ | ✓ |
| Marsden *et al.* (1981)#2 | 2160 | 34 | 15, 18, 21, 24, 27, 30 | G | B | ✓ | ✓ | ✓ |
| Marsden *et al.* (1987)#1 | 2160 | 34 | 15, 18, 21, 24, 27, 30 | G | W | ✓ | | |
| Marsden *et al.* (1987)#2 | 2160 | 34 | 15, 18, 21, 24, 27, 30 | G | B | ✓ | | |
| Marsden *et al.* (1987)#3 | 1080 | 61 | 18, 22.5, 27 | G | W | ✓ | | |
| Marsden *et al.* (1987)#4 | 1080 | 61 | 18, 22.5, 27 | G | B | ✓ | | |
| Miller and Sunde (1975)#1 | 12 | 12 | 10, 21 | S | W | ✓ | | ✓ |
| Miller and Sunde (1975)#2 | 162 | 13 | 10, 21, 32 | S | W | ✓ | ✓ | |
| Ota (1960)[+] | * | * | -5[a], 3, 8, 13, 18, 24, 29 | S | B | ✓ | | |
| Payne (1966*a*) | 72 | 16 | 18, 24, 30 | G | W | ✓ | ✓ | |
| Peguri and Coon (1985) | 1440 | 16 | 16.1, 18.9, 22.2, 24.4, 27.8, 31.1 | G | W | ✓ | | |
| Smith (1970) | 42 | 14 | 21, 32, 38[a] | S | W | ✓ | ✓ | ✓ |
| Stockland and Blaylock (1974) | 432 | 26 | 18.3, 29.4 | G | W | ✓ | | ✓ |
| Wilson *et al.* (1972) | 120 | 24 | 10, 23, 34 | G | W | ✓ | | ✓ |

[+] Information not available.

[a] Data available but were not used in the statistical analysis.

conclusion. There is a need to examine the responses of birds kept in diurnally cycling ambient temperatures to evaluate the effect on their egg laying performance. The objectives of this study were, first, to quantitatively describe the relationship between different constant environmental temperatures and the egg production characteristics of laying hens by a statistical analysis and assessment of the published literature. Second, to compare the effect of different cycling environmental temperatures on the egg production characteristics of laying hens.

#### 2.2.2.1 Methods of selection and analysis of data

A detailed literature search was conducted for all published experiments in which two or more constant temperatures had been maintained for at least 3 weeks. The experiments selected had to include 21°C within their temperature treatment range and at least one temperature had to be within ± 3°C of 21°C. Twenty-nine experiments were found that had used white feathered birds (a total of 27487 birds) and ten experiments were found that used brown-feathered birds (a total of 24594 birds). Twenty-three experiments used birds that were kept in groups in cages (a total of 50873 birds) and sixteen experiments used birds caged singly (a total of 1208 birds) (*Table 2.4*).

The selected experiments spanned 30 years and included a large number of production methods and different strains of laying hen kept under varied management conditions. There were, therefore, large variations between experiments in the egg production characteristics of the laying hens, so the temperature treatment differences were described as a proportion of the treatment group within that experiment that were kept at 21°C. This temperature has been suggested to be the optimum ambient

temperature for caged laying hens (Emmans and Charles, 1977; Charles, 1985). All the reported egg production variables of the treatment group were expressed as a proportion (%) of the values obtained from the layers kept at 21°C in that experiment. If the experiment did not contain a treatment exactly at 21°C then a predicted performance was estimated linearly using the nearest treatment groups above and below 21°C. As one of these treatment groups was within 3°C of 21°C, any inaccuracies of this method were considered to be only relatively small. Only the reported temperatures were used in the analysis and no adjustments were made for stocking rate, humidity, air speeds or any other factors that may affect the response to temperature.

Regression analyses were conducted on these data using temperature as the explanatory variable. Linear, quadratic and exponential models were fitted to the data sets and the model that gave the lowest residual standard deviation was selected to describe the temperature responses. The two classifications of white and brown feathered birds and multiple or singly caged birds were included in the regression analyses as grouping factors and tests of whether these factors significantly reduced the residual standard deviation were conducted.

A second statistical analysis examined the egg laying performance of hens kept under fluctuating temperature regimens. Data were available from eight published experiments (a total of 1032 birds) in which a constant temperature of 21°C (±3°C) was used and compared to cyclic temperature regimens (*Table 2.5*). A number of calculations were made to describe the responses of the birds to the diurnally fluctuating temperatures. First, the mean of the daily cycling temperatures was calculated,

Equation 1: mean temperature = (low temperature x hours at this temperature + high temperature x hours at this temperature)/24

A second calculation was used to test the hypothesis that the responses of birds given two diurnally fluctuating temperatures were equivalent to the predicted response of birds in each of the temperatures multiplied by the proportion of the day spent at that temperature. The response of the birds to the high and low temperatures were separately predicted using the equations previously derived in this paper for the birds given different constant temperatures. The predicted responses of the birds at each temperature were then multiplied by the proportion of the day (number of hours/24) that the high and low temperatures were given.

Equation 2: Predicted response to a diurnally cycling temperature regimen with two temperatures = (proportion of time at high temperature each day x predicted response of birds kept constantly at this high temperature) + (proportion of time at low temperature each day x predicted response of birds kept constantly at this low temperature).

The cycling temperature regimen was further described by recording the proportions of time at the high and low temperatures and the amplitude of the high and low temperatures (high temperature minus low temperature).

A regression technique was used to compare the closeness of fit of the responses of the birds given the cycling temperature regimens to that of the birds given the equivalent mean temperature constantly. Equation 1 was used to describe the mean single temperature given to the birds.

The poor correlation between the egg laying performance of the birds given cycling temperatures and their predicted response using the mean daily temperature (equation1)

resulted in the second equation being devised. A multiple regression analysis was used to examine the characteristics of the diurnal cycling temperature regimens that significantly explained the variation in the egg production responses. The following explanatory variables were used to describe the responses relative to the birds kept at a constant 21°C; the mean predicted response to different temperatures each day (equation 2), the mean temperature (equation 1), the high temperature, the proportion of time at the high temperature (number of hours/24), the low temperature, the proportion of time at the low temperature (number of hours/24) and the temperature amplitude (high temperature minus low temperature).

### 2.2.2.2 Results

#### 1. Constant Temperature

None of the responses to temperature of any of the measured variables of egg production, egg composition and quality were affected ($P>0.05$) by bird strain (brown or white feathered) or caging method (group or single caged). Therefore, no further references to these possible causes of variation are made in describing the results.

#### Egg laying performance

The data for the effect of constant temperature on egg laying performance are shown in *Figures 2.17 and 2.18.* The results obtained show that increases in environmental temperature gave relatively small decreases in all measured variables (except feed

**Figure 2.17** The effect of ambient temperature for laying hens on their egg laying performance (expressed as a % response relative to 21°C); egg mass, egg weight and egg number. Data given for single caged birds (Δ) and group caged birds (O) but regression equations are given for the combined data

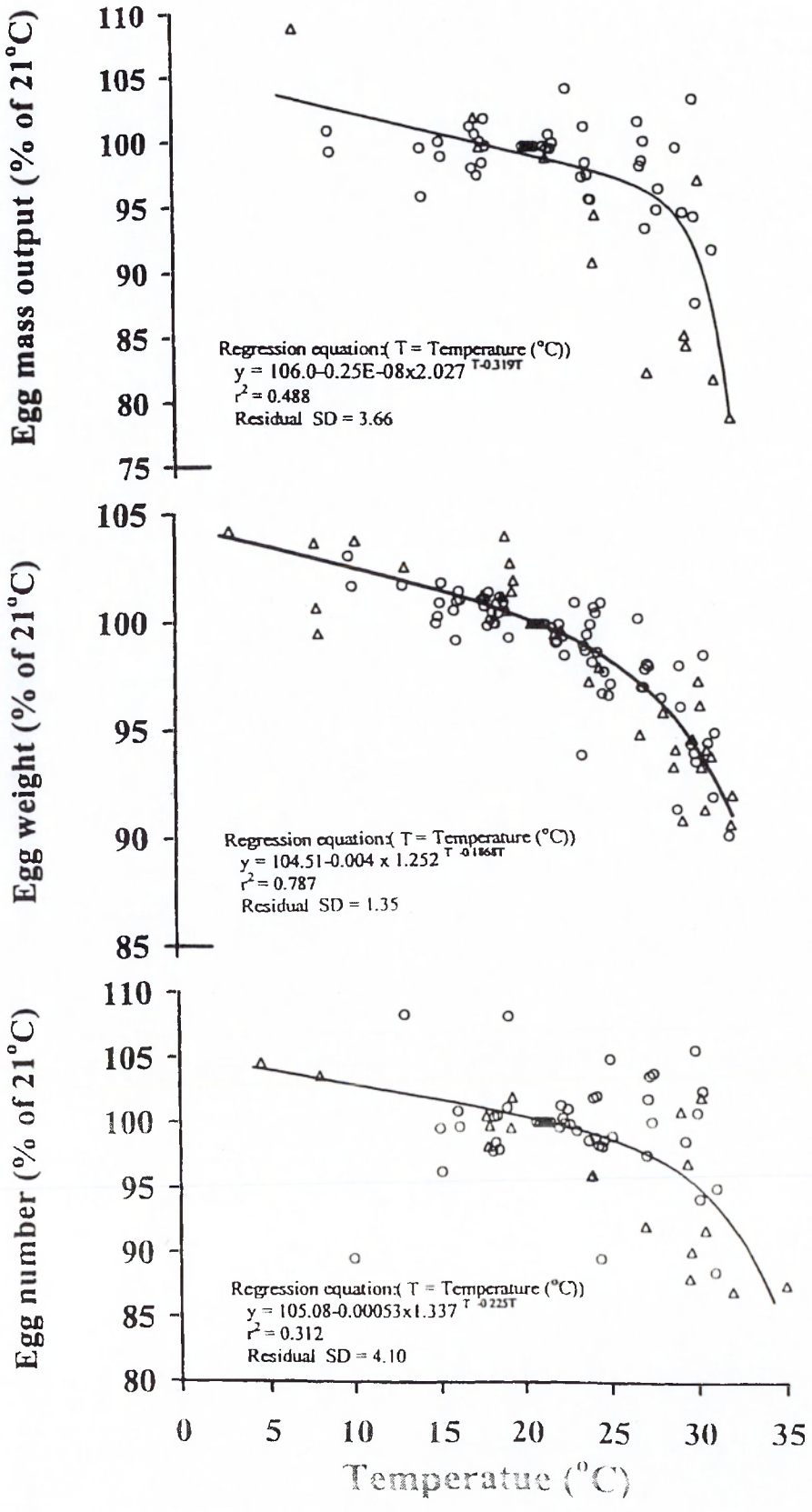
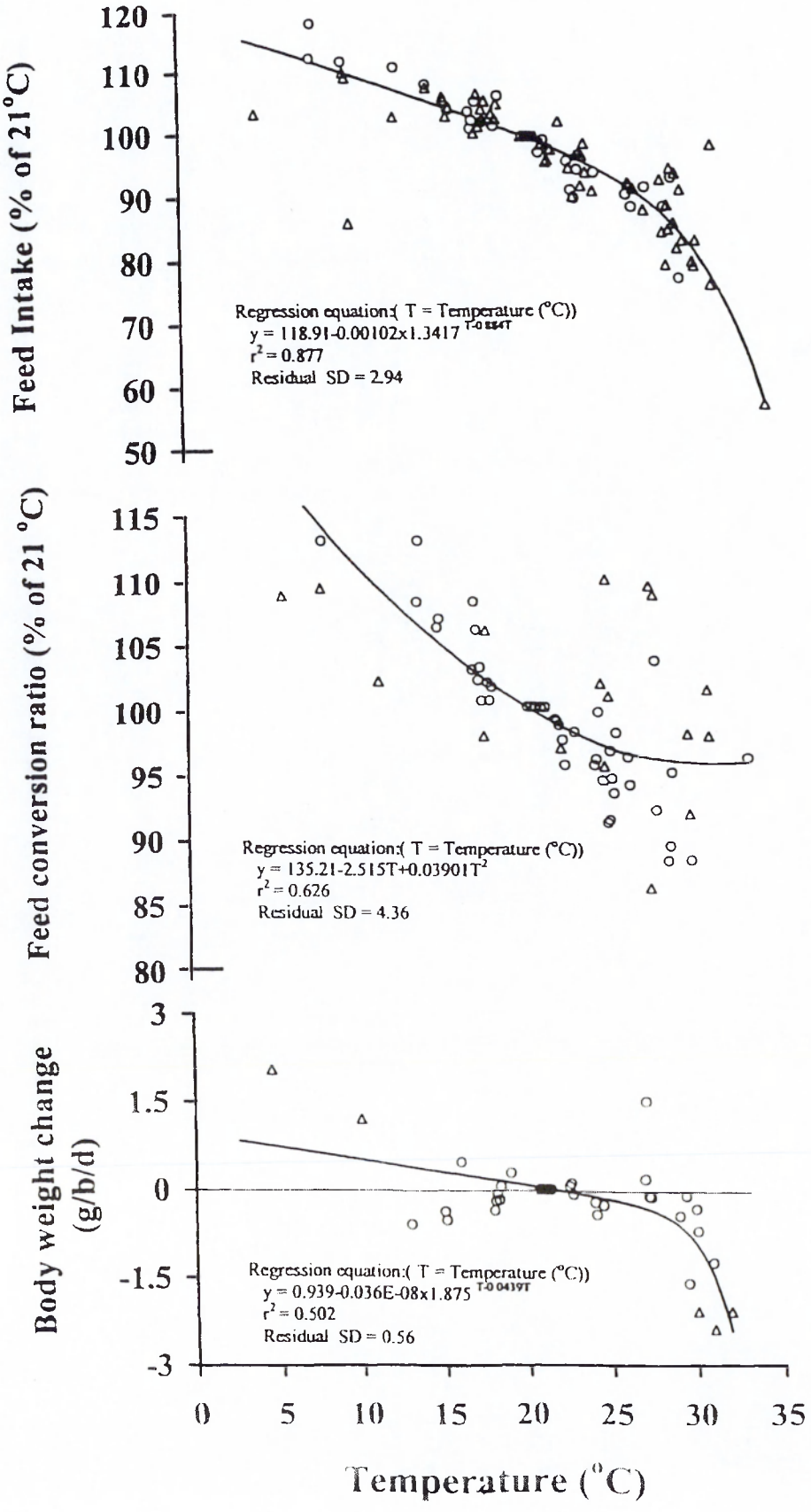


**Figure 2.18** The effect of ambient temperature for laying hens on their egg laying performance (expressed as a % response relative to 21°C); feed intake, feed conversion ratio and body weight change. Data given for single caged birds (Δ) and group caged birds (O) but regression equations are given for the combined data



conversion ratio) of the laying hens until a critical temperature was reached. Thereafter, there were large decreases in these egg production variables. The lowest residual standard deviations for these relationships were obtained using a curvilinear exponential model with the addition of a linear trend;

$$y = a + ( b \times r^{(T + (c \times T))} )$$

Where y = egg laying response (% of birds kept at a constant 21°C), T= ambient temperature (°C) and b, r and c are constants.

Increasing temperature decreased feed conversion ratios, although there was evidence that a minimum value was reached at high ambient temperatures. The lowest residual standard deviation for this relationship was obtained using a quadratic model;

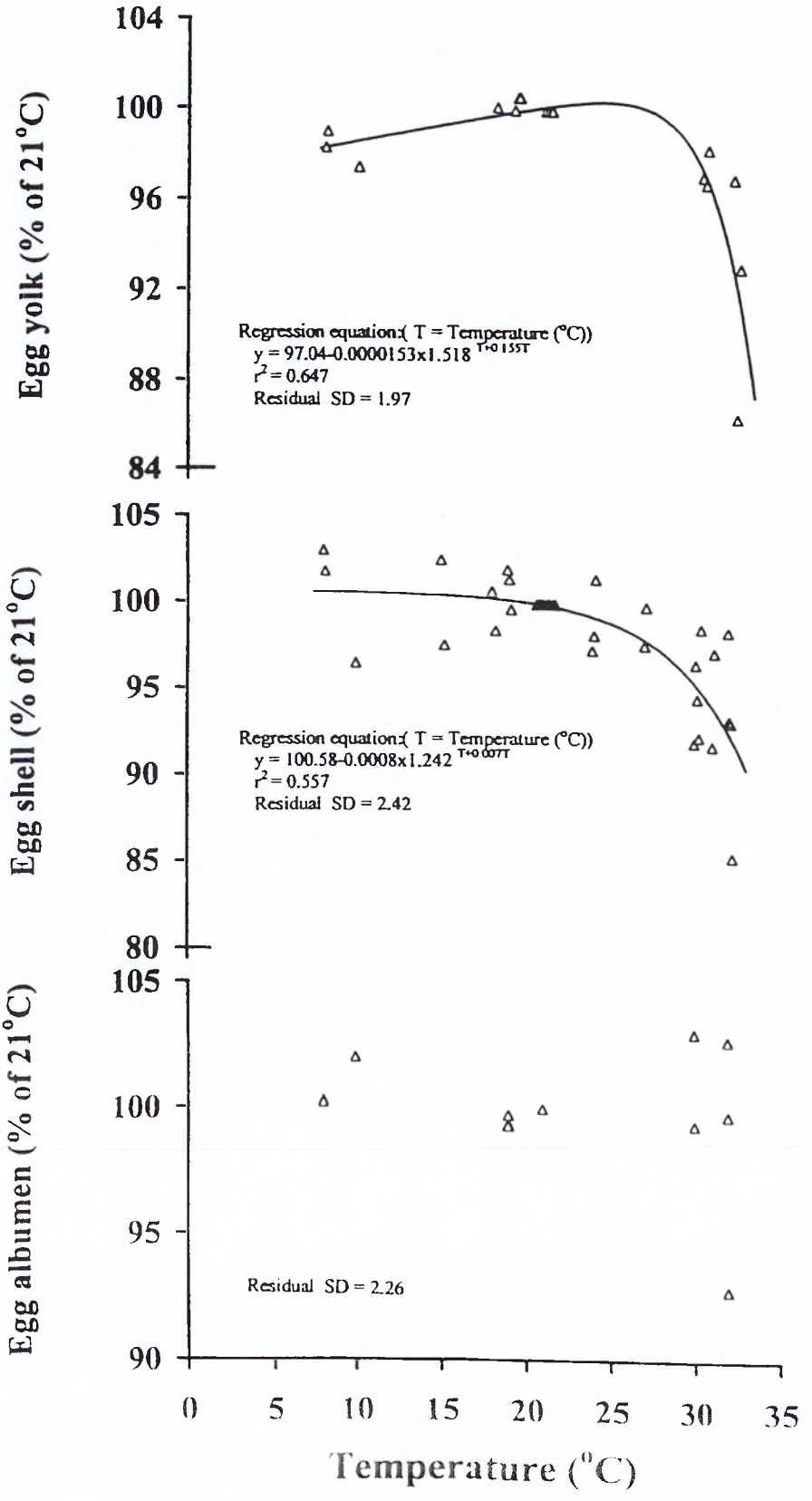
$$y = a + ( b \times T) + (c \times T^2 )$$

Where y = feed conversion ratio response (% of birds kept at a constant 21°C), T= ambient temperature (°C) and b and c are constants.

- EGG COMPOSITION

The data for egg composition (egg yolk, egg shell and egg albumen) as functions of temperature are shown in *Figure 2.19.* The results obtained show that increases in environmental temperature gave relatively small decreases in the egg yolk and egg shell of laying hens until a critical temperature was reduced. Thereafter, there were large

**Figure 2.19** The effect of ambient temperature for laying hens on their egg laying performance (expressed as a % response relative to 21°C); egg shell, egg yolk and egg albumen.



decreases in these egg composition variables. There was no statistically significant relationship ($P>0.05$) of egg albumen with increasing temperature. The lowest residual standard deviations for the relationships of the proportions of yolk and shell with different temperatures were again obtained using a curvilinear exponential model with the addition of a linear trend.

- EGG QUALITY

The data for egg laying for egg shell strength (egg shell thickness, egg deformation and specific weight) and Haugh unit as functions of temperature are shown in *Figure 2.20.* There were negative linear relationships of egg shell thickness and specific weight of the eggs with increasing environmental temperatures, and a positive linear response with egg deformation;

$$y = a + ( b \times T )$$

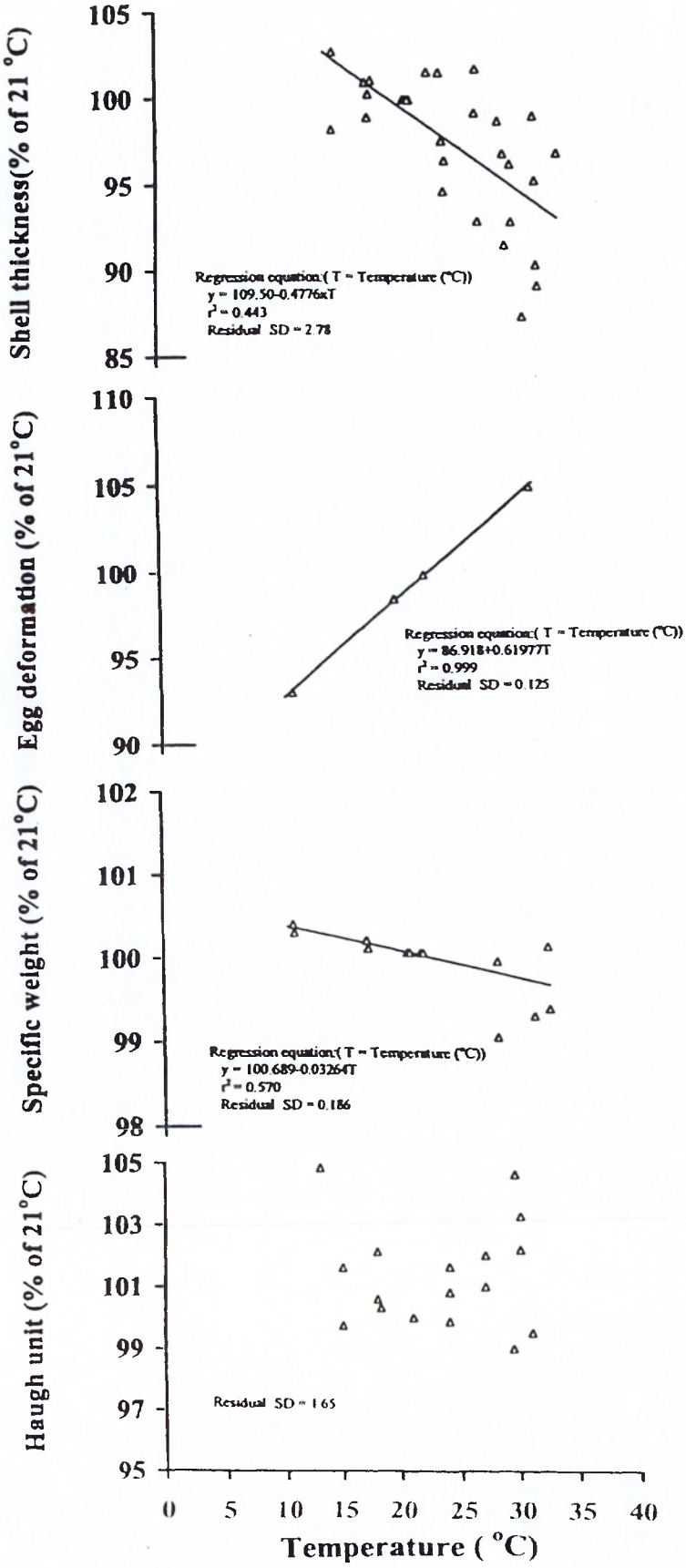
Where y= egg shell thickness, egg deformation or specific weight responses (% of birds kept at a constant 21°C), T= ambient temperature (°C) and a and b are constants.

There was no statistically significant effect ($p>0.05$) of temperature on Haugh units.

**2. Fluctuating Temperature**

A first hypothesis was tested that the egg laying responses of layers given a cycling temperature regimen was equivalent to the response of layers kept at a constant temperature that was the mean of the fluctuating temperatures. The results indicated that, apart from feed conversion ratio, this approach consistently over-estimated the

**Figure 2.20** The effect of ambient temperature for laying hens on their egg laying performance (expressed as a % response relative to 21°C); egg shell thickness, egg deformation, specific weight and Haugh unit.



reduction in egg laying performance of high diurnally fluctuating temperatures compared to high constant temperatures (*Figure 2.21*).

A second hypothesis was tested that the egg laying responses of layers given a cycling temperature regimen was equivalent to the sum of the two predicted responses of the high and low diurnal temperatures multiplied by the proportion of the day at these temperatures (Equation 2). The results similarly indicated that this approach consistently over-estimated the reduction in egg laying performance at the diurnally cycling temperatures. The third hypothesis tested that the egg laying responses were explained by a number of explanatory variables that included the predicted responses equation and a number of measurable characteristics of the diurnally varying temperature regimen. The multiple regression analysis indicated that four variables explained ($P<0.05$) the variation in egg production; these were the predicted response (Equation 2), the low temperature, proportion of time at the low temperature and amplitude of the temperature regimen (*Table 2.5*).

### 2.2.2.3 Discussion

Temperature is a major environmental variable that can affect the profitability of a commercial egg laying enterprise due to its effects on the egg production characteristics and egg quality of laying hens. Caged laying hen egg production in hot climates can be conducted in low-cost, open-sided houses where the birds can be subjected to high ambient temperatures. Controlled environment buildings can reduce the heat load on caged laying hens but these houses are more expensive and so their use increases the fixed capital costs of an egg production enterprise. Cooling systems within controlled environment buildings can reduce internal ambient temperatures but their installation

**Table 2.5** Relationship between experimentally determined egg laying performance of hens given cycling temperature regimens with their predicted response (weighted according to the proportion of time spent at each temperature) and three characteristics of the cycling temperature regimen

| Dependent variable observed experimental response ( % of constant 21°C ) | Independent variables (±SE) | | | | Residual standard deviation | % Variance accounted for |
|---|---|---|---|---|---|---|
| | Predicted response (% of constant 21°C) [$] | Low temperature ( °C ) [§] | Proportion of time at low temperature [†] | Temperature amplitude (°C ) [‡] | | |
| Food intake | 0.4364 (0.0870) | 1.163 (0.454) | 30.1 (20.9) | 1.582 (0.736) | 6.04 | 45.1*** |
| Food conversion ratio | 1.00752 (0.00561) | -0.0055 (0.0126) | - 0.051 (0.565) | - 0.1559 (0.0211) | 0.140 | 99.5*** |
| Egg number | 0.7434 (0.0797) | 0.800 (0.211) | 5.6 (10.7) | 0.820 (0.371) | 2.82 | 4.9*** |
| Egg weight | 0.8699 (0.0391) | 0.3852 (0.0980) | 2.70 (4.95) | 0.502 (0.171) | 1.28 | 89.6*** |
| Egg mass | 0.5340 (0.0739) | 1.160 (0.391) | 18.9 (17.7) | 1.407 (0.641) | 5.26 | 75.4*** |

Sources of data used in multiple regression analysis: Cowan and Michie (1980) ,DeAndrade *et al.* (1976), Emery *et al.* (1984), Miller and Sunde (1975) #1, Miller and Sunde (1975) #2, Payne (1966*a*) #1, Payne (1966*a*) #2, Payne (1966*a*) #3.

Where [$] Predicted response (% of constant 21°C) = proportion of time at high temperature x predicted response of birds at this constant temperature + proportion of time at low temperature x predicted response of birds at this constant temperature.

[§] Lowest temperature in cycling regimen.

[†] Proportion of 24 hours at low temperature.

[‡] Temperature amplitude = high temperature – low temperature.

*** P<0.001

**Figure 2.21** Comparison of the predicted and actual responses of laying birds to different ambient temperatures when kept either in constant or diurnally fluctuating temperature regimens. The predicted responses were calculated using the equations previously determined in this paper for birds kept under constant temperatures. A single ambient temperature was determined for the diurnally fluctuating temperatures by calculating the mean daily temperature.

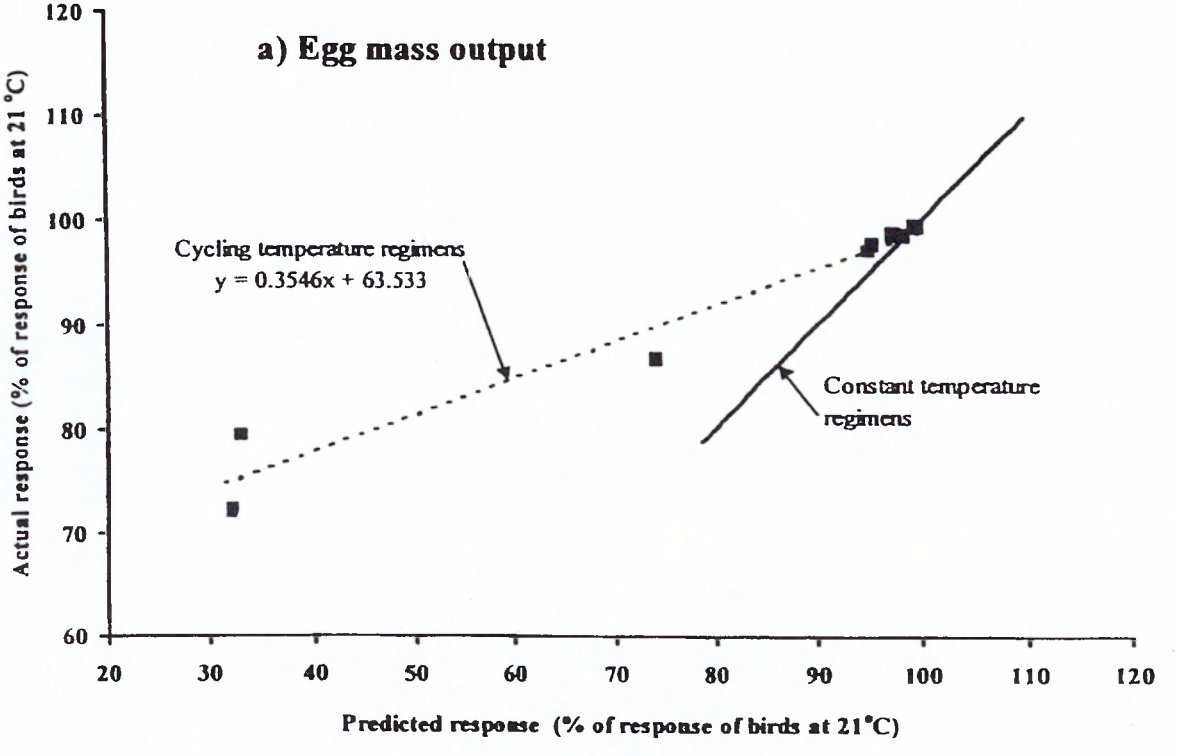
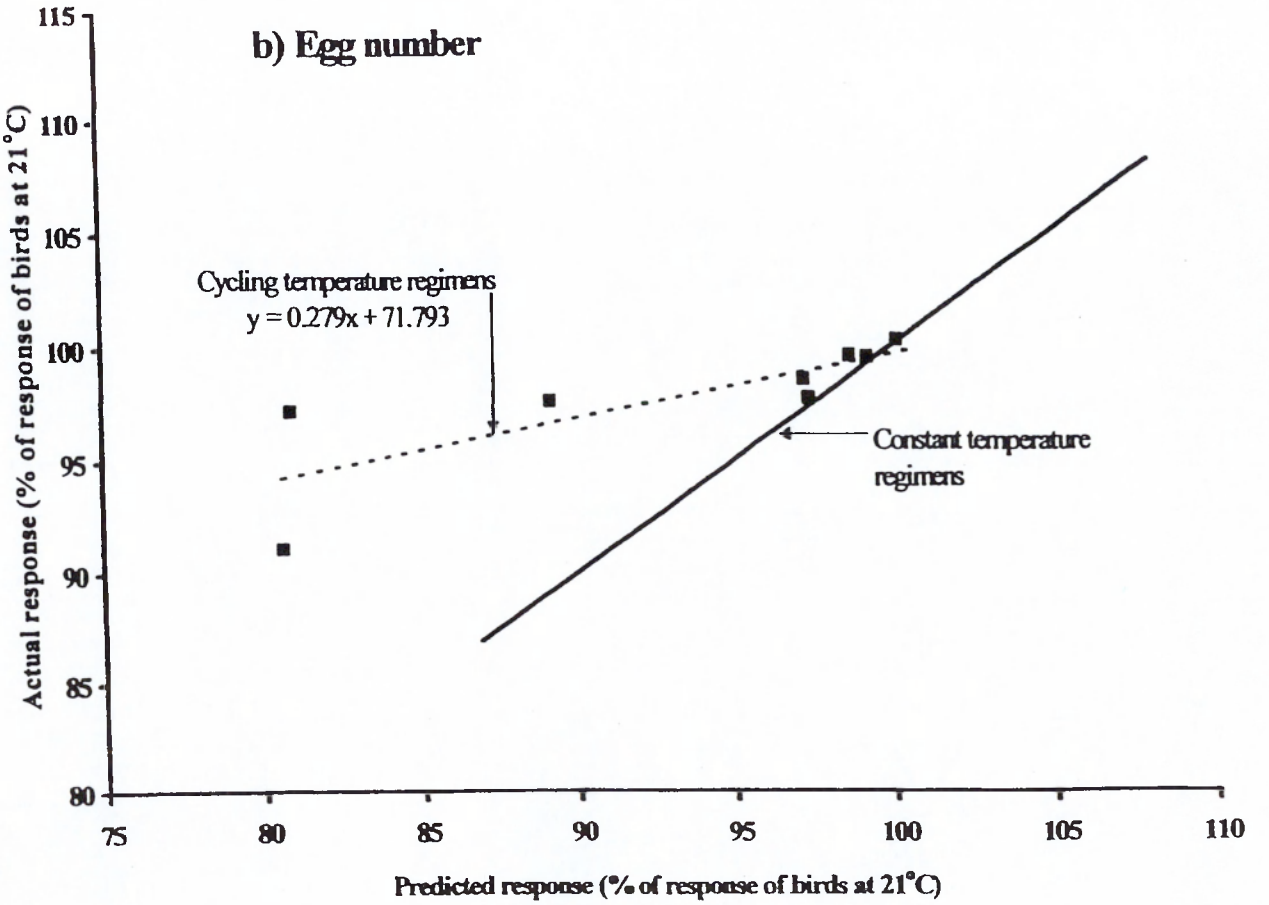


**Figure 2.21** *(Continue)*



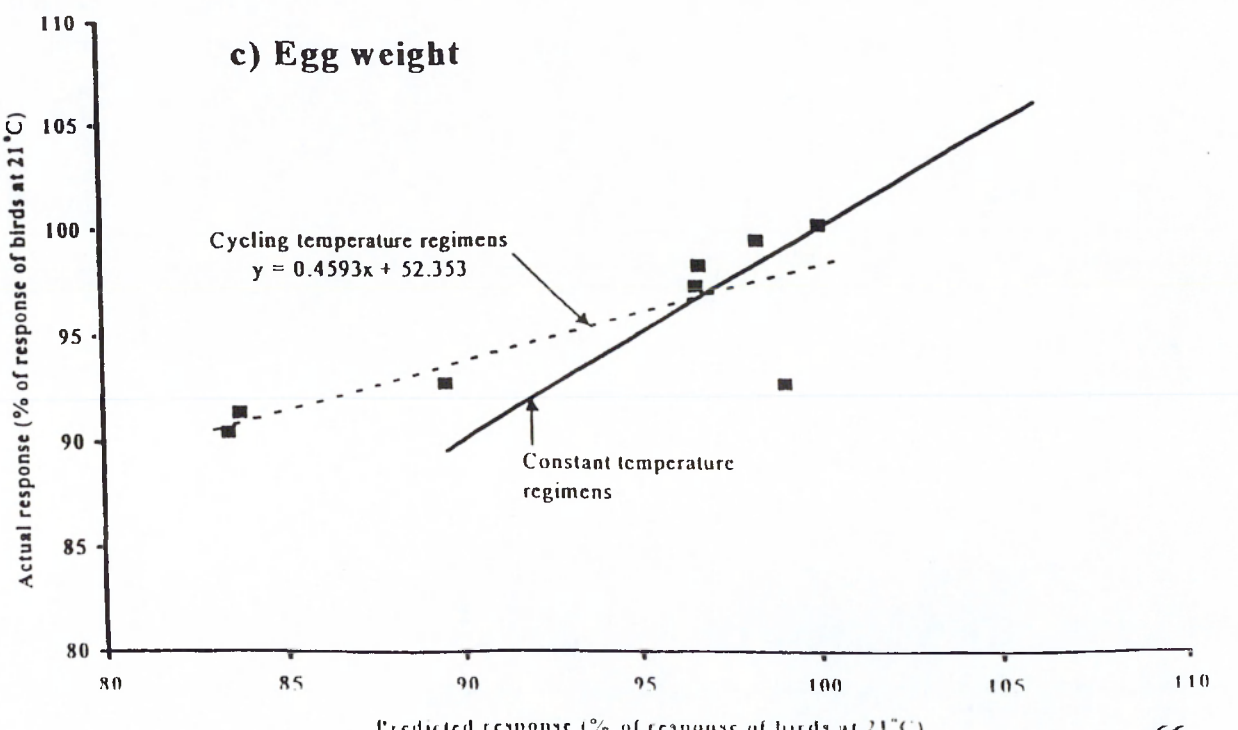
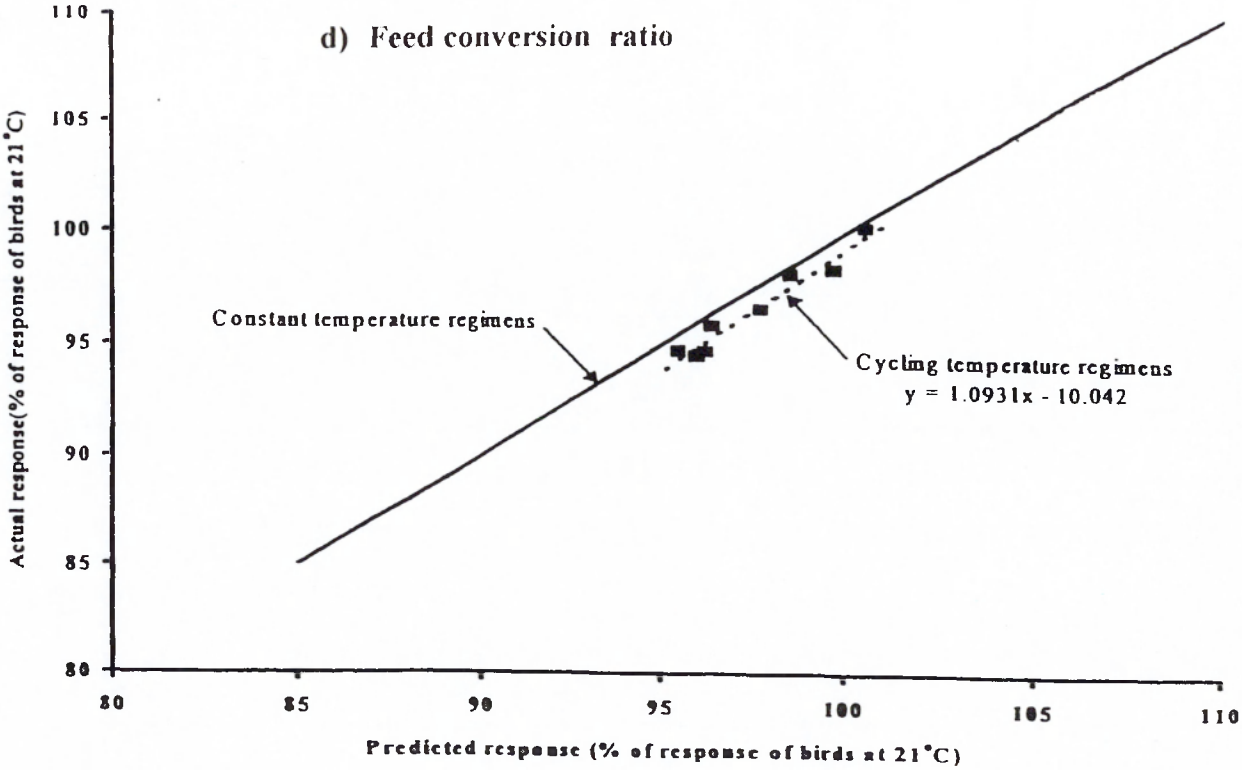
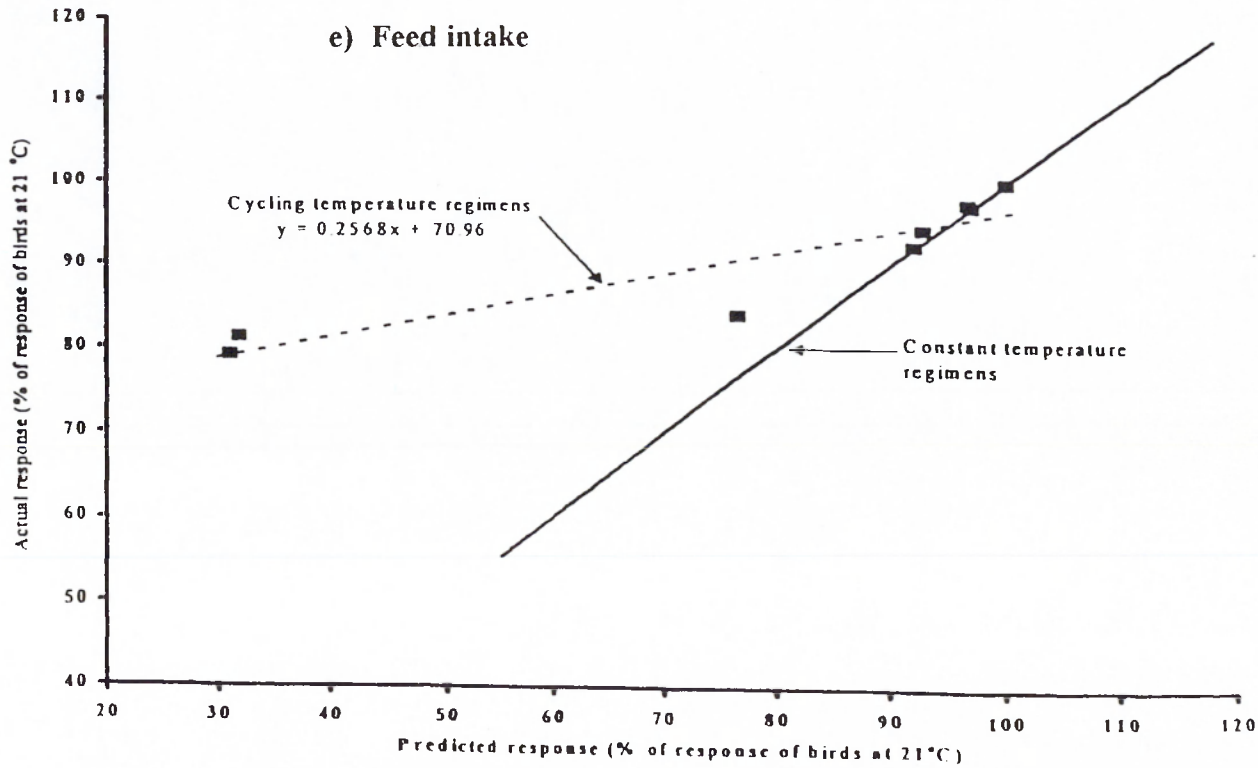



**Figure 2.21** *(Continue)*



costs are high and the running costs are expensive. The choice of building type and the possibility of installing cooling equipment are thus important economic decisions that can only be correctly made if the improvement of egg production characteristics can be accurately quantified for a proposed reduction in ambient temperature for the layers.

Caged egg production in cold climates is frequently conducted in controlled environment buildings that, if suitable building construction and material and ventilation design are implemented, can use bird heat to maintain a wide range of possible temperatures. The actual set temperature that is used in these situations is an economic decision that also depends on accurately quantifying the comparison of increased egg production characteristics against the increased cost of higher feed intakes.

The objective of this section was to review all published literature to give a precise description of the effect of changing temperatures on the egg production, egg quality and feed intakes of laying hens. The curves used to describe these egg laying response not only mathematically explain the high proportion of the observed variation but also give models that are biologically relevant: The fitted curve shows a rapid fall in egg production, feed intakes and shell strength at approximately 28 to 30°C and this is the temperature where panting and the acute effects of heat stress would begin to occur. Decreasing temperature below this critical point gave a relatively small, linear increase in most egg production variables, shell strength and feed intakes. Increasing heat loss to the environment would increase the energy requirements of the birds for thermogenesis and so they would increase their feed intakes to meet their increased energy requirements. The increased feed intakes also increase their intakes of other essential nutrients and so this may cause a relatively small increase in egg mass output and shell strength.

The equations derived from this approach to reviewing the literature on temperature have given practically consistent results. For example, Payne (1964) estimated that a one degree decrease in ambient temperature below the point of heat stress would give a 0.90% increase in feed intake. The feed intake equation derived from the present study indicates that a one degree decrease in temperature in this zone would result in a 0.95% increase in feed intakes. The regression equations indicate that, below the point of heat stress, the weight of increased feed intake is approximately three times greater than the weight of egg output. These regression equations therefore give the egg production industry the quantitative information that they require to make economic decisions about the cost-effectiveness of temperature reduction costs.

Although the effects of cycling temperatures on laying hens has should be have often been examined, no general model has been proposed that would enable their responses to be predicted. DE Andrade *et al.* (1977) observed that there was a poor relationship if the mean daily temperature was computed and the predicted response was considered to be equivalent to birds given this temperature constantly. The present study has confirmed the poor prediction using this approach in determining the egg laying responses of birds kept in daily fluctuating temperatures. In general, the reduction in egg laying performance and feed intakes of birds kept at high average temperatures was over-estimated by this approach. Birds change their eating behaviour to be more active during cooler parts of the day (DE Andrade *et al.* 1977). This study indicates that birds are able to tolerate periods of high temperature during the day without markedly affecting their productive performance.

A second approach used to predict the responses to cycling temperatures was to consider that there were separate responses to each of the temperatures that occur during the day. It was proposed that the response to each temperature could be predicted from

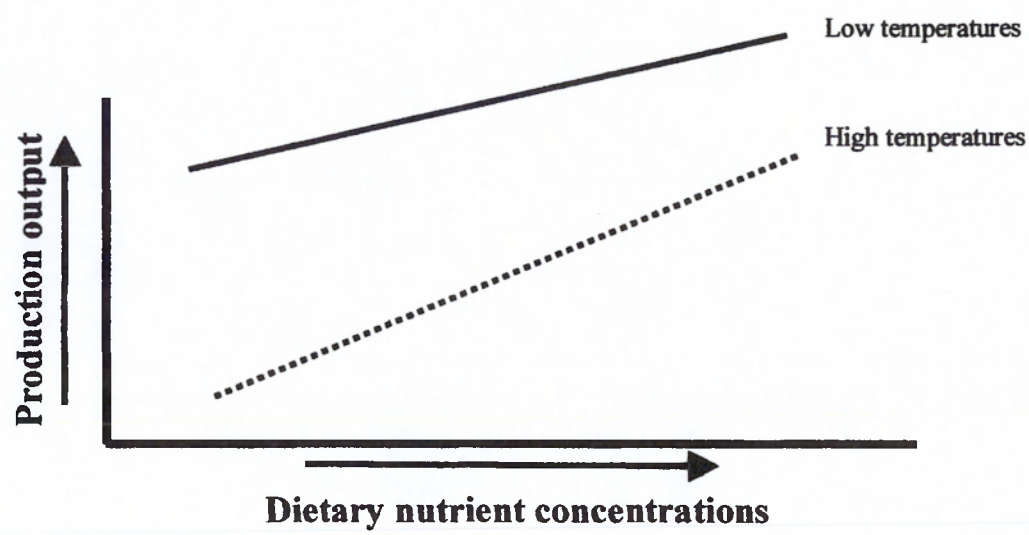
the response of birds kept at constant temperatures and that their overall daily responses were the sum of these individual responses weighted for the proportion of the day at each temperature. The results of the statistical analysis showed that this method gave an improved accuracy in predicting the response compared to the first method, but there was still relatively poor precision.

The multiple regression approach considered a large number of explanatory variables but only four were highly significant in explaining the variation in the egg laying responses of laying hens in fluctuating temperatures. The results indicate that the mean of the responses to the individual temperatures must be computed but also the responses are affected by the characteristics of the daily fluctuating temperature: Information on the lowest temperature, the amount of time at this temperature and the amplitude of the cycling temperature are required to effectively describe the birds' responses. These results therefore give a model for egg producers to predict the egg laying responses of hens kept in hot, diurnally fluctuating temperatures when changes in housing construction or design or investments in cooling equipment are being contemplated.

## 2.2.2 Nutrient and temperature interactions

Section 2.2 has quantified the response of laying hens to ambient temperature. Numerous authors have postulated because poultry reduce their voluntary feed intakes at high temperatures, there should be an increase in nutrient density of the diets. The interaction of ambient temperature x nutrient concentration shown in *Figure 2.22* has been proposed.

**Figure 2.22** The postulated relationship between nutrient concentrations and egg outputs under low and high environmental ambient temperatures.




The aims of this section are to explore whether such temperature x nutrient interactions have been demonstrated in the published literature.

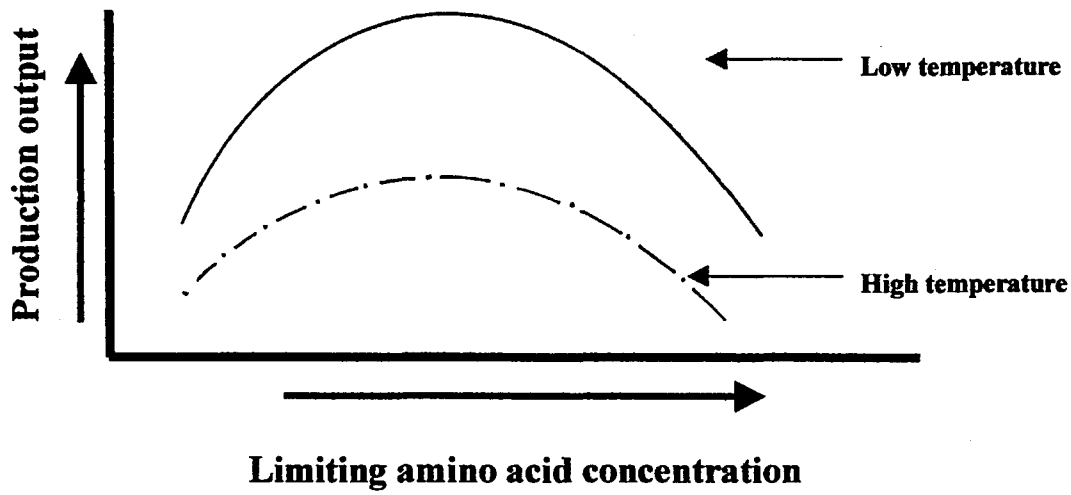
## 1. Total dietary protein concentrations

Although a number of studies have shown increased productive output from increasing dietary protein concentration, there is little published evidence of a greater, or lesser, response at different temperatures. Emmans and Charles (1977), Cowan and Michie (1980) and Marsden *et al.* (1987) have shown that there were no significant dietary protein concentration x temperature interactions on hen egg output. Emmans and Charles (1977) reported an interaction in one experiment, but there were no significant interactions in a second experiment. The further lack of dietary protein concentration x temperature interactions is supported in growing chickens by Cowan and Michie (1978) and Charles *et al.* (1981) and in growing turkeys by Hurwitz *et al.* (1980) and Rose and Michie (1987). Swiergiel and Ingram (1986) concluded that there was no dietary protein concentration x temperature interactions on thermoregulatory behaviour of piglets.These data provide no evidence of an interaction between temperature and dietary protein concentration.

## 2. Amino acid balance

There are few previously published experiments that have examined amino acid balance x temperature in laying birds. However, published experimental evidence in growing poultry indicates that the response to amino acid balance depends on ambient temperature. At low temperatures there is a large increase in productive output with the increase of a limiting amino acid up to an optimum concentration (*Figure 2.23*). At high temperatures there is a large decrease in productive output with the decrease of a limiting amino acid.

**Figure 2.23** A model to demonstrate the responses between limiting amino acid concentrations and production output kept at low and high temperatures.




These amino acid balance x temperature interaction responses have been demonstrated in experiments with lysine in growing broiler chickens (Rose and Salah Uddin, 1997), with lysine, methionine and threonine in growing turkeys (Veldkamp *et al.*, 2000a) and arginine and lysine balance in growing turkeys (Veldkamp *et al.*, 2000b). Schench *et al.* (1992) showed that there were dietary lysine concentration x temperature interactions on body weight gain and feed efficiency of pigs.

These effects have not always been demonstrated and there are published experiments where no amino acid balance x temperature interactions have been observed (Sinurat and Balnave, 1985; Hsu *et al.*,1998; Al-saffar and Rose, 2000b).

### 3. Energy density

Increasing the energy density of practical diets is generally achieved by increasing the fat content. High fat diets have a lower heat increment of digestion than high carbohydrate or high protein feeds that supply the same concentration of metabolizable energy. Addition of fat to diets has therefore generally been demonstrated to increase the feed intakes (Carew and Hill, 1964) and productive output (Fuller and Mora, 1973; Dale and Fuller, 1979, 1980) of birds kept at high temperatures compared to birds kept at low temperatures. Experiments by Daghir (1987) showed there were temperature x fat interactions in feed intake and shell thickness of laying hens. Adding 5% fat to laying hen diets improved their feed intakes and shell thickness by 17.2% and 13.5% respectively, at high temperature (30°C) compared to 4.5% and -2.6% respectively, at low temperatures.

These effects have not always been demonstrated and there are published experiments where no dietary energy balance x temperature interactions have been observed (Ahmad *et al.*, 1974; De Albuquerque *et al.*, 1978; Dale and Fuller, 1980; Keshavars and Fuller, 1980; Hurwitz *et al.*, 1987; Peguri and Coon, 1991; Howlider and Rose 1992).

### 4. Vitamins

Published literature indicates that high environmental ambient temperatures increase the requirement of antioxidant vitamins in laying hens. Cheng *et al.* (1990); Utomo *et al.* (1994); Bollengier *et al.* (1997, 1998); Whitehead *et al.* (1998) have observed that the depression in egg production in laying hens caused by heat stress can be partially

prevented by dietary supplementation of vitamin E. Ascorbic acid supplementation had no effect on the body temperature of laying hens maintained in a moderate environment, *i.e.*, 24.4 to 26.1°C. Elevating the ambient temperature to 37.8 °C resulted in significantly lower body temperatures in ascorbic acid supplemented laying hens (Thornton, 1962; Ahmad *et al.*, 1967). Hunt and Aitken, (1962); Attia (1976) reported that blood ascorbic acid was significantly depressed in Leghorn hens maintained at 35 °C and in broiler chickens (Datta and Gangwar, 1981). Perek and Kendler (1962, 1963); Pardue *et al.*(1985); Gross (1988) demonstrated that supplementation with ascorbic acid provided marked protection against the lethal effect of acute exposure to 43 °C in fowls. Pardue *et al.*(1985); Stiborn *et al.*(1988); McKee *et al.* (1997) reported that there was no ascorbic acid beneficial on broiler growth or feed efficiency under heat stress.

Thornton and Moreng (1959); El-Boushy (1966); El-Boushy and Van Albada (1970) reported a temperature x ascorbic acid interaction in laying hens. Increased dietary concentrations improved egg production and egg weight, reduced egg breakage and improved shell percentage, hatchability and fertility of laying hens in a hot environment. McKee *et al.* (1997) detected temperature x ascorbic acid interaction in which heat-exposed birds expressed lower ($P<0.10$) respiratory quotients when consuming the ascorbic acid supplemented diet of broiler chicks.

**5. Minerals**

Evidence from published literatures indicates that the response to minerals is affected by heat stress. Dietary mineral x temperature interactions responses were demonstrated in experiments with phosphorus in laying hens (Daghir, 1987; Orban and Roland, 1990) and with molybdenum in turkeys (Bailey *et al.*, 1983). Rose *et al.* (1998) observed a

trend ($P<0.1$) for a similar response with potassium in broiler chickens. However, no dietary sodium x temperature interactions were observed in laying hens (El-Boushy, 1966).

## 2.3 General conclusions of the literature review

- Although the biochemistry of the major limiting amino acids is well-defined, the effects on their dietary supply to poultry is not fully understood.

- There is a curvilinear ($P<0.001$) response in egg number, egg mass outputs of laying hens given increased dietary amino acids. Increases in dietary amino acid concentrations gave small increases in egg numbers, egg weights and egg mass outputs until an asymptote was reached.

- The proposal of Morris *et al.* (1999) that it is preferable to express amino acid requirements as a proportion of the crude protein is supported by the literature. Expressing dietary amino acid concentrations as a proportion of the crude protein supply reduced the unexplained variation in the published laying hen response data compared to expressing as a proportion of the total diet. However, there was no further advantage to expressing the amino acid concentration as a proportion of the ideal protein supply.

- There is little published information on the responses of broiler breeder flocks to amino acid supply.

- There was a curvilinear ($P<0.001$) response in egg weight and egg mass output of egg-laying strains of hen to different ambient temperatures. These variables decreased with increasing temperature but the decrease accelerated at temperatures

above 27°C. There was a positive linear relationship ($P<0.001$) between egg shell strength and temperature.

- Cycling temperature regimens gave greater egg mass outputs by the egg-laying hen strains than would be predicted from the mean of the daily temperatures.

- There is little published information on the responses of broiler breeder flocks to ambient temperatures.

- Temperature x single limiting amino acid interactions have been determined to exist in poultry, although the effects on laying broiler breeder flocks have not been studied.

# Chapter 3

# 3. Experimental

## 3.1 Experiment 1

*The effect of four ambient temperatures and dietary lysine concentrations on the reproductive performance of broiler breeders.*

### 3.1.1 Introduction

The previous chapter has quantified the response of laying hens to ambient temperature. However, there is a lack of information on whether laying broiler breeders respond similarly. Broiler breeders have a much greater body weight in relation to their egg output, also they are restrictedly fed in commercial practice. These differences with egg laying strains could result in significant differences in response.

Section 2.1.3 quantified to response of laying hens to variations in a limiting dietary amino acid. There is a lack of information as to whether these responses are similar in broiler breeders and whether these responses interact with ambient temperature.

The specific objectives of the experiment were first to examine and explain the effects of four different ambient temperatures 21, 26, 29 and 32°C on the response of the egg laying performance, egg composition and hatchability of the eggs from laying broiler breeders. Second, to examine their response of four dietary lysine concentrations (35, 50, 65 and 90 g/kg crude protein). Third, to examine whether there were temperature x lysine concentration interactions in the responses of broiler breeders.

## 3.1.2. Materials and Methods

### 3.1.2.1 Diets and Measurements

A single wheat-based, lysine deficient diet that contained 151 g/kg crude protein was formulated (*Table 3.1*). The concentration of all amino acids and nutrients met or exceeded the requirements of broiler breeder hens according to the National Research Council (1994) and Ross Breeders Limited (1998). Three further dietary levels of lysine concentration were achieved by adding L-lysine-HCl to the deficient diet, in replacement for maize starch, to give four concentrations of lysine (35, 50, 65 and 90 g/kg crude protein). All experimental diets were stored in the same environmental conditions. A daily feed restriction programme was followed; they were given 150 g/day bird, at the start of the experiment followed by a reduction of 2.5 g feed/ bird after each 28 day period (Ross Breeders Limited, 1998). After the completion of each period in the experiment, birds were given a standard broiler breeder feed (*Table 7.1A* (Appendix 1) ) for one week before the start of the next period (*Figure 3.1*).

### 3.1.2.2 Laboratory Analysis

At the beginning of each period, a lysine deficient diet sample was collected for amino acid analysis. The samples were treated with 6M hydrochloric acid in sealed bottles at 110°C for 22 hours in order to hydrolyse the protein chains. For the analysis of cystine and methionine, the samples were oxidised with performic acid at 2-8°C for 16 hours prior to acid hydrolysis. The resulting hydrolysate was diluted and filtered. An aliquot was then adjusted to pH 2.2, and a known quantity of norleucine was added as an

**Table 3.1** Composition of the broiler breeder lysine deficient diet fed in experiment 1 ( 29 - 50 weeks of age).

| Ingredients | Lysine deficient diet (kg)† |
|---|---|
| Wheat | 750.0 |
| Maize gluten meal (600g/kg CP) | 50.0 |
| Dehulled soya bean meal | 60.0 |
| Full fat soya | 10.0 |
| Maize starch | 20.8 |
| Limestone | 70.0 |
| Dicalcium phosphate | 10.0 |
| Sodium bicarbonate | 2.0 |
| Salt | 2.0 |
| DL-methionine | 2.0 |
| L-threonine | 1.7 |
| L-tryptophan | 1.5 |
| Vitamin-Mineral Supplement‡ | 20.0 |
| Total | 1000.0 |
| **Nutrient Composition:** | |
| Determined chemical analysis | |
| Crude Protein ( CP) (g/kg) | 151.0 |
| Lysine (g\kg CP) | 35.0 |
| Methionine (g\kg CP) | 30.0 |
| Tryptophan (g\kg CP) | 19.2 |
| Methionine+ Cystine (g\kg CP) | 46.5 |
| Threonine (g\kg CP) | 41.5 |
| Isoleucine (g\kg CP) | 44.0 |
| Arginine (g\kg CP) | 65.0 |
| Valine (g\kg CP) | 47.0 |
| Histidine (g\kg CP) | 24.0 |
| Calculated analysis | |
| Metabolisable Energy (MJ/kg) | 11.8 |
| Calcium (g\kg) | 33.6 |
| Phosphorus (g\kg) | 5.0 |
| Sodium (g\kg) | 1.8 |
| Potassium (g\kg) | 4.8 |
| Linoleic acid (g\kg) | 12.0 |
| Choline (mg\kg ) | 1000 |

†The other 3 experimental diets included additional (L-lysine HCl) in replacement for maize starch.

‡Supplied per kg of diet: *trans*-retinol(A), 4.8 mg; cholecalciferol(D3), 125µg; α-tocopherol acetate(E), 183.8 mg; thiamine(B1), 3 mg; riboflavin(B2), 10 mg; pyridoxine(B6), 5 mg; vitamin B12, 12 mg; nicotinic acid, 60 mg; pantothenic acid, 15 mg; folic acid, 2.5 mg; biotin, 205 µg; choline chloride, 500 mg; Fe, 20 mg; Co,1 mg; Mn, 100 mg; Cu, 10 mg; Zn, 80 mg; I, 2 mg; Se, 0.2 mg; Mo, 0.5 mg; Ca, 206g/kg; P, 100.5g/kg; Na, 50g/kg; Ash , 882g/kg.

**Figure 3.1** Pens randomization of the broiler breeder in experiment ( 1 ) . Four diets (lysine concentrations: 35, 50, 65 and 90 g\ kg CP) and four temperatures: 21, 26, 29 and 32°C .

Period #1

| | | | | |
|---|---|---|---|---|
| 50 | 35 | 65 | 90 | 32°C |
| 90 | 50 | 35 | 65 | 21°C |
| 65 | 90 | 50 | 35 | 29°C |
| 35 | 65 | 90 | 50 | 26°C |

Period #2

| | | | | |
|---|---|---|---|---|
| 65 | 90 | 35 | 50 | 29°C |
| 35 | 65 | 50 | 90 | 32°C |
| 50 | 35 | 90 | 65 | 26°C |
| 90 | 50 | 65 | 35 | 21°C |

Period #3

| | | | | |
|---|---|---|---|---|
| 90 | 65 | 50 | 35 | 21°C |
| 65 | 35 | 90 | 50 | 26°C |
| 35 | 50 | 65 | 90 | 32°C |
| 50 | 90 | 35 | 65 | 29°C |

Period #4

| | | | | |
|---|---|---|---|---|
| 35 | 50 | 90 | 65 | 26°C |
| 50 | 90 | 65 | 35 | 29°C |
| 90 | 65 | 35 | 50 | 21°C |
| 65 | 35 | 50 | 90 | 32°C |

internal standard before making up to volume. The method was not suitable for the analysis of tryptophan and did not distinguish between the D and L forms of the amino acids.

### 3.1.2.3 Birds and Housing

Two hundred and twenty-four 29-week old hens (308 Broiler Breeder, Ross Breeder Ltd., Newbridge, Midlothian, Scotland) were randomly allocated to 16 identical pens within four environmentally controlled rooms in a facility (*Figure 3.1*) at Harper Adams University College, Shropshire. Two male birds were chosen at random and also placed in each pen. Each pen was equipped with two galvanized metal nest boxes with an alighting bar. Additionally one cup drinker allowed *ad libitum* access to water and two single feeders (*Figure 3.2*). The daily lighting programme was maintained at 16 hours of artificial illumination with a mean light intensity of 60 lux throughout the experimental period. A data-logging (thermohygrogragh) computer programme continuously recorded temperature and relative humidity.

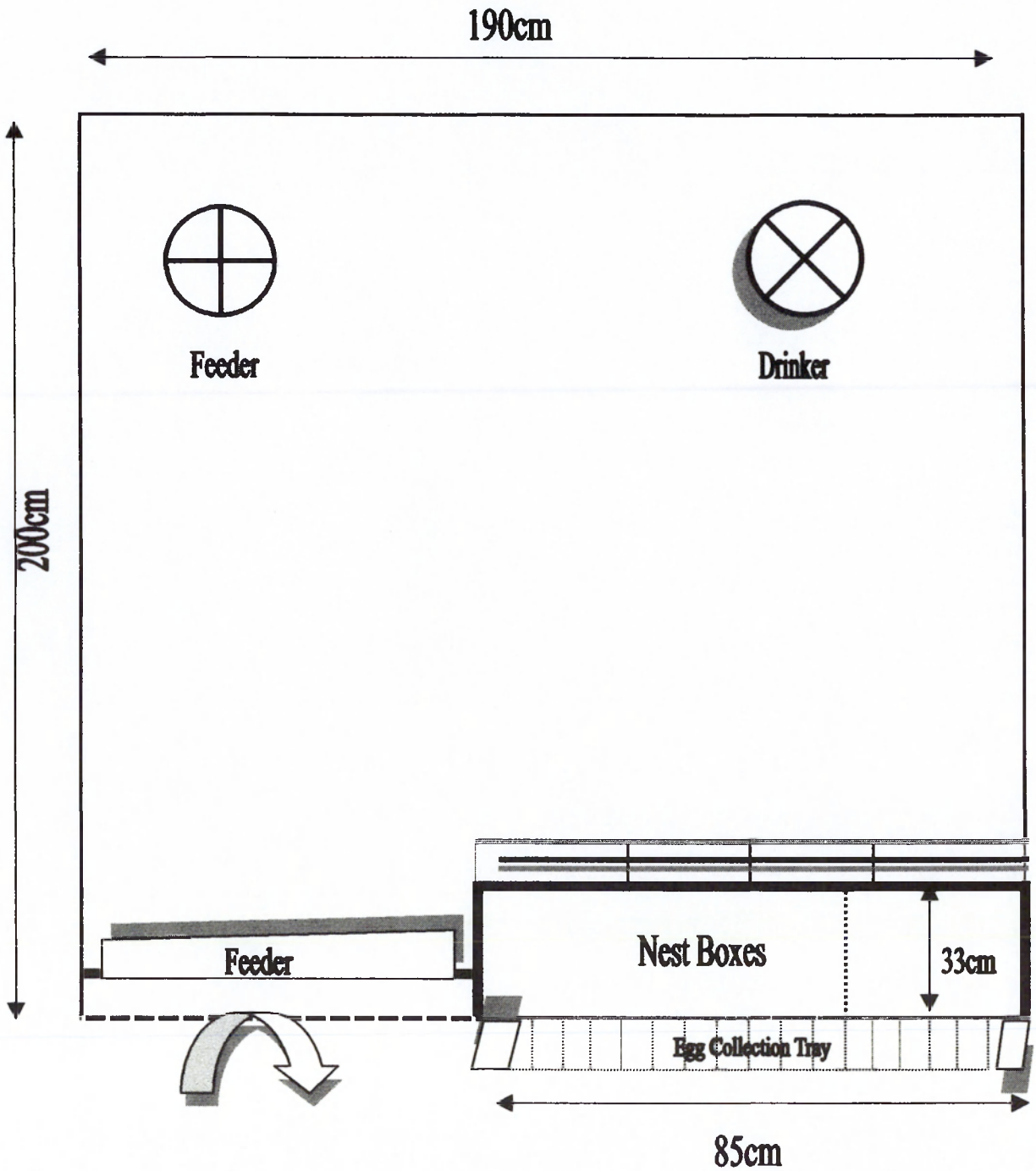
The body weight change of each pen of birds was recorded at the end of each period and expressed as g/bird day. Males were swapped between pens within each room at the end of each 28 days period to maintain fertility in each pen.

### 3.1.2.4 Temperatures

There were four different temperatures used in the experiment (21, 26, 29 and 32°C). Each of the four rooms were given one of the four constant temperatures at the beginning of each period (*Figure 3.1*). After the completion of each 28 day period, all

**Figure 3.2** Broiler breeder pen layout used in experiment 1 and 2

16 birds per pen



temperature treatments were immediately changed for three days to 21°C and 70-75% relative humidity. On the fourth day, if necessary, a gradual daily change in temperature was made so that the ambient temperature applied in the next period could be reached (*Section 3.1.2.1*). Each temperature was given to each room of birds over the four 28 day periods in a latin square design.

### 3.1.2.5 Egg production, composition and shell strength

Total egg numbers were recorded daily during the experimental feeding periods. All the eggs were weighed and egg mass output was calculated for each pen of broiler breeders. Additionally, all the eggs (single yolk only) produced on one day in the final week of each period were collected and the proportion of yolk, albumen and shell were measured. Shell strength was determined by two methods;

**Shell deformation:**

Shell deformation is a method of determination of the resistance of the shell against deformation without damaging the shell. It was measured using an apparatus designed by Schoorl and Boersma (1962). This apparatus measures the deformation of the egg shell under a certain load (500g). The deformation was measured on the equator of the egg.

**Shell weight *per* unit area:**

Eggs were broken, the inner contents were removed and yolk was separated using an egg separation tool and weighed. Shells were rinsed carefully with luke warm water to

remove the traces of albumen. The shells were then dried at 103°C for 3 hours and weighed. Shell weight *per* unit area was calculated as follows using the equation given by Romanoff and Romanoff (1949):

$$\text{Shell weight/surface area (mg/cm}^2\text{)}$$

$$\text{Where Surface area} = (4.558 \times W^{2/3}) \times 1000$$

W= egg weight (g)

### 3.1.2.6 Egg fertility, hatchability and chick viability

All the eggs produced on one day in the final week of each period were collected from all pens. A collection was made every two hours throughout this day. Each egg was carefully cleaned by sandpaper to remove any dirt on the shell. The eggs were then stored at 14.4°C and 73.1% relative humidity in a light-proof room for one day. On the next day, the eggs were candled and any cracked shells were discarded. The eggs were weighed and then fumigated with formaldehyde (Alphagen Prills) for two hours at 23.9°C. The eggs were returned to the storage room for one more day where they were kept under the same temperature and relative humidity conditions.

Approximately six hours prior to placing eggs in the setter they were moved from the storage room and allowed to warm to room temperature (22°C). Eggs were transferred into the single stage incubator. The incubator temperature was set at 37-38°C, and each egg was numbered and placed with the broad end up. Eggs were candled on the 6$^{th}$ day to check mid-term embryonic development (fertility). Samples of eggs were selected that represented each dietary treatment that had been placed in randomly selected sites

within the incubator. Their weights were measured daily to monitor the percentage weight loss during incubation. The incubation conditions were adjusted to give a mean daily egg weight loss of (12/21) % (equivalent to an approximately 12% weight loss throughout the 21 day incubation period.

At day 18, eggs were candled to check the late embryonic development. Fertile eggs were then transferred to the hatcher. The hatcher temperature was set at 36.7°C. At hatching, male and female birds were identified by wing feather shape, and chicks (males and females) from each pen were weighed and wing-banded.

Chicks were reared in one group for 7 days under standard commercial conditions. A localised brooder temperature of approximately 32°C was provided by radiant heaters. Chicks were allowed *ad libitum* access to water and a proprietary broiler starter diet. Body weight gain and survival rates were determined for both males and females.

### 3.1.2.7 Experimental Design

**Statistical analysis**

A split-plot design was used in which four main plots (rooms) were kept at four constant temperatures each for a 28 day period. The temperature of the room was then changed to another constant experimental temperature decided upon in a latin-square design. Within each main plot, four sub-plots (pens) were each fed the four different diets each for a 28 day period. Data were compared by analysis of variance of the

measured and calculated variables, using a split-plot design that examined the effects of temperature (main-plot) and the effect of diet and the lysine x temperature interactions within the sub-plot (*GENSTAT* statistical package, Lawes Agricultural Trust, 1998). For both the temperature and lysine treatments, the treatment sums of squares was partitioned into their linear and non-linear (quadratic) effects. Data for egg numbers, egg weight and egg mass output were additionally collected for each pen in the week prior to the commencement of the 28 day feeding periods. These data were used to adjust the egg numbers, egg weight and egg mass data respectively for that experimental unit in that experimental period using analysis of covariance.

### 3.1.3. Results

Throughout the experiment the allocated amounts of feed were always eaten. The mean mortality during the experiment was 2.3% (6 birds in total), which was not associated with particular treatments. The overall mean egg output of the flock was low relative to commercial standards but this was probably a reflection of the experimental treatments applied.

Increasing temperatures gave linear decreases in egg weight ($P$=0.027) and numbers of eggs laid (*Table 3.3*, $P$=0.002). Additionally, increasing temperature gave linear decreases in the proportion of shell in the eggs (*Table 3.4*, $P$=0.049) and shell weight *per* unit area (*Table 3.5*, $P$=0.026). Egg deformation linearly increased (*Table 3.5*, $P$=0.001) with increasing temperatures. Male body weight gain was decreased linearly ($P$<0.001) by increasing temperature, but not ($P$>0.05) for female body weight gain (*Table 3.2*). There were no ($P$>0.05) effects of temperature on egg fertility, hatchability or chick viability.

There were no significant differences ($P$>0.05) between the different lysine concentration levels in any variable of egg composition, shell strength, egg fertility and hatchability, chick viability or body weight gain variables (*Tables 3.3, 3.4, 3.5, 3.6*). There tended (P=0.085) to be a curvilinear relationship between egg mass output and increasing dietary lysine concentrations. Similarly, there were no consistent ($P$>0.05) temperature x lysine concentration interactions.

**Table 3.2** Effects of four dietary lysine levels on body weight gain in both female and male broiler breeders (29- 50 weeks of age) kept at 21, 26, 29 or 32 °C (Experiment 1)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | Mean temperature effects |
|---|---|---|---|---|---|---|
| | | **35** | **50** | **65** | **90** | |
| **21** | | | | | | |
| | Female weight gain (g / d) | 0.55 | 0.15 | 1.78 | 2.19 | 1.17 |
| | Male weight gain (g / d) | 16.70 | 17.28 | 19.24 | 16.28 | 17.37 |
| **26** | | | | | | |
| | Female weight gain (g / d) | -0.20 | -0.94 | 2.95 | 0.47 | 0.57 |
| | Male weight gain (g / d) | 17.58 | 15.71 | 17.71 | 17.09 | 17.02 |
| **29** | | | | | | |
| | Female weight gain (g / d) | -0.68 | -0.73 | 0.57 | 1.54 | 0.17 |
| | Male weight gain (g / d) | 17.42 | 16.68 | 16.30 | 16.74 | 16.78 |
| **32** | | | | | | |
| | Female weight gain (g / d) | 3.42 | 1.20 | -0.72 | 2.71 | 1.65 |
| | Male weight gain (g / d) | 15.40 | 16.99 | 14.96 | 15.03 | 15.60 |
| | Mean lysine concentration effects | | | | | |
| | Female weight gain (g / d) | 0.77 | -0.08 | 1.14 | 1.73 | |
| | Male weight gain (g / d) | 16.77 | 16.67 | 17.05 | 16.28 | |

| | Statistical significance and SEM of treatment means[†] | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=4) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=4) | |
| | P | SEM | P | SEM | P | SEM |
| Female weight gain (g / d) | P>0.10 | 1.756 | P>0.10 | 0.782 | P>0.10 | 1.614 |
| Male weight gain (g / d) | Linear (P<0.001) Quadratic (P=0.007) | 0.166 | P>0.10 | 0.418 | Quadratic.Quadratic (P=0.07) | 0.729 |

[†] Any P<0.10 is indicated

**Table 3.3** Effects of four dietary lysine levels on egg laying performance*; total egg mass output, egg weight and egg number of broiler breeders (29- 50 weeks of age) kept at 21, 26, 29 or 32 °C (Experiment 1)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | Mean Temperature effects |
|---|---|---|---|---|---|---|
| | | **35** | **50** | **65** | **90** | |
| **21** | | | | | | |
| | Egg mass (g / hen / d) | 33.56 | 32.60 | 34.34 | 27.20 | 31.92 |
| | Egg weight (g / d) | 66.62 | 66.28 | 65.35 | 65.04 | 65.82 |
| | Egg number (no./ hen / d) | 0.50 | 0.50 | 0.53 | 0.42 | 0.49 |
| **26** | | | | | | |
| | Egg mass (g / hen / d) | 35.03 | 35.59 | 30.01 | 30.40 | 32.76 |
| | Egg weight (g / d) | 63.64 | 65.33 | 66.23 | 65.02 | 65.06 |
| | Egg number (no./ hen / d) | 0.56 | 0.55 | 0.45 | 0.48 | 0.51 |
| **29** | | | | | | |
| | Egg mass (g / hen / d) | 22.75 | 33.70 | 31.23 | 29.38 | 29.27 |
| | Egg weight (g / d) | 64.42 | 65.46 | 65.48 | 65.24 | 65.15 |
| | Egg number (no./ hen / d) | 0.36 | 0.52 | 0.49 | 0.46 | 0.46 |
| **32** | | | | | | |
| | Egg mass (g / hen / d) | 29.63 | 31.07 | 29.51 | 27.05 | 29.06 |
| | Egg weight (g / d) | 64.23 | 62.97 | 65.07 | 66.56 | 64.70 |
| | Egg number (no./ hen / d) | 0.47 | 0.50 | 0.44 | 0.42 | 0.46 |
| | Mean lysine concentration effects | | | | | |
| | Egg mass (g / hen/d) | 30.24 | 33.24 | 31.02 | 28.51 | |
| | Egg weight (g / d) | 64.73 | 65.01 | 65.53 | 65.47 | |
| | Egg number (no./ hen / d) | 0.47 | 0.52 | 0.48 | 0.44 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=4) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=4) | |
| | P | SEM | P | SEM | P | SEM |
| Egg mass (g / hen / d) | Linear (P<0.001) Quadratic (P=0.035) | 0.360 | Quadratic (P=0.085) | 1.367 | P>0.10 | 2.422 |
| Egg weight (g / d) | Linear (P=0.027) | 0.259 | P>0.10 | 0.397 | Linear. Linear (P=0.008) Quadratic.Quadratic (P=0.025) | 0.734 |
| Egg number (no./ hen / d) | Linear (P=0.002) Quadratic (P=0.020) | 0.006 | P>0.10 | 0.021 | P>0.10 | 0.038 |

*Total egg mass output and egg numbers were adjusted by analysis of covarience using the egg numbers each pen in the pre-experimental week.

**Table 3.4** Effects of four dietary lysine levels on egg composition ( expressed as a proportion of the egg weight ); egg shell, egg yolk and egg albumen of broiler breeders (29- 50 weeks of age) kept at 21, 26, 29 or 32°C ( Experiment 1)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | Mean temperature effects |
|---|---|---|---|---|---|---|
| | | **35** | **50** | **65** | **90** | |
| **21** | | | | | | |
| | Egg shell | 0.090 | 0.090 | 0.096 | 0.092 | 0.092 |
| | Egg yolk | 0.296 | 0.318 | 0.311 | 0.305 | 0.307 |
| | Egg albumen | 0.615 | 0.592 | 0.593 | 0.603 | 0.601 |
| **26** | | | | | | |
| | Egg shell | 0.092 | 0.090 | 0.090 | 0.090 | 0.091 |
| | Egg yolk | 0.304 | 0.305 | 0.306 | 0.311 | 0.307 |
| | Egg albumen | 0.604 | 0.605 | 0.604 | 0.598 | 0.603 |
| **29** | | | | | | |
| | Egg shell | 0.090 | 0.089 | 0.090 | 0.091 | 0.090 |
| | Egg yolk | 0.311 | 0.310 | 0.305 | 0.303 | 0.307 |
| | Egg albumen | 0.599 | 0.600 | 0.605 | 0.607 | 0.603 |
| **32** | | | | | | |
| | Egg shell | 0.088 | 0.089 | 0.089 | 0.088 | 0.088 |
| | Egg yolk | 0.313 | 0.305 | 0.307 | 0.307 | 0.308 |
| | Egg albumen | 0.599 | 0.606 | 0.605 | 0.605 | 0.604 |
| | Mean lysine concentration effects | | | | | |
| | Egg shell | 0.090 | 0.089 | 0.091 | 0.091 | |
| | Egg yolk | 0.306 | 0.310 | 0.307 | 0.306 | |
| | Egg albumen | 0.604 | 0.601 | 0.602 | 0.603 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=4) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=4) | |
| | P | SEM | P | SEM | P | SEM |
| Egg shell | Linear (P=0.049) | 0.0010 | P>0.10 | 0.0009 | P>0.10 | 0.0018 |
| Egg yolk | P>0.10 | 0.0034 | P>0.10 | 0.0023 | Linear.Quadratic (P=0.009) | 0.0052 |
| Egg albumen | P>0.10 | 0.0037 | P>0.10 | 0.0027 | Linear.Quadratic (P=0.013) | 0.0059 |

**Table 3.5** Effects of four dietary lysine levels on egg shell strength; egg deformation, shell weight per unit area and total egg weight loss (expressed as percentage of egg weight) during incubation of broiler breeders (29- 50 weeks of age) kept at 21, 26, 29 or 32 °C (Experiment 1)

| Temperature (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | Mean temperature effects |
|---|---|---|---|---|---|---|
| | | **35** | **50** | **65** | **90** | |
| **21** | | | | | | |
| | Egg deformation (μm) | 25.4 | 25.2 | 23.1 | 24.3 | 24.5 |
| | Shell weight *per* unit area (mg/cm$^2$) | 79.6 | 79.8 | 83.7 | 81.9 | 81.3 |
| | Egg weight loss ( %) | 10.9 | 10.4 | 10.6 | 10.8 | 10.7 |
| **26** | | | | | | |
| | Egg deformation (μm) | 23.9 | 26.5 | 25.1 | 25.4 | 25.2 |
| | Shell weight *per* unit area (mg/cm$^2$) | 81.4 | 79.7 | 79.8 | 79.4 | 80.1 |
| | Egg weight loss ( %) | 10.8 | 10.6 | 11.5 | 11.2 | 11.0 |
| **29** | | | | | | |
| | Egg deformation (μm) | 27.5 | 25.3 | 25.6 | 24.3 | 25.7 |
| | Shell weight *per* unit area (mg/cm$^2$) | 78.1 | 79.4 | 80.0 | 80.5 | 79.5 |
| | Egg weight loss ( %) | 10.8 | 11.2 | 10.7 | 10.9 | 10.9 |
| **32** | | | | | | |
| | Egg deformation (μm) | 26.6 | 27.6 | 25.9 | 27.1 | 26.8 |
| | Shell weight *per* unit area (mg/cm$^2$) | 76.6 | 77.2 | 77.9 | 78.6 | 77.6 |
| | Egg weight loss ( %) | 10.9 | 11.2 | 11.1 | 11.2 | 11.1 |
| | Mean lysine concentration effects | | | | | |
| | Egg deformation (μm) | 25.9 | 26.2 | 24.9 | 25.3 | |
| | Shell weight *per* unit area (mg/cm$^2$) | 78.9 | 79.0 | 80.3 | 80.1 | |
| | Egg weight loss ( %) | 10.9 | 10.9 | 11.0 | 11.0 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=4) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=4) | |
| | P | SEM | P | SEM | P | SEM |
| Egg deformation (μm) | Linear (P=0.001) | 0.22 | P>0.10 | 0.47 | Linear (P=0.043) | 0.84 |
| Shell weight *per* unit area (mg/cm$^2$) | Linear (P=0.026) | 0.88 | P>0.10 | 0.67 | P>0.10 | 1.45 |
| Egg weight loss ( %) | P>0.10 | 0.20 | P>0.10 | 0.16 | P>0.10 | 0.34 |

**Table 3.6** Effects of four dietary lysine levels on eggs fertility, hatchability, and chicks weight (day one and 0-7 d of age), of broiler breeders (29- 50 weeks of age) kept at 21, 26, 29 or 32 °C (Experiment 1)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | Mean temperature effects |
|---|---|---|---|---|---|---|
| | | 35 | 50 | 65 | 90 | |
| 21 | | | | | | |
| | Fertility ( % ) | 77.0 | 74.9 | 86.4 | 75.3 | 78.4 |
| | Hatchability ( % ) | 82.7 | 79.8 | 82.7 | 82.7 | 82.0 |
| | Chick weight (female g –day one) | 46.4 | 47.3 | 46.1 | 45.7 | 46.4 |
| | Chick weight (male g –day one) | 45.6 | 45.5 | 46.1 | 45.4 | 45.6 |
| | 7 days chick weight gain (female g/d) | 15.7 | 17.6 | 15.9 | 15.5 | 16.2 |
| | 7 days chick weight gain (male g / d ) | 15.8 | 16.4 | 17.9 | 15.6 | 16.4 |
| 26 | | | | | | |
| | Fertility ( % ) | 77.5 | 86.4 | 79.0 | 79.0 | 79.2 |
| | Hatchability ( % ) | 65.4 | 82.4 | 73.5 | 86.4 | 76.9 |
| | Chick weight (female g –day one) | 44.5 | 45.2 | 46.1 | 45.5 | 45.3 |
| | Chick weight (male g –day one) | 44.6 | 45.8 | 45.6 | 46.4 | 45.8 |
| | 7 days chick weight gain (female g/d) | 16.7 | 16.5 | 15.7 | 15.5 | 16.1 |
| | 7 days chick weight gain (male g / d ) | 16.8 | 14.3 | 16.9 | 15.8 | 15.9 |
| 29 | | | | | | |
| | Fertility ( % ) | 72.4 | 71.6 | 65.7 | 65.7 | 71.4 |
| | Hatchability ( % ) | 71.8 | 81.6 | 82.4 | 72.4 | 77.1 |
| | Chick weight (female g –day one) | 30.8 | 44.0 | 42.7 | 45.6 | 40.8 |
| | Chick weight (male g –day one) | 44.2 | 46.1 | 43.5 | 46.4 | 45.1 |
| | 7 days chick weight gain (female g/d) | 16.5 | 16.1 | 16.0 | 15.9 | 16.1 |
| | 7 days chick weight gain (male g / d ) | 16.6 | 16.4 | 15.6 | 16.2 | 16.2 |
| 32 | | | | | | |
| | Fertility ( % ) | 79.0 | 69.4 | 82.7 | 74.8 | 76.5 |
| | Hatchability ( % ) | 82.1 | 82.7 | 78.4 | 86.4 | 82.4 |
| | Chick weight (female g –day one) | 44.1 | 44.0 | 44.6 | 46.8 | 44.9 |
| | Chick weight (male g –day one) | 44.8 | 45.0 | 43.4 | 46.4 | 44.9 |
| | 7 days chick weight gain (female g/d) | 16.0 | 15.7 | 15.9 | 15.9 | 16.0 |
| | 7 days chick weight gain (male g / d ) | 15.3 | 17.2 | 15.2 | 15.5 | 15.8 |
| | Mean lysine concentration effects | | | | | |
| | Fertility ( % ) | 76.5 | 75.6 | 78.5 | 75.0 | |
| | Hatchability ( % ) | 75.5 | 81.6 | 79.3 | 82.0 | |
| | Chick weight (female g –day one) | 46.4 | 45.3 | 40.8 | 44.9 | |
| | Chick weight (male g –day one) | 44.8 | 45.6 | 44.6 | 46.1 | |
| | 7 days chick weight gain (female g/d) | 16.2 | 16.5 | 15.9 | 15.8 | |
| | 7 days chick weight gain (male g / d ) | 16.1 | 16.0 | 16.4 | 15.8 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=4) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=4) | |
| | P | SEM | P | SEM | P | SEM |
| Fertility ( % ) | P>0.10 | 3.07 | P>0.10 | 3.27 | P>0.10 | 6.45 |
| Hatchability ( % ) | P>0.10 | 2.77 | P>0.10 | 2.41 | P>0.10 | 5.01 |
| Chick weight (female g –day one) | P>0.10 | 1.41 | Linear (P=0.049) | 1.37 | P>0.10 | 2.76 |
| Chick weight (male g –day one) | P>0.10 | 0.34 | P>0.10 | 0.59 | P>0.10 | 1.08 |
| 7 days chick weight gain (female g/d) | P>0.10 | 0.31 | P>0.10 | 0.33 | P>0.10 | 0.65 |
| 7 days chick weight gain (male g / d ) | P>0.10 | 0.60 | P>0.10 | 0.37 | Quadratic.Quadratic (P=0.059) | 0.87 |

### 3.1.4 Discussion

Temperature was shown to be an important environmental variable that affected the egg production characteristics of the breeder hens. The experiment has shown that the responses of the breeder hens to different ambient temperatures in egg production variables and shell strength characteristics were similar with that of egg laying strains. *Figures 3.3, 3.4* compares the four treatment means for egg production and eggshell strength variables that were obtained in this experiment with the response curve obtained from the analysis of the published literature (Section 2.2). The treatment differences due to temperature for egg numbers and egg mass output were similar to those of the published literature. Although mean egg weights decreased ($P<0.05$) with increasing temperature, the rate of decrease was less than expected. The relatively small decrease in egg weights with increasing temperature would be of benefit to hatching egg producers in hot climates who would find that, although the total numbers of hatching eggs from their flocks are decreased, there would not be an economically important increase in the proportion of these eggs that are too small to set.

There is published evidence that high environmental temperatures influence the fertility and hatchability of eggs in broiler breeders (Heywang, 1944). However, in the present study, there was no evidence that ambient temperature affected these reproductive variables.

**Figure 3.3** A comparison of the four temperature treatment means from the present experiment (expressed as a % response relative to 21°C) for egg production with the published data describing the responses of laying hens (Chapter 1)

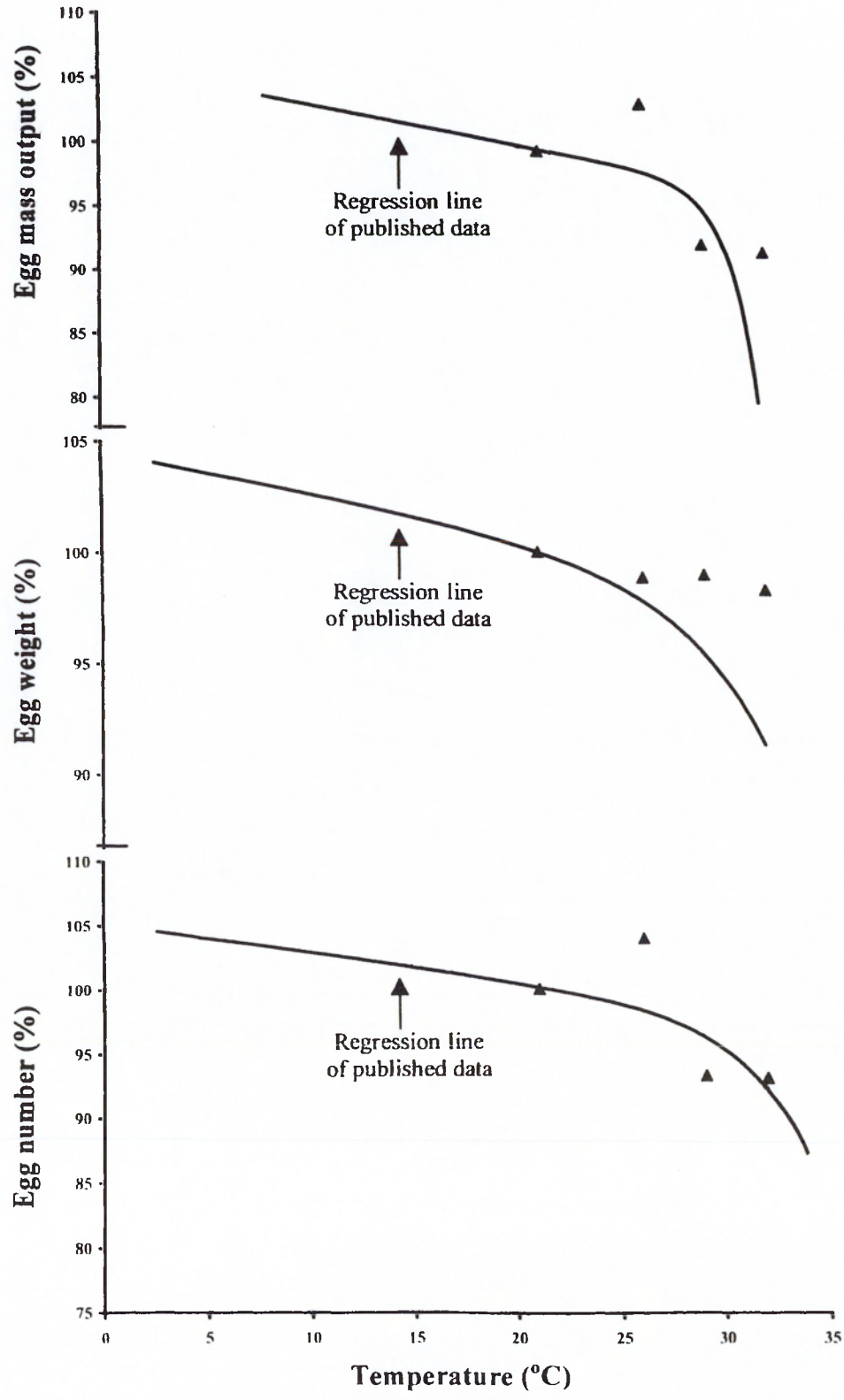
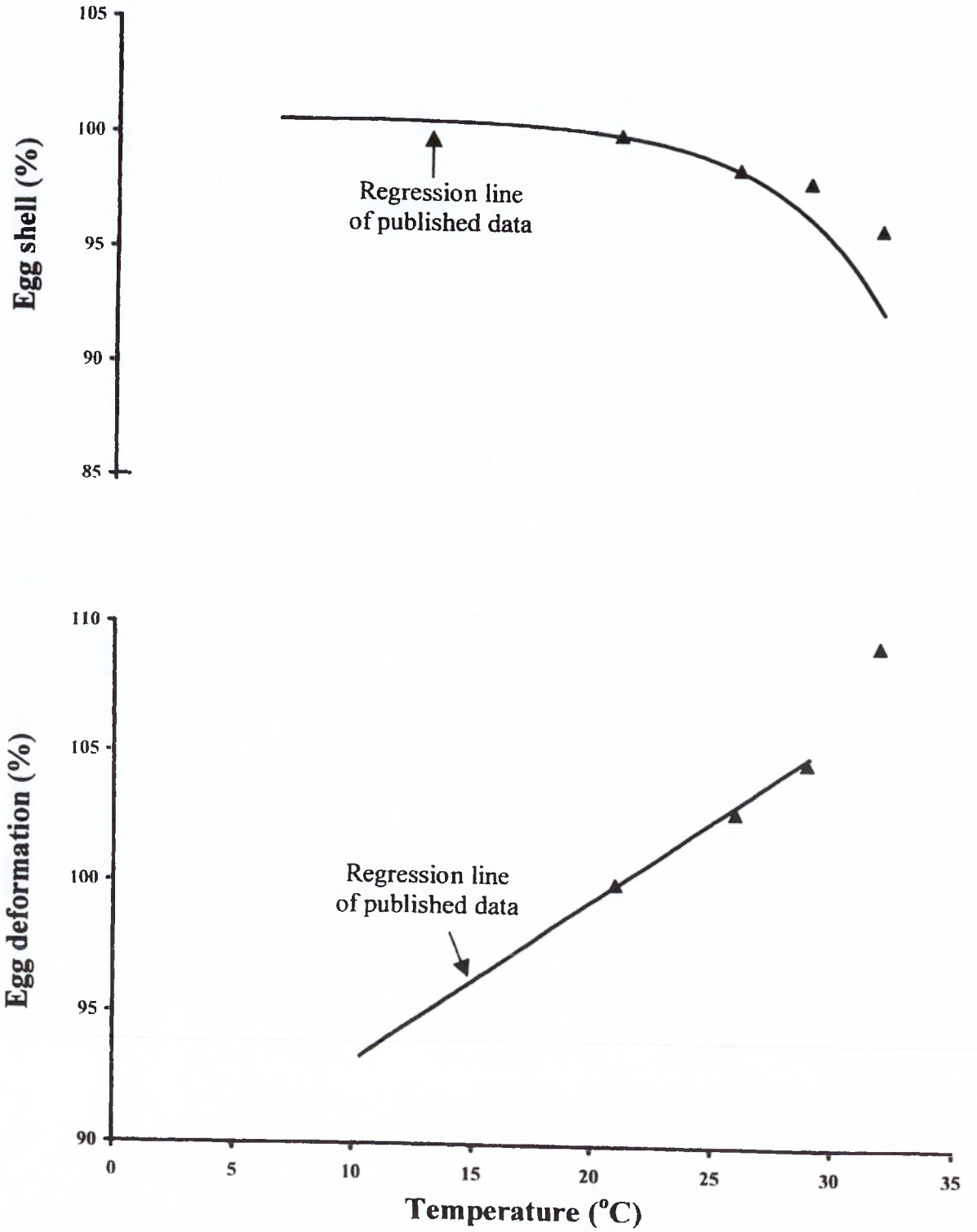


**Figure 3.4** A comparison of the four temperature treatment means from the present experiment (expressed as a % response relative to 21°C) for egg shell strength with the published data describing the responses of laying hens (Chapter 1)



This inconsistency with the literature may have been due to a number of factors;

- Short-term storage of fertile eggs at high temperatures can reduce their hatchability (Mayes and Takeballi, 1984; Meijerhof, 1992). However, the egg collection protocol used in the present experiment ensured that all eggs were collected within two hours of being laid and were immediately cooled.
- The frequency of mating is reduced in high temperatures (Huston and Carmon, 1958; Boone and Huston, 1963). A high male:female ratio was used in this present experiment and group sizes were low, thus the effect of temperature on this reproductive behaviour may have been less important.
- The fertilizing capacity of males declines at high temperatures (Parker and McSpadden, 1943; Huston and Wheeler, 1949). However, this has only been demonstrated above 35°C (Vo *et al.*, 1980).

It may have been that the temperatures used in the present experiment were not high enough to have a direct effect on male fertility. The egg weight differences due to temperature did not appear to be large enough to have any effect on chick hatching weight or on chick viability (Watt *et al.*, 1985). The eggs laid by birds kept at high temperatures had a lower proportion of egg shell and the high deformation values indicated a lower shell thickness. Shell thickness is a factor that determines the conductance of egg shells (Ross Breeder Manual, 1998). A sufficiently high total number of pores in relation to shell thickness is required for normal development and hatching (Rahn and Ar, 1980). All the eggs from the present experiment were set in the same incubator and so there was a possibility that different weight losses may have accrued between the treatment groups. However, no treatment differences in hatchability or weight loss occurred ($P>0.05$). This indicates that the differences in egg

shells between the temperature treatments were not big enough to affect water vapour conductance or gas exchange.

There were no significant differences ($P>0.05$) in any experimental variables with different dietary lysine concentrations, although there tended ($P=0.085$) to be a curvilinear response in egg mass output with the maximum response indicated to be at approximately 50g/kg CP. The magnitude of the numerical differences ($P=0.085$) between the four dietary lysine concentrations was similar to that expected from the review of the literature (Section 2.1.3). The shape of the response curve in the present experiment was, however, different in that there was some evidence that high concentrations of dietary lysine gave a decrease in egg mass output. High dietary lysine concentrations did not cause a decrease in egg mass outputs in egg-laying strains of hens (*Figure 2.9*) although broiler chicken growth does decrease at high lysine concentrations (Abebe and Morris, 1990a). The 35g/kg lysine diet provided a deficiency of lysine as defined by the NRC (1994) recommendations. A greater difference between the 35g/kg and 50g/kg lysine concentrations would therefore have been expected.

The lysine concentrations in the four experimental feeds were checked and they contained the correct concentrations of lysine. Similarly, the concentrations of all other amino acids were present in the correct amounts. The biological availability of the amino acids to the birds were not determined and there could have been a problem with the availability of one or more amino acids using this particular diet formulation. It was concluded that the effects of dietary lysine concentration should be re-examined using a different lysine-deficient diet formulation.

In conclusion, this experiment has given clear effects of different ambient temperatures on the hatching egg production of broiler breeders. These responses were

similar to that previously demonstrated for egg-laying strains. Although increasing ambient temperature decreases ($P<0.049$) the proportion of shell in the hatching eggs, there were no effects ($P>0.05$) on hatchability or chick quality. The egg laying responses of the broiler breeders to dietary lysine were somewhat unexpected, and a re-examination of this effect with a different diet formulation was considered necessarily.

## 3.2 Experiment 2

*The interaction of dietary lysine and temperature on the reproductive performance of broiler breeders.*

### 3.2.1. Introduction

The objectives of the previous experiment were to quantify the response of broiler breeders to dietary lysine when kept at different ambient temperature. Although there were clear temperature treatment effects, the experiment gave an unexpected response curve (P=0.085) of the change in egg mass output to dietary lysine supply. A second experiment was therefore designed to further examine these main objectives using a different diet formulation to achieve a lysine deficient diet. The temperature effects were clear from experiment 1, so only two temperatures were compared in order to examine if there were temperature x lysine concentration interactions. This allowed for a greater replication of the individual temperature and lysine concentration treatments.

The specific objectives of the present experiment were first to examine and explain the effects of two different ambient temperatures 21 °C and 32°C on the response of the egg laying performance, egg composition and hatchability of the broiler breeders. Second, to examine the response of four dietary lysine concentrations (40, 52.5, 65 and 90 g/kg crude protein). Third, to examine whether there were temperature x lysine concentration interactions in the responses of the broiler breeders.

## 3.2.2. Materials and Methods

### 3.2.2.1 Experimental treatments

A single lysine deficient diet that contained 158 g/kg crude protein (*Table 3.7*) was formulated based on wheat . The concentration of all other amino acids and nutrients met or exceeded the requirements of the broiler breeder hens according to National Research Council (1994) and Ross Breeders Limited (1998). Amino acid analysis of the prepared diet was undertaken prior to feeding to confirm the calculated composition (Section 3.1.2.2). Three further dietary levels of lysine concentration were achieved by adding lysine-HCl to the deficient diet in replacement for maize starch, to give four dietary concentrations of lysine (40, 52.5, 65 and 90 g/kg crude protein). All experimental diets were stored under the same environmental conditions.

Two hundred and twenty-four 34-week old hens (308 Broiler Breeder, Ross Breeders Ltd., Newbridge, Midlothian, Scotland) were randomly placed in one of 16 identical floor pens within four environmentally controlled rooms (*Figure 3.1*) at Harper Adams University College, Shropshire. Two male birds were each also randomly placed in each pen. The pen descriptions and bird management details were as described in section 3.1.2.3. A daily feed restriction programme was followed, 150 g/bird d then a reduction of 2.5 g/ bird / 28 d period (Ross Breeders Limited 1998). After the completion of each period in the experiment, the diet was changed and a new diet introduced for the next period in the experiment (*Figure 3.5*).

There were two different temperatures used in the experiment (21 °C and 32°C). Each room was randomly allocated a temperature at the beginning of the experiment

**Table 3.7** Composition of the broiler breeder lysine deficient diet fed in experiments 2† and 3‡ ( 34-51 and 28-40 weeks of age respectivily).

| Ingredients | Lysine deficient diet (kg)†‡ |
|---|---|
| Wheat | 680.0 |
| Maize gluten meal (600g/kg CP) | 68.0 |
| Sunflower meal (330 g/kg protein) | 83.0 |
| Full fat soya | 20.0 |
| Maize starch | 18.9 |
| Soya oil | 10.0 |
| Limestone | 80.0 |
| Dicalcium phosphate | 10.0 |
| Sodium bicarbonate | 2.0 |
| Salt | 2.0 |
| DL-methionine | 2.8 |
| L-threonine | 1.7 |
| L-tryptophan | 1.6 |
| Vitamin-Mineral Supplement[$] | 20.0 |
| Total | 1000.0 |
| **Nutrient Composition:** | |
| Determined chemical analysis | |
| Crude Protein ( CP) (g/kg) | 158.0 |
| Lysine (g\kg CP) | 40.0 |
| Methionine (g\kg CP) | 32.0 |
| Tryptophan (g\kg CP) | 17.2 |
| Methionine+ Cystine (g\kg CP) | 49.5 |
| Threonine (g\kg CP) | 41.5 |
| Isoleucine (g\kg CP) | 40.0 |
| Arginine (g\kg CP) | 65.5 |
| Valine (g\kg CP) | 48.0 |
| Histidine (g\kg CP) | 23.0 |
| Calculated analysis | |
| Metabolisable Energy (MJ/kg) | 11.6 |
| Calcium (g\kg) | 37.5 |
| Phosphorus (g\kg) | 5.2 |
| Sodium (g\kg) | 1.8 |
| Potassium (g\kg) | 4.3 |
| Linoleic acid (g\kg) | 14.0 |
| Choline (mg\kg ) | 1100 |

† The other 3 experimental diets included additional (L-lysine HCl) in replacement for maize starch.

‡ The other 5 experimental diets included additional (L-lysine HCl) in replacement for maize starch.

[$]Supplied per kg of diet: *trans*-retinol(A), 4.8 mg; cholecalciferol(D3), 125µg; α-tocopherol acetate(E), 183.8 mg; thiamine(B1), 3 mg; riboflavin(B2), 10 mg; pyridoxine(B6), 5 mg; vitamin B12, 12 mg; nicotinic acid, 60 mg; pantothenic acid, 15 mg; folic acid, 2.5 mg; biotin, 205 µg; choline chloride, 500 mg; Fe, 20 mg; Co,1 mg; Mn, 100 mg; Cu, 10 mg; Zn, 80 mg; I, 2 mg; Se, 0.2 mg; Mo, 0.5 mg; Ca, 206g/kg; P, 100.5g/kg; Na, 50g/kg; Ash , 882g/kg.

**Figure 3.5** Pen randomizations in experiment (2). Four diets (lysine concentrations: 40, 52.5, 65 and 90 g\ kg CP) and two temperatures: 21°C and 32°C were compared.

Period #1

| | | | |
|---|---|---|---|
| 52.5 | 90 | 40 | 65 |
| 65 | 52.5 | 90 | 40 |
| 40 | 65 | 52.5 | 90 |
| 90 | 40 | 65 | 52.5 |

Period #2

| | | | | |
|---|---|---|---|---|
| 32°C Room 1 | 65 | 40 | 52.5 | 90 |
| 21°C Room 2 | 40 | 90 | 65 | 52.5 |
| 32°C Room 3 | 90 | 52.5 | 40 | 65 |
| 21°C Room 4 | 52.5 | 65 | 90 | 40 |

Period #3

| | | | |
|---|---|---|---|
| 40 | 65 | 90 | 52.5 |
| 90 | 40 | 52.5 | 65 |
| 52.5 | 90 | 65 | 40 |
| 65 | 52.5 | 40 | 90 |

Period #4

| | | | | |
|---|---|---|---|---|
| 32°C Room 1 | 90 | 52.5 | 65 | 40 |
| 21°C Room 2 | 52.5 | 65 | 40 | 90 |
| 32°C Room 3 | 65 | 40 | 90 | 52.5 |
| 21°C Room 4 | 40 | 90 | 52.5 | 65 |

(*Figure 3.5*) and kept at this temperature until the completion of the whole experiment. Relative humidity was kept in the range of 70-75%.

### 3.2.2.2 Experimental measurements

The methods of data collection were as described in experiment 1 (Sections 3.1.2.5 and 3.1.2.6). However after incubation, hatchlings were separated into males and females and weighed but not grown for a further seven days.

### 3.2.2.3 Experimental Design

#### Statistical analysis

A split-plot design was used in which four main plots (rooms) were kept at one of two constant temperatures throughout the whole experimental period. Within each main plot, four sub-plots (pens) were fed each of the four different diets each for a 28 day period. The diet received by each pen in each of four 28 day periods was arranged in a latin-square design. Data were compared by analysis of variance of the measured and calculated variables using a split-plot design that examined the temperature, dietary lysine concentration and the diet x temperature interactions (*GENSTAT* statistical package, Lawes Agricultural Trust, 1998). The treatment sums of squares of the dietary lysine concentrations were partioned into their linear and non-linear (quadratic) effects. Egg fertility data were not normally distributed so an arcsine transformation was applied prior to statistical comparison of the treatment effects.

### 3.2.3 Results

Throughout the experiment the allocated amounts of feed were always eaten. The mean mortality during the experiment was 1.8% (4 birds in total), which was not associated with particular treatments. The overall mean egg output of the flock was low relative to commercial standards but this was probably a reflection of the experimental treatments applied.

Increasing temperature decreased the shell weight *per* unit area (*Table 3.11*, P=0.048). Additionally, egg fertility decreased (*Table 3.12*, P=0.007) with increasing temperature. The numerical differences (P>0.05) between temperatures in any body weight change, egg output, egg composition, shell strength, egg hatchability or chick viability variables were not demonstrated to be statistically significant (*Tables 3.8, 3.9, 3.10, 3.11, 3.12*).

Female body weight gain was increased linearly (P<0.001) by increasing lysine concentration, but male body weight gain was not affected (P>0.05) (*Table 3.8*). Also, increasing lysine concentration gave a linear increase in egg weight (*Table 3.9*, P= 0.017). There were no significant differences (P>0.05) between different lysine concentration levels in any other variables of egg output, egg composition, shell strength, egg fertility and hatchability or chick viability (*Tables 3.9, 3.10, 3.11, 3.12*). Similarly, there were no consistent (P>0.05) temperature x lysine concentration interactions.

**Table 3.8** Effects of four dietary lysine levels on body weight change in both female and male broiler breeders (33- 49 weeks of age) kept at 21 or 32 °C (Experiment 2)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | Mean temperature effects |
|---|---|---|---|---|---|---|
| | | **40** | **52.5** | **65** | **90** | |
| **21** | | | | | | |
| | Female weight gain (g / d) | 0.77 | 2.39 | 4.86 | 5.02 | 3.26 |
| | Male weight gain (g / d) | 8.07 | 10.21 | 6.00 | 4.68 | 7.24 |
| **32** | | | | | | |
| | Female weight gain (g / d) | 2.92 | 3.66 | 5.15 | 5.34 | 4.27 |
| | Male weight gain (g / d) | 11.99 | 13.17 | 10.90 | 9.34 | 11.35 |
| | Mean lysine concentration effects | | | | | |
| | Female weight gain (g / d) | 1.84 | 3.03 | 5.00 | 5.18 | |
| | Male weight gain (g / d) | 10.03 | 11.69 | 8.45 | 7.01 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=2) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=8) | |
| | P | SEM | P | SEM | P | SEM |
| Female weight gain (g / d) | P=0.033 | 0.132 | Linear (P<0.001) Quadratic (P=0.085) | 0.610 | P>0.10 | 0.759 |
| Male weight gain (g / d) | P=0.52 | 0.690 | Linear (P=0.084) | 1.618 | P>0.10 | 2.098 |

**Table 3.9** Effects of four dietary lysine levels on egg laying performance; total egg mass output, egg weight and egg number of broiler breeders (33- 49 weeks of age) kept at 21 or 32 °C (Experiment 2).

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | Mean temperature effects |
|---|---|---|---|---|---|---|
| | | **40** | **52.5** | **65** | **90** | |
| **21** | | | | | | |
| | Egg mass (g / hen / d) | 31.84 | 35.40 | 30.84 | 33.30 | 32.85 |
| | Egg weight (g / d) | 64.06 | 64.72 | 65.07 | 65.29 | 64.79 |
| | Egg number (no./ hen / d) | 0.50 | 0.55 | 0.47 | 0.51 | 0.51 |
| **32** | | | | | | |
| | Egg mass (g / hen / d) | 30.03 | 31.36 | 28.25 | 32.40 | 30.48 |
| | Egg weight (g / d) | 61.91 | 62.85 | 63.19 | 66.12 | 63.51 |
| | Egg number (no./ hen / d) | 0.49 | 0.50 | 0.45 | 0.49 | 0.48 |
| | Mean lysine concentration effects | | | | | |
| | Egg mass (g / hen / d) | 30.92 | 33.36 | 29.50 | 32.85 | |
| | Egg weight (g / d) | 62.98 | 63.79 | 64.13 | 65.70 | |
| | Egg number (no./ hen / d) | 0.49 | 0.52 | 0.46 | 0.50 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=2) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=8) | |
| | P | SEM | P | SEM | P | SEM |
| Egg mass (g / hen / d) | P>0.10 | 1.985 | P>0.10 | 1.411 | P>0.10 | 2.632 |
| Egg weight (g / d) | P>0.10 | 0.571 | Linear (P=0.017) | 0.797 | P>0.10 | 1.131 |
| Egg number (no./ hen / d) | P>0.10 | 0.027 | P>0.10 | 0.023 | P>0.10 | 0.038 |

**Table 3.10** Effects of four dietary lysine levels on egg composition (expressed as a proportion of the egg weight); egg shell, egg yolk and egg albumen of broiler breeders (33- 49 weeks of age) kept at 21 or 32 °C (Experiment 2)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | Mean temperature effects |
|---|---|---|---|---|---|---|
| | | **40** | **52.5** | **65** | **90** | |
| **21** | | | | | | |
| | Egg shell | 0.093 | 0.092 | 0.093 | 0.092 | 0.092 |
| | Egg yolk | 0.300 | 0.300 | 0.303 | 0.303 | 0.301 |
| | Egg albumen | 0.607 | 0.608 | 0.605 | 0.606 | 0.606 |
| **32** | | | | | | |
| | Egg shell | 0.090 | 0.088 | 0.091 | 0.089 | 0.090 |
| | Egg yolk | 0.299 | 0.300 | 0.303 | 0.304 | 0.302 |
| | Egg albumen | 0.611 | 0.612 | 0.606 | 0.607 | 0.609 |
| | Mean lysine concentration effects | | | | | |
| | Egg shell | 0.091 | 0.090 | 0.092 | 0.090 | |
| | Egg yolk | 0.300 | 0.300 | 0.303 | 0.304 | |
| | Egg albumen | 0.609 | 0.610 | 0.605 | 0.606 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=2) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=8) | |
| | P | SEM | P | SEM | P | SEM |
| Egg shell | P>0.10 | 0.0006 | P>0.10 | 0.0006 | P>0.10 | 0.0009 |
| Egg yolk | P>0.10 | 0.0026 | P>0.10 | 0.0024 | P>0.10 | 0.0039 |
| Egg albumen | P>0.10 | 0.0029 | P>0.10 | 0.0023 | P>0.10 | 0.0041 |

**Table 3.11** Effects of four dietary lysine levels on egg shell strength; egg deformation, shell weight per unit area and total egg weight loss (expressed as percentage of egg weight) during incubation of broiler breeders (33- 49 weeks of age) kept at 21 or 32 °C (Experiment 2)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | Mean temperature effects |
|---|---|---|---|---|---|---|
| | | **40** | **52.5** | **65** | **90** | |
| **21** | | | | | | |
| | Egg deformation (μm) | 24.3 | 23.7 | 24.0 | 24.1 | 24.0 |
| | Shell weight *per* unit area (mg/cm$^2$) | 81.3 | 81.5 | 81.9 | 80.7 | 81.4 |
| | Egg weight loss ( %) | 11.7 | 12.3 | 11.5 | 11.7 | 11.8 |
| **32** | | | | | | |
| | Egg deformation (μm) | 26.5 | 27.1 | 26.2 | 26.6 | 26.6 |
| | Shell weight *per* unit area (mg/cm$^2$) | 78.2 | 77.1 | 79.9 | 77.9 | 78.3 |
| | Egg weight loss ( %) | 12.1 | 12.0 | 11.6 | 11.5 | 11.8 |
| | Mean lysine concentration effects | | | | | |
| | Egg deformation (μm) | 25.4 | 25.4 | 25.1 | 25.3 | |
| | Shell weight *per* unit area (mg/cm$^2$) | 78.2 | 77.1 | 79.9 | 77.9 | |
| | Egg weight loss ( %) | 11.9 | 12.2 | 11.6 | 11.6 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=2) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=8) | |
| | P | SEM | P | SEM | P | SEM |
| Egg deformation (μm) | P>0.10 | 0.63 | P>0.10 | 0.36 | P>0.10 | 0.77 |
| Shell weight *per* unit area (mg/cm$^2$) | P=0.048 | 0.50 | P>0.10 | 0.56 | P>0.10 | 0.84 |
| Egg weight loss (%) | P>0.10 | 0.40 | P>0.10 | 0.26 | P>0.10 | 0.51 |

**Table 3.12** Effects of four dietary lysine levels on egg fertility, hatchability, and chicks weights ( day one and 0-7 d of age), from broiler breeders (33- 49 weeks of age) kept at 21 or 32 °C (Experiment 2)

| Temperatures ( °C ) | Variables | Lysine concentrations ( g/kg Crude Protein ) | | | | Mean temperature effects |
|---|---|---|---|---|---|---|
| | | **40** | **52.5** | **65** | **90** | |
| **21** | | | | | | |
| | Fertility (%) | 100.0 | 98.7 | 98.7 | 98.7 | 99.0 |
| | Hatchability ( % ) | 91.3 | 95.0 | 95.0 | 93.2 | 93.6 |
| | Hatching chick weight- female (g ) | 45.5 | 45.9 | 45.3 | 46.3 | 45.7 |
| | Hatching chick weight -male (g) | 46.5 | 46.0 | 45.7 | 47.2 | 46.4 |
| **32** | | | | | | |
| | Fertility (%) | 86.1 | 86.2 | 95.0 | 92.1 | 89.9 |
| | Hatchability ( % ) | 95.6 | 98.6 | 94.7 | 96.0 | 96.3 |
| | Hatching chick weight- female (g ) | 42.6 | 44.9 | 44.3 | 45.0 | 44.2 |
| | Hatching chick weight -male (g) | 44.5 | 44.5 | 44.9 | 44.0 | 44.5 |
| Mean lysine concentration effects | | | | | | |
| Fertility (%) | | 93.1 | 92.5 | 96.9 | 95.4 | |
| Hatchability ( % ) | | 93.5 | 96.8 | 94.9 | 94.6 | |
| Hatching chick weight- female (g ) | | 44.0 | 45.4 | 44.8 | 45.7 | |
| Hatching chick weight -male (g) | | 45.5 | 45.2 | 45.3 | 45.6 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ( *n*=2 ) | | Lysine ( *n*=16) | | Temperature x lysine interaction ( *n*=8 ) | |
| | P | SEM | P | SEM | P | SEM |
| Fertility (%) | P=0.007 | 0.01† | P>0.10 | 0.06† | P>0.10 | 0.07† |
| Hatchability ( % ) | P>0.10 | 1.56 | P>0.10 | 1.72 | P>0.10 | 2.62 |
| Hatching chick weight- female (g ) | P>0.10 | 0.88 | P>0.10 | 0.66 | P>0.10 | 1.19 |
| Hatching chick weight –male (g) | P>0.10 | 0.34 | P>0.10 | 0.57 | P>0.10 | 0.78 |

†These data were not normally distributed. The quoted SEM is from the analysis of variance of the transformation using arcsine.

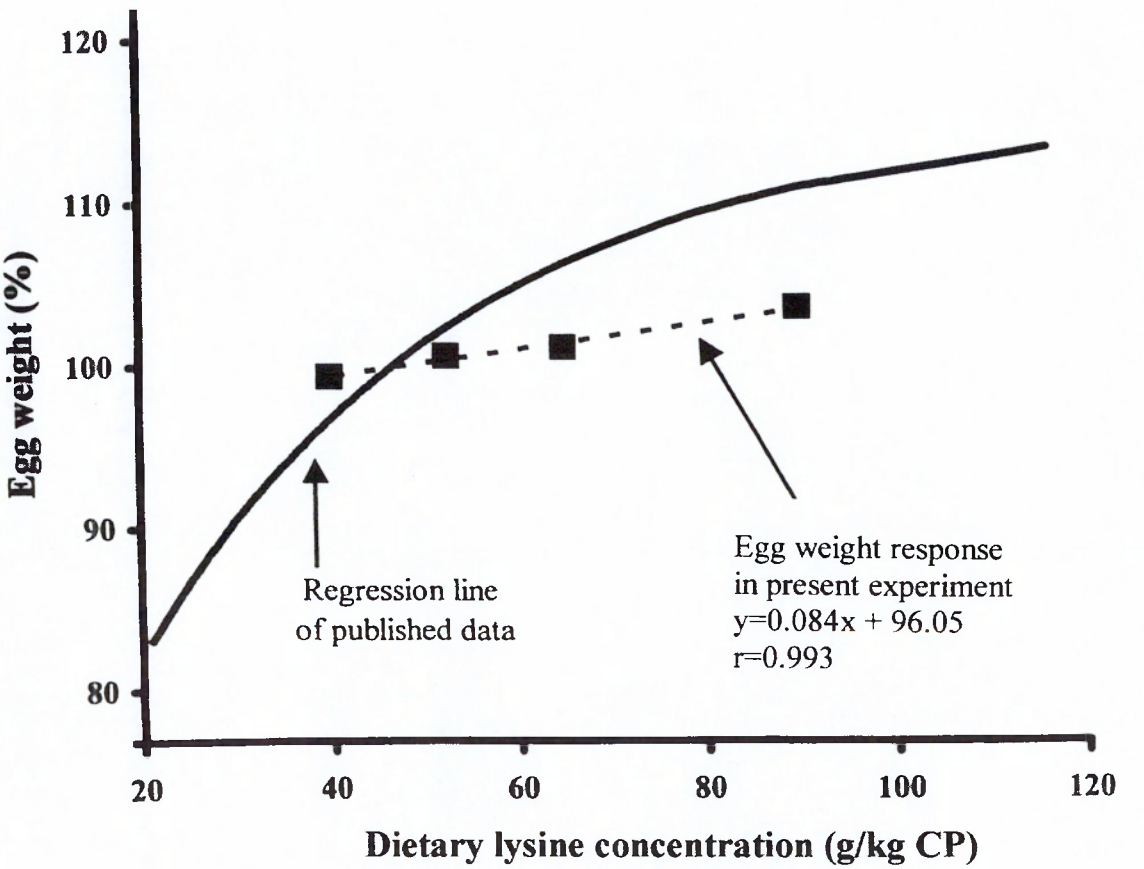
### 3.2.4 Discussion

The objectives of this experiment were to examine the effects on reproductive characteristics of four diets that differed in their lysine concentrations (40, 52.5, 65 and 90 g/kg crude protein) fed to broiler breeders when kept at two ambient temperatures (21 and 32°C).

The experiment demonstrated that increasing dietary lysine concentrations increased ($P<0.05$) the mean egg weights from the broiler breeders although there were no significant effects ($P>0.05$) on egg mass outputs. The comparison with published evidence in *Figure 3.6* indicates that an effect of dietary lysine on egg weights was expected, but the magnitude of the response was much lower than expected. The amino acid levels in the feeds were monitored during the feed formulation and throughout the experimental period. It does therefore appear that lysine was the limiting amino acid in the experimental diets and that there was only a small egg weight response to differences in lysine supply. Experiment 1 used a different basal diet formulation and there was a numerical increase in egg weight with increasing dietary lysine concentrations, although these differences were not statistically significant.

The difference between the dietary lysine responses of the broiler breeders in the present experiment and the published responses of egg laying strain hens agrees with the data of Karunajeewa (1974) and Bowmaker and Gous (1991).

**Figure 3.6** A comparison of the four temperature treatment means from the present experiment (expressed as a % response relative to 46g/kg CP) for egg weight with the published data describing the responses of laying hens (Chapter 2).




The feed intakes of the broiler breeders were restricted in the present experiment and all pens ate their feed allocations. It may be that the larger egg weight responses of egg laying strains were due to differences in feed intakes with increasing lysine concentrations. The egg mass outputs of the broiler breeders were also much lower as a proportion of daily feed intake and body mass compared to egg layer strains.

There was a tendency (P=0.085) for a quadratic response in egg mass output to increasing dietary lysine concentration in experiment 1 but no evidence of this effect in the present experiment. The relatively small dietary lysine effects on the egg production characteristics of the breeder hens were not expected. The different dietary lysine concentrations were given to the pens of breeder hens for 28 day periods. It may be that a longer feeding period may have given a larger effect on the egg weight and egg mass output of the breeders. There was a large increase ($P<0.05$) in body weight gain in the present experiment with increasing dietary lysine concentrations indicating a better feed utilization. The body energy reserves of the birds may have become more depleted with a longer feeding period.

Dietary lysine concentration had no effect on egg composition, shell strength or any incubation variables. This agrees with the results obtained in experiment 1 and the egg composition data of Chi and Speers (1976) and Prochaska *et al.* (1996) and the hatchability data of Ingram *et al.* (1950) and Wilson and Harms (1984).

The present experiment demonstrated differences between the two temperature treatment groups that were consistent with the previous experiment. The numerical differences in egg mass output and mean egg weight were not, however, demonstrated to be statistically significant. This experiment had only two replicate rooms for each temperature so it was unlikely that statistically significant differences would be demonstrated. Nevertheless, the high ambient temperature treatment increased ($P<0.05$) shell weight *per* unit area and decreased ($P<0.01$) hatching egg fertility. The effect on fertility was not observed in the first experiment although it would be expected that reduced bird activity in the high temperature would reduce the mating frequency and so have an effect on egg fertility.

There was no statistical evidence of a temperature x lysine concentration interac. any of the measured variables. However, there was a trend (P=0.085) for the differences in weight gain between the dietary lysine concentrations to be greater at the lower ambient temperature.

In conclusion, this experiment has confirmed the effect of different ambient temperatures on the hatching egg production of broiler breeders. Increasing dietary lysine increased egg weights and increased female weight gain. The responses were small relative to published responses of egg-laying strains.

## 3.3 Experiment 3

*Effect of six dietary lysine concentrations on the reproductive physiology of caged broiler breeders kept at two different temperatures.*

### 3.3.1 Introduction

Experiments 1 and 2 have given important quantitative information on the responses of broiler breeders to different temperatures and dietary lysine concentrations. Both experiments had protocols that used replicated small flocks of birds kept in floor pens. However, the experimentation with flocks of birds did not allow detailed physiological explanations of the effects of dietary lysine and temperature to be elucidated. There is a need to understand the physiological mechanisms that control the responses of broiler breeders to dietary lysine at different temperatures. Individual bird data could therefore enable the physiological mechanisms to be explained.

Experiments 1 and 2 were unable to detect large effects of dietary lysine concentrations on the egg production of the breeder hens. It is possible that the relatively short feeding periods (28 days) used in these experiments were not sufficient to enable egg production responses to manifest. There is a need to examine the dietary effects using a longer experimental feeding period.

A disadvantage of caging breeder hens is that it is not possible for mating activity to occur. Although artificial insemination was possible, it was decided

not to further examine differences in hatching egg fertility and hatchability because of the lack of treatment effects observed in experiment 1 and 2.

The objectives of this experiment were;

- To examine and explain the effects of two different ambient temperatures (21°C and 32°C), six dietary lysine concentrations (40, 50, 60, 70, 80 and 90 g/kg CP) and their interactions on the physiological changes in individually caged laying broiler breeders.
- To examine whether nutrient availability was affected by temperature, dietary lysine supply and their interactions.

## 3.3.2 Materials and methods

### 3.3.2.1 Experimental treatments

A single lysine deficient wheat-based diet that contained 158 g/kg crude protein (*Table 3.7*) was formulated. The concentration of all other amino acids and nutrients met or exceeded the requirements of the broiler breeder hens according to National Research Council (1994) and Ross Breeders Limited (1998). Amino acid analyses of the prepared diet were undertaken prior to feeding to confirm the calculated compositions (Section 3.1.2.2). Five further dietary levels of lysine concentration were achieved by adding lysine-HCl to the deficient diet in replacement for maize starch, to give six dietary concentrations of lysine (40, 50,

60, 70, 80 and 90 g/kg crude protein). All experimental diets were stored under the same environmental conditions.

Ninety-six 28-week old hens (308 Broiler Breeder, Ross Breeders Ltd.) were randomly placed in one of 12 identical cages within eight environmentally controlled rooms (*Figure 3.7*) at Harper Adams University College, Shropshire. A daily feed restriction programme was followed, 156 g/bird d then a reduction of 2.5 g/ bird / 28 day period following a recommended feeding schedule (Ross Breeders Limited 1998). There were two different temperatures used in the experiment (21 °C and 32°C) and each was applied in four replicate rooms. Each room was randomly allocated to a temperature at the beginning of the experiment and kept at this temperature until the completion of the experiment. Relative humidity was maintained in the range of 70-75%.

### 3.3.2.2 Experimental measurements

The data measurements of egg production, composition and shell strength (shell deformation and shell weight *per* unit area), were completed using methods as described in section 3.1.2.5.

#### 3.3.2.2.1 Blood collection and compositional measurements

**Sampling procedure**

96 birds were killed and 20 ml of whole blood was collected into a screw-capped 50 ml tube containing 2 ml of EDTA and rotated gently. The samples were placed in a shaking water bath for 5 minutes (75 strokes/ minutes), then the tubes were placed in a tray kept at 41°C until sampling had been completed.

**A. Packed cell volume**

PVC capillary tubes were filled up to 2mm (from the top of the tube) with plasma and placed in a micro centrifuge. The centrifuge was run at 2000 rpm for 4 minutes to separate red blood cell from plasma. A PCV auto-reader was used to calculate the percentage of red blood cells.

**B. Plasma amino acid determination**

Deproteinization of the plasma was carried out within 1 hour of blood collection. Each plasma sample was placed in a centrifuge and spun at 2000 rpm for 4 minutes. The supernatant (4.5ml) was then placed in an eppendorf tube with 50µl of 35% 5-sulphosalicylic acid and 'Vortex' mixed for 5 seconds, then centrifuged at 11200 rpm for 5 minutes. Finally, the supernatant was removed and placed into an eppendorf tube and stored at –80°C.

Samples were removed from the freezer and allowed to defrost at room temperature and centrifuged at 11200 rpm for two minutes to remove any further particulate matter. 300 mg of plasma was transferred by pipette into a clean hydrolysis tube. 400 µl of 1 % phenol in water and 1000 µl of 50 % HCl solution were added into the sample tubes. Each sample tube was evacuated by vacuum pump for 1 minute and flushed with nitrogen for 5 seconds. Tubes were placed into a pre-heated heating block, set at 110°C, and digested overnight (approximately 17 hours). Sample tubes were removed from the heating block and allowed to cool and 'Vortex' mixed for two seconds. Contents were transferred individually by a pasteur pipette into a 7 ml bijou tube. Tubes were rinsed with 3 x 1-ml portions of HPLC-grade water and the washings were transferred into the bijou tubes. The contents were mixed thoroughly by using a magnetic stirrer. The sample pH was adjusted between 6 and 8 with 40 % sodium hydroxide (approx. 15-20 drops) and checked using a pH meter.

Each tube content was transferred into a 10ml volumetric flask and made up to volume with HPLC-grade water. Finally, samples were filtered through a 0.2 micro syringe filter into 2ml auto sampler vials, crimped and stored at 4°C for the HPLC analysis.

**C. Chromatography**

The samples were analyzed using high performance liquid chromatography at Harper Adams University College laboratories (Newport, Shropshire, UK. TF10

8NB). An Agilent Technologies (HP) Model 1100 liquid chromatograph equipped with an autosampler and diode array ultraviolet detector at 338/10 nm was used in this analysis. Analysis of samples (from section B) was performed by using two mobile phases A and B on a 200 x 2.1mm (inner diameter) AminoQuant column at 40°C oven temperature. Mobile phase A consisted of 20 mMol of sodium acetate tri-hydrate and 0.018% of triethylamine adjusted to pH 7.2 with 1-2 acetic acid. Mobile phase B consisted of 20% of 100 mMol sodium acetate tri-hydrate adjusted to pH 7.2 with 1-2% acetic acid, 40% acetonitrile and 40% methanol. All reagents, including deionized water, were HPLC-grade.

#### 3.3.2.2.2 Carcass composition measurements

At the end of the feeding period, a single bird that had laid at least an egg during the past seven days was selected from each diet per room. The birds were weighed then killed by cervical dislocation. Ovaries were dissected from the body cavity and weighed and the oocyte number (matured yolks only) counted. The oviduct and abdominal fat (defined as the abdominal leaf fat and the fat surrounding the gizzard and lower intestine) was dissected out and weighed. The carcasses were scalded and plucked and, after chilling, were weighed, cut, and minced individually until homogeneous.

Homogeneous samples (100 g) were freeze-dried for 72 hours to determine water content. The dried material was analysed for nitrogen content by the Kjeldahl procedure using a nitrogen conversion factor of 6.25, for ash content by

combustion at 500°C and for fat by extraction with petroleum ether and 3 g of sand (AOAC 2000).

**3.3.2.2.3 Other measurements**

- For the last two days of the feeding period, in each room each for the replicate cages were of the hens fed the deficient lysine diet (40 g lysine/ kg crude protein) had trays inserted under the cage for two days and all excreta were collected. The excreta were oven-dried at 60°C for two days. Dry samples were ground and treated with 6M hydrochloric to determine amino acid composition (Section 3.1.2.2). A further representative sample of the oven-dried excreta was taken and its gross energy determined using an adiabatic bomb calorimeter (Parr Instrument Company, USA). The AME of the feed was calculated by deducting the amount of gross energy contained in the collected excreta output from the gross energy intakes of the hens over the two day period.
- Body temperature (rectal temperature) was measured in all birds at three time points during a single day by inserting a digital clinical thermometer in the rectum to a depth of 5 cm for 1 min at 0900, 1300 and 1600 h.
- In the last week of feeding period, feeding activity was determined by measuring the feed intakes of all birds in the experiment every 30 minutes during a single feeding day until a full daily allocation of feed was eaten. Feed intakes were determined by measuring the feed disappearance from the feed troughs at timed intervals.

### 3.3.2.2.4 Experimental design

**Statistical analysis**

A split-plot design was used in which four rooms (main plots), were kept at one of two constant temperatures. Within each room, 12 cages (sub-plots) were fed six different diets. Data were compared by analysis of variance of the measured and calculated variables, using a split-plot design that examined the effects of temperature of the rooms and the effect of diet and the diet x temperature interactions within the sub-plots (cages) (*GENSTAT* statistical package, Lawes Agricultural Trust, 1998).

### 3.3.3 Results

Throughout the experiment the allocated amounts of feed were always eaten. The mean mortality during the experiment was 8.3% (8 birds in total), which was not associated with particular treatments.

There was a significant difference ($P<0.001$) between different temperatures in body weight change (*Table 3.13*). Additionally, increasing temperature gave a decrease ($P<0.05$) in egg mass, egg weight and egg number (*Table 3.14*). Increasing temperature decreased the proportion of egg shell (*Table 3.15*, $P= 0.014$), decreased shell weight *per* unit area and increased egg deformation (*Table* 3.16, $P<0.05$). Also, increasing temperature gave an increase ($P=0.024$) in blood lysine concentration (*Table 3.20*). Carcass and ovary weight decreased with increasing temperature (*Table 3.18*, $P=0.044$, $P=0.016$ respectively).

There were effects of dietary lysine but they interacted with ambient temperature. However, increasing lysine level concentration tended to give a quadratic increase in egg mass output and egg number and a linear increase in egg weight (*Table 3.14*; $P=0.080$, $P=0.070$, $P=0.053$ respectively) at 21°C but not at 32°C. Also, increasing lysine concentration tended to give a linear increase (*Table 3.20*; $P= 0.064$) in blood lysine concentration at 21°C but not at 32°C.

There were significant ($P<0.05$) temperature x lysine concentration interactions in PCV and blood lysine concentration at 21°C but not at 32°C. Also, there were temperature x lysine concentration interactions in carcass composition, linear trend (*Table 3.17*; $P>0.05$) in DM and fat content and ($P<0.05$) in N amount at 21°C but not at 32°C.

**Table 3.13** Effects of six dietary lysine levels on body weight change of female broiler breeders (28-40 weeks of age) kept at 21 or 32 °C (Experiment 3)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|
| | | **40** | **50** | **60** | **70** | **80** | **90** | |
| **21** | | | | | | | | |
| | Female weight gain (g / d) | 0.66 | 4.26 | 3.34 | 4.04 | 2.88 | 3.32 | 3.08 |
| **32** | | | | | | | | |
| | Female weight gain (g / d) | -0.46 | 0.45 | -3.91 | 2.08 | 1.17 | 1.34 | 0.11 |
| | Mean lysine concentration effects | | | | | | | |
| | Female weight gain (g / d) | 0.10 | 2.35 | -0.29 | 3.06 | 2.03 | 2.33 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=4) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=8) | |
| | P | SEM | P | SEM | P | SEM |
| Female weight gain (g / d) | P<0.001 | 0.626 | P>0.10 | 1.011 | P>0.10 | 1.447 |

**Table 3.14** Effects of six dietary lysine levels on egg laying performance; total egg mass output, egg weight and egg number of broiler breeders (28- 40 weeks of age) kept at 21 or 32 °C (Experiment 3)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|
| | | **40** | **50** | **60** | **70** | **80** | **90** | |
| **21** | | | | | | | | |
| | Egg mass (g / hen /d ) | 48.52 | 53.03 | 50.22 | 52.38 | 49.46 | 51.88 | 50.92 |
| | Egg weight (g / d ) | 58.60 | 59.53 | 59.88 | 58.57 | 61.03 | 61.29 | 59.82 |
| | Egg number ( no. / hen / d ) | 0.83 | 0.89 | 0.84 | 0.90 | 0.82 | 0.85 | 0.85 |
| **32** | | | | | | | | |
| | Egg mass (g / hen /d ) | 40.94 | 40.66 | 44.14 | 45.46 | 44.27 | 41.33 | 42.80 |
| | Egg weight (g / d ) | 53.49 | 52.70 | 54.92 | 57.62 | 55.44 | 54.53 | 54.78 |
| | Egg number ( no. / hen / d ) | 0.76 | 0.77 | 0.80 | 0.78 | 0.80 | 0.75 | 0.78 |
| | Mean lysine concentration effects | | | | | | | |
| | Egg mass (g / hen /d ) | 44.73 | 46.85 | 47.18 | 48.92 | 46.87 | 46.61 | |
| | Egg weight (g / d ) | 56.04 | 56.11 | 57.40 | 58.10 | 58.23 | 57.91 | |
| | Egg number ( no. / hen / d ) | 0.79 | 0.83 | 0.82 | 0.84 | 0.81 | 0.80 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ( $n$=4 ) | | Lysine ( $n$=16) | | Temperature x Lysine interaction ( $n$=8) | |
| | P | SEM | P | SEM | P | SEM |
| Egg mass (g / hen /d ) | P=0.020 | 1.833 | Quadratic (P=0.080) | 1.315 | P>0.10 | 2.499 |
| Egg weight (g / d ) | P=0.013 | 1.012 | Linear (P=0.053) | 0.994 | P>0.10 | 1.634 |
| Egg number ( no. / hen / d ) | P=0.045 | 0.022 | Quadratic (P=0.070) | 0.019 | P>0.10 | 0.033 |

**Table 3.15** Effects of six dietary lysine levels on egg composition (expressed as a proportion of the egg weight); egg shell, egg yolk and egg albumen of broiler breeders (28-40 weeks of age) kept at 21 or 32 °C (Experiment 3)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|
| | | **40** | **50** | **60** | **70** | **80** | **90** | |
| **21** | | | | | | | | |
| | Egg shell | 0.092 | 0.093 | 0.089 | 0.095 | 0.096 | 0.095 | 0.094 |
| | Egg yolk | 0.318 | 0.324 | 0.319 | 0.321 | 0.315 | 0.317 | 0.319 |
| | Egg albumen | 0.590 | 0.583 | 0.591 | 0.585 | 0.589 | 0.588 | 0.588 |
| **32** | | | | | | | | |
| | Egg shell | 0.091 | 0.088 | 0.088 | 0.084 | 0.084 | 0.087 | 0.087 |
| | Egg yolk | 0.317 | 0.310 | 0.308 | 0.296 | 0.317 | 0.302 | 0.308 |
| | Egg albumen | 0.591 | 0.603 | 0.604 | 0.620 | 0.600 | 0.611 | 0.605 |
| | Mean lysine concentration effects | | | | | | | |
| | Egg shell | 0.092 | 0.091 | 0.089 | 0.089 | 0.090 | 0.091 | |
| | Egg yolk | 0.318 | 0.317 | 0.314 | 0.308 | 0.316 | 0.310 | |
| | Egg albumen | 0.591 | 0.593 | 0.598 | 0.602 | 0.594 | 0.599 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=4) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=8) | |
| | P | SEM | P | SEM | P | SEM |
| Egg shell | P=0.014 | 0.1344 | P>0.10 | 0.0018 | Linear (P=0.030) | 0.0027 |
| Egg yolk | P=0.056 | 0.3200 | P>0.10 | 0.0046 | P>0.10 | 0.0068 |
| Egg albumen | P=0.012 | 0.3450 | P>0.10 | 0.0047 | P>0.10 | 0.0070 |

**Table 3.16** Effects of six dietary lysine levels on egg shell strength; egg deformation and shell weight per unit area of broiler breeders (28-40 weeks of age) kept at 21 or 32 °C (Experiment 3)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|
| | | **40** | **50** | **60** | **70** | **80** | **90** | |
| **21** | | | | | | | | |
| | Egg deformation (μm) | 24.1 | 24.4 | 26.7 | 25.0 | 23.1 | 23.5 | 24.5 |
| | Shell weight *per* unit area (mg/cm$^2$) | 77.7 | 79.2 | 75.7 | 80.7 | 83.2 | 82.3 | 79.8 |
| **32** | | | | | | | | |
| | Egg deformation (μm) | 29.1 | 29.5 | 30.0 | 31.4 | 32.2 | 30.3 | 30.4 |
| | Shell weight *per* unit area (mg/cm$^2$) | 77.6 | 71.9 | 72.7 | 70.5 | 69.2 | 72.4 | 72.0 |
| | Mean lysine concentration effects | | | | | | | |
| | Egg deformation (μm) | 26.6 | 26.9 | 28.4 | 28.2 | 27.6 | 26.9 | |
| | Shell weight *per* unit area (mg/cm$^2$) | 76.7 | 75.6 | 74.2 | 75.6 | 76.2 | 77.3 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=4) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=8) | |
| | P | SEM | P | SEM | P | SEM |
| Egg deformation (μm) | P=0.011 | 1.17 | P>0.10 | 1.18 | P>0.10 | 1.92 |
| Shell weight *per* unit area (mg/cm$^2$) | P=0.003 | 1.10 | P>0.10 | 1.69 | Linear (P=0.022) | 2.44 |

**Table 3.17** Effects of six dietary lysine levels on chemical carcass composition of female broiler breeders (28-40 weeks of age) kept at 21 or 32 °C (Experiment 3)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|
| | | **40** | **50** | **60** | **70** | **80** | **90** | |
| **21** | | | | | | | | |
| | DM (g/kg) | 384 | 382 | 375 | 369 | 378 | 382 | 378 |
| | N (g/kg) | 74 | 79 | 81 | 84 | 79 | 79 | 80 |
| | Fat (g/kg) | 422 | 393 | 391 | 372 | 386 | 410 | 396 |
| | Ash (g/kg) | 93 | 100 | 92 | 97 | 99 | 98 | 97 |
| **32** | | | | | | | | |
| | DM (g/kg) | 367 | 378 | 375 | 385 | 380 | 397 | 380 |
| | N (g/kg) | 83 | 76 | 80 | 76 | 80 | 72 | 78 |
| | Fat (g/kg) | 385 | 420 | 385 | 432 | 412 | 448 | 413 |
| | Ash (g/kg) | 93 | 93 | 96 | 90 | 97 | 96 | 94 |
| Mean lysine concentration effects | | | | | | | | |
| | DM (g/kg) | 375 | 380 | 375 | 377 | 379 | 389 | |
| | N (g/kg) | 79 | 78 | 80 | 80 | 79 | 76 | |
| | Fat (g/kg) | 403 | 407 | 388 | 402 | 399 | 429 | |
| | Ash (g/kg) | 93 | 96 | 94 | 94 | 98 | 97 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=4) | | Lysine ($n$=8) | | Temperature x Lysine interaction ($n$=4) | |
| | P | SEM | P | SEM | P | SEM |
| DM (g/kg) | P>0.10 | 7.3 | P>0.10 | 5.9 | Linear (P= 0.057) | 10.6 |
| N (g/kg) | P>0.10 | 2.2 | P>0.10 | 1.9 | Linear (P= 0.031) | 3.3 |
| Fat (g/kg) | P>0.10 | 18.4 | P>0.10 | 15.3 | Linear (P= 0.099) | 27.0 |
| Ash (g/kg) | P>0.10 | 2.4 | P>0.10 | 4.6 | P>0.10 | 6.4 |

**Table 3.18** Effects of six dietary lysine levels on carcass composition of female broiler breeders (28-40 weeks of age) kept at 21 or 32 °C (Experiment 3)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|
| | | **40** | **50** | **60** | **70** | **80** | **90** | |
| **21** | | | | | | | | |
| | Live weight (g) | 3370 | 3620 | 3690 | 3642 | 3385 | 3665 | 3562 |
| | Carcass weight (g) | 3114 | 3354 | 3465 | 3398 | 3166 | 3393 | 3315 |
| | Abdominal fat weight (g) | 100.8 | 106.7 | 110.3 | 95.6 | 103.5 | 117.7 | 99.0 |
| | Oviduct weight (g) | 81.3 | 79.7 | 79.0 | 80.2 | 72.4 | 74.3 | 77.8 |
| | Ovary weight (g) | 53.9 | 68.5 | 65.2 | 60.5 | 57.2 | 58.8 | 60.7 |
| | Oocyte number | 4.8 | 4.8 | 5.3 | 4.8 | 4.8 | 5.3 | 4.9 |
| **32** | | | | | | | | |
| | Live weight (g) | 3312 | 3222 | 2855 | 3462 | 3467 | 3487 | 3301 |
| | Carcass weight (g) | 3089 | 2971 | 2649 | 3232 | 2993 | 3186 | 3020 |
| | Abdominal fat weight (g) | 67.8 | 111.6 | 75.8 | 136.1 | 89.7 | 113.1 | 99.0 |
| | Oviduct weight (g) | 79.6 | 55.3 | 62.1 | 73.5 | 66.7 | 75.7 | 68.8 |
| | Ovary weight (g) | 51.0 | 45.8 | 46.4 | 55.1 | 55.3 | 45.2 | 49.8 |
| | Oocyte number | 4.5 | 3.3 | 4.5 | 4.8 | 4.5 | 3.7 | 4.2 |
| | Mean lysine concentration effects | | | | | | | |
| | Live weight (g) | 3341 | 3421 | 3273 | 3552 | 3426 | 3576 | |
| | Carcass weight (g) | 3101 | 3163 | 3057 | 3317 | 3079 | 3290 | |
| | Abdominal fat weight (g) | 84.3 | 109.2 | 93.0 | 116.0 | 96.6 | 115.4 | |
| | Oviduct weight (g) | 80.4 | 67.5 | 70.5 | 76.9 | 69.5 | 75.0 | |
| | Ovary weight (g) | 52.4 | 57.1 | 55.8 | 57.8 | 56.3 | 52.0 | |
| | Oocyte number | 4.6 | 4.0 | 4.9 | 4.8 | 4.6 | 4.5 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=4) | | Lysine ($n$=8) | | Temperature x Lysine interaction ($n$=4) | |
| | P | SEM | P | SEM | P | SEM |
| Live weight (g) | P=0.061 | 80.2 | P>0.10 | 133.6 | P>0.10 | 190.2 |
| Carcass weight (g) | P=0.044 | 89.0 | P>0.10 | 118.2 | P>0.10 | 173.2 |
| Abdominal fat weight (g) | P>0.10 | 10.37 | P>0.10 | 11.16 | P>0.10 | 17.75 |
| Oviduct weight (g) | P=0.089 | 3.15 | P>0.10 | 4.62 | P>0.10 | 6.75 |
| Ovary weight (g) | P=0.016 | 2.32 | P>0.10 | 3.77 | P>0.10 | 5.40 |
| Oocyte number[1] | P>0.10 | 0.08 | P>0.10 | 0.08 | P>0.10 | 0.13 |

[1] Standard errors of means used square root of means values of birds kept at 21°C or 32°C.

**Table 3. 19** Effects of six dietary lysine levels on carcass composition (expressed as a proportion of the bird live weight) of female broiler breeders (28-40 weeks of age) kept at 21 or 32 °C (Experiment 3)

| Temperatures (°C) | Variables | Lysine concentrations ( g/kg Crude Protein ) | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|
| | | **40** | **50** | **60** | **70** | **80** | **90** | |
| **21** | | | | | | | | |
| | Carcass [1] | 0.923 | 0.926 | 0.940 | 0.933 | 0.935 | 0.925 | 0.930 |
| | Abdominal fat | 0.029 | 0.029 | 0.030 | 0.026 | 0.030 | 0.032 | 0.030 |
| | Oviduct | 0.024 | 0.022 | 0.021 | 0.022 | 0.022 | 0.020 | 0.022 |
| | Ovary | 0.016 | 0.019 | 0.018 | 0.017 | 0.017 | 0.016 | 0.017 |
| **32** | | | | | | | | |
| | Carcass | 0.931 | 0.921 | 0.928 | 0.934 | 0.874 | 0.925 | 0.917 |
| | Abdominal fat | 0.019 | 0.034 | 0.026 | 0.039 | 0.026 | 0.033 | 0.030 |
| | Oviduct | 0.025 | 0.017 | 0.022 | 0.021 | 0.019 | 0.021 | 0.021 |
| | Ovary | 0.016 | 0.014 | 0.016 | 0.016 | 0.016 | 0.013 | 0.015 |
| | Mean lysine concentration effects | | | | | | | |
| | Carcass | 0.927 | 0.924 | 0.934 | 0.934 | 0.904 | 0.920 | |
| | Abdominal fat | 0.024 | 0.032 | 0.028 | 0.033 | 0.028 | 0.033 | |
| | Oviduct | 0.025 | 0.020 | 0.022 | 0.022 | 0.020 | 0.021 | |
| | Ovary | 0.016 | 0.016 | 0.017 | 0.016 | 0.017 | 0.014 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ( $n$=4 ) | | Lysine ( $n$=8) | | Temperature x lysine interaction ( $n$=4) | |
| | P | SEM | P | SEM | P | SEM |
| Carcass | P>0.10 | 0.0922 | P>0.10 | 0.1292 | P>0.10 | 0.1906 |
| Abdominal fat | P>0.10 | 0.0261 | P>0.10 | 0.0275 | Quadratic (P=0.096) | 0.0441 |
| Oviduct | P>0.10 | 0.0101 | P>0.10 | 0.0143 | P>0.10 | 0.0210 |
| Ovary | P>0.10 | 0.0081 | P>0.10 | 0.0092 | P>0.10 | 0.0144 |

[1] Plucked and bled weight

**Table 3.20** Effects of six dietary lysine levels on blood packed cell volume (PCV) and blood lysine content of female broiler breeders (28-40 weeks of age) kept at 21 or 32 °C (Experiment 3)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|
| | | **40** | **50** | **60** | **70** | **80** | **90** | |
| **21** | | | | | | | | |
| | PCV (%) | 38.8 | 41.5 | 43.3 | 44.9 | 41.7 | 39.5 | 41.6 |
| | Lysine (mMol /L) | 0.25 | 0.28 | 0.30 | 0.32 | 0.38 | 0.36 | 0.33 |
| **32** | | | | | | | | |
| | PCV (%) | 36.1 | 37.1 | 32.8 | 32.1 | 34.8 | 33.6 | 34.4 |
| | Lysine (mMol /L) | 0.43 | 0.34 | 0.29 | 0.42 | 0.37 | 0.41 | 0.38 |
| | Mean lysine concentration effects | | | | | | | |
| | PCV (%) | 37.5 | 39.3 | 38.1 | 38.5 | 38.2 | 36.5 | |
| | Lysine (mMol /L) | 0.34 | 0.31 | 0.29 | 0.37 | 0.37 | 0.38 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=4) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=8) | |
| | P | SEM | P | SEM | P | SEM |
| PCV (%) | P>0.10 | 2.93 | P>0.10 | 1.47 | Quadratic (P= 0.026) | 3.49 |
| Lysine (mMol /L) | P=0.024 | 0.036 | linear (P= 0.064) | 0.024 | linear (P= 0.044) | 0.046 |

**Table 3.21** Effects of six dietary lysine levels on body temperatures of female broiler breeders (28-40 weeks of age) kept at 21 or 32 °C (Experiment 3)

| Temperatures (°C) | Variables | Lysine concentrations (g/kg Crude Protein) | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|
| | | **40** | **50** | **60** | **70** | **80** | **90** | |
| **21** | | | | | | | | |
| | Daily mean of body temperature (°C) | 41.7 | 41.7 | 41.7 | 41.7 | 41.7 | 41.7 | 41.7 |
| | Range of body temperature(°C) † | 0.6 | 0.5 | 0.7 | 0.5 | 0.6 | 0.7 | 0.6 |
| **32** | | | | | | | | |
| | Daily mean of body temperature (°C) | 42.0 | 42.1 | 41.8 | 42.3 | 41.8 | 42.1 | 42.0 |
| | Range of body temperature (°C) | 0.4 | 0.4 | 0.5 | 0.6 | 0.3 | 0.5 | 0.4 |
| | Mean lysine concentration effects | | | | | | | |
| | Daily mean of body temperature (°C) | 41.8 | 41.9 | 41.7 | 42.0 | 41.7 | 41.9 | |
| | Range of body temperature (°C) | 0.5 | 0.5 | 0.6 | 0.5 | 0.4 | 0.6 | |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=4) | | Lysine ($n$=16) | | Temperature x lysine interaction ($n$=8) | |
| | P | SEM | P | SEM | P | SEM |
| Daily mean of body temperature (°C) | P=0.045 | 0.09 | P>0.10 | 0.08 | P>0.10 | 0.15 |
| Range of body temperature (°C) | P>0.10 | 0.08 | P>0.10 | 0.05 | Quadratic (P= 0.055) | 0.11 |

† Range temperature = highest temperature – lowest temperature of the diurnal change in body temperature

**Table 3.22** Effects of six dietary lysine levels on birds eating time (expressed as number of hens to eat all their daily allocation of feed within 3 time periods) of female broiler breeders (28-40 weeks of age) kept at 21°C or 32 °C (Experiment 3)

| Lysine concentrations ( g/kg Crude Protein ) | Number of hens to eat all their daily allocation of feed | | | $\chi^2$ | *P* |
|---|---|---|---|---|---|
| | **<2 hrs.** | **2.1-10 hrs.** | **>10.1 hrs.** | | |
| **40** | 5 | 6 | 3 | | |
| **50** | 9 | 2 | 4 | | |
| **60** | 7 | 2 | 3 | | |
| **70** | 6 | 7 | 3 | | |
| **80** | 8 | 6 | 2 | | |
| **90** | 7 | 4 | 2 | | |
| | | | | 6.64 | P>0.05 |
| Temperatures (°C) | | | | | |
| **21** | 41 | 6 | 0 | | |
| **32** | 1 | 21 | 18 | | |
| | | | | 64.28 | P<0.001 |

**Table 3. 23** Apparent metabolisable energy (AME, MJ/Kg DM), total amino acid digestibility and amino acid digestibility of a basal diet fed to female broiler breeders (28-40 weeks of age) kept at 21 or 32 °C (Experiment 3)

| Variables[†] | Amino acid digestibility | | SEM of amino acid digestibility | *P* |
|---|---|---|---|---|
| | 21°C | 32°C | | |
| Arginine | 0.775 | 0.754 | 0.042 | P>0.05 |
| Cystine | 0.828 | 0.757 | 0.033 | P>0.05 |
| Histidine | 0.785 | 0.785 | 0.035 | P>0.05 |
| IsoLeucine | 0.759 | 0.742 | 0.050 | P>0.05 |
| Leucine | 0.837 | 0.820 | 0.031 | P>0.05 |
| Lysine | 0.669 | 0.661 | 0.054 | P>0.05 |
| Methionine | 0.880 | 0.861 | 0.021 | P>0.05 |
| Phenylalanine | 0.828 | 0.810 | 0.031 | P>0.05 |
| Threonine | 0.758 | 0.759 | 0.037 | P>0.05 |
| Tyrosine | 0.794 | 0.783 | 0.030 | P>0.05 |
| Valine | 0.727 | 0.736 | 0.051 | P>0.05 |
| Alanine | 0.720 | 0.694 | 0.051 | P>0.05 |
| Aspartic acid | 0.762 | 0.762 | 0.036 | P>0.05 |
| Glutamic acid | 0.912 | 0.900 | 0.016 | P>0.05 |
| Glycine | 0.637 | 0.616 | 0.081 | P>0.05 |
| Proline | 0.909 | 0.895 | 0.016 | P>0.05 |
| Serine | 0.770 | 0.777 | 0.031 | P>0.05 |
| Total amino acid digestibility | 0.823 | 0.811 | 0.159 | P>0.05 |
| AME | 11.34 | 11.18 | 0.065 | P>0.05 |

[†]Tryptophan values were not determined.

### 3.3.4 Discussion

The major objectives of this experiment were to examine the effects on physiological characteristics of six diets that differed in their lysine concentrations (40, 50, 60, 70, 80 and 90 g/kg crude protein) fed to broiler breeders when kept at two ambient temperatures (21 and 32°C). The egg production data were consistent with the previous experiments in this project. For example, there was a numerical decrease ($P>0.05$) of 19% in egg mass output in the birds kept at 32°C. The quadratic response in egg mass output to increasing dietary lysine concentrations support the results of experiment1. In the previous experiments, birds were kept in group pens, which gave less variability in egg production characteristics, but did not enable detailed physiological variables to be examined.

The present experiment shows that there were no significant ($P>0.05$) effects of temperature on total amino acid digestibility or apparent metabolisable energy availability of the basal diet fed to the broiler breeders. Farrell and Swain (1977) found a small significant effect of temperature on the metabolisability of the diets by broilers and reported a 0.4 MJ/kg decrease in metabolisability of a broiler feed in birds kept at 35°C compared to 22°C, although they did not observe any decrease in birds kept at 30°C. The present experiment used a constant 32°C with adult birds, so it is possible that no significant effect on metabolisability would occur.

High ambient temperatures in the present experiment had a profound effect on the diurnal pattern of feeding activity of broiler breeders. The birds kept at 21°C ate their daily allowance of feed in approximately two hours (87% of the birds ate their

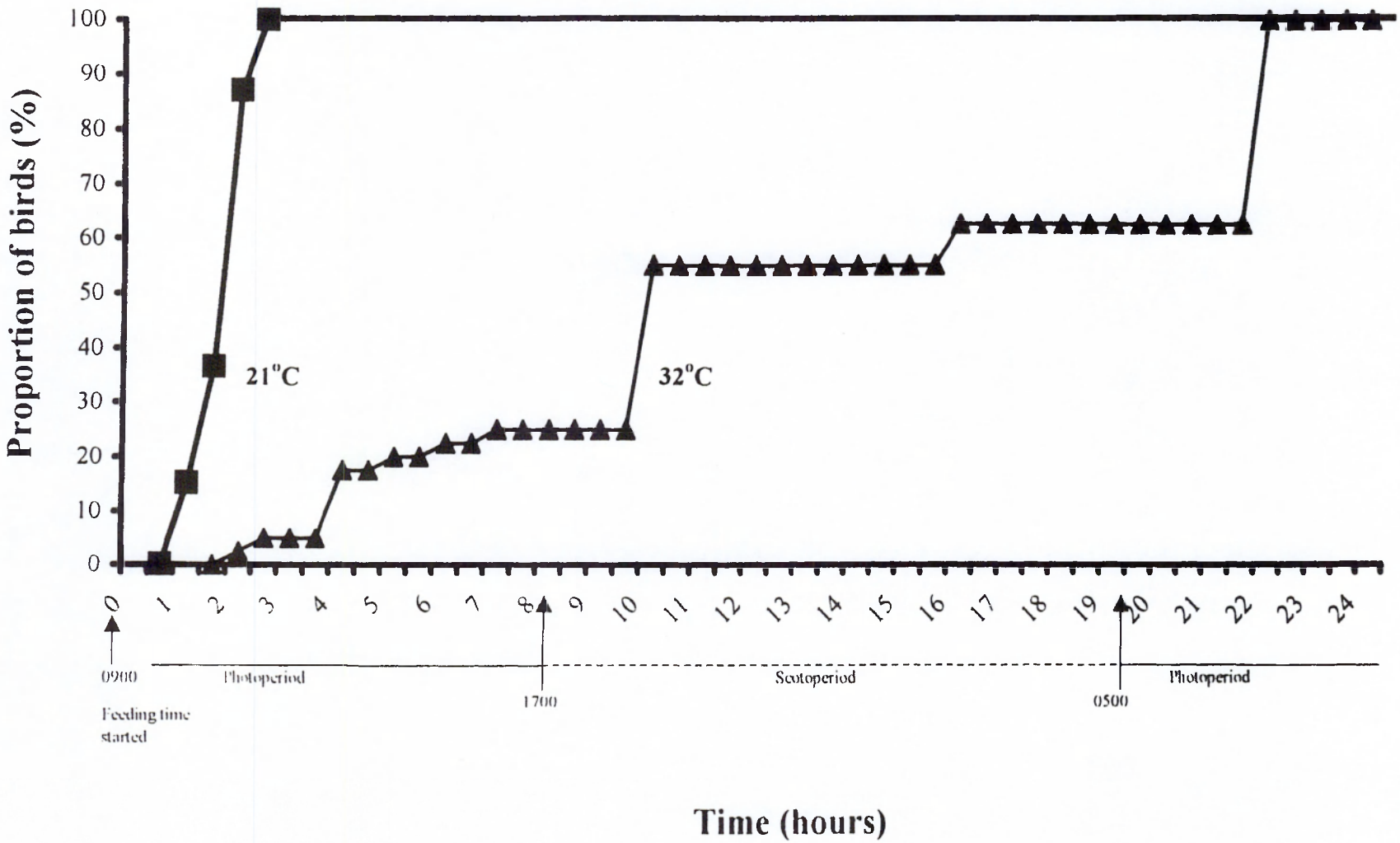
allocation in less than two hours). However, the birds kept at 32°C ate their feed allocation much more slowly, with only one bird eating its feed allocation in less than two hours and 45% of the birds taking greater than 10 hours (*Figure 3.7*). It is possible that these large differences in diurnal feeding activity may have themselves affected the breeder hens' responses to dietary nutrient composition.

There was a rise in rectal temperature in the birds kept at 32°C with increased ambient temperatures of 0.34°C. A similar increase (0.40°C) in body temperatures was observed by Hocking *et al.*, (1994) in broiler breeders kept at 32°C compared to 20°C.

The observed effects of temperature on blood components confirm observations in the literature of birds kept at high temperatures. Huston (1960, 1965); Washburn and Huston (1968); Donkoh (1989); Zhou *et al.*,(1998) in broilers and Parker and Boone (1971) in adult turkeys and Vo *et al.*, (1978) in laying hens found that the haematocrit value was decreased in birds kept at high temperatures.

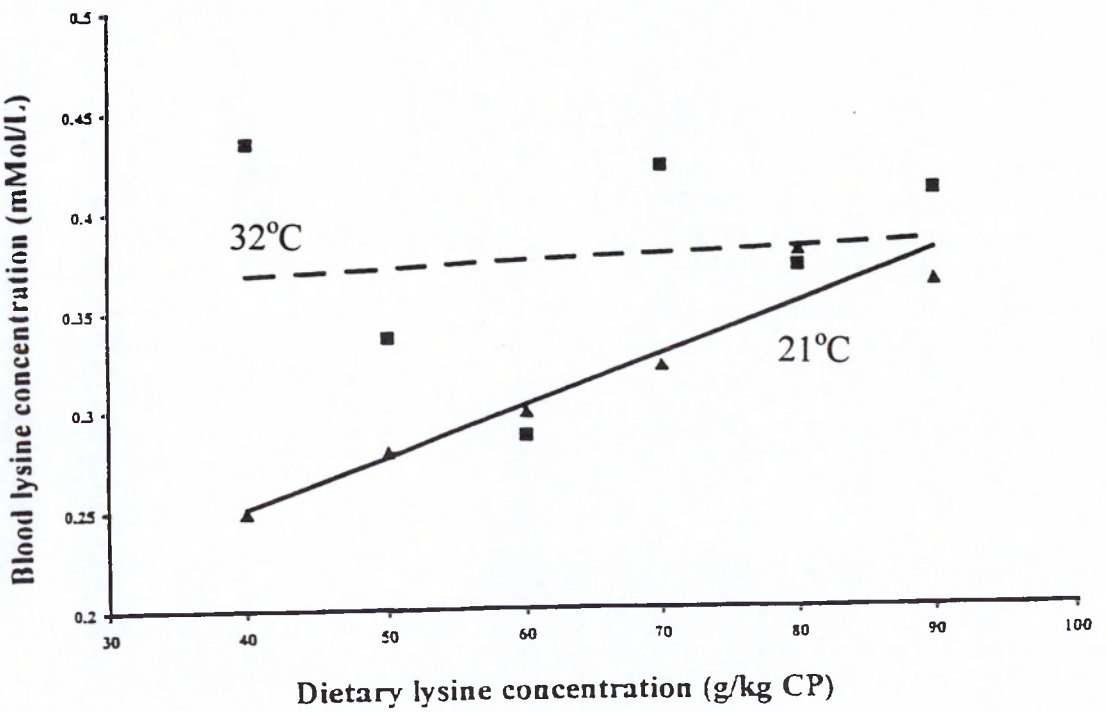
The experiment demonstrated that dietary lysine concentration affected a number of physiological parameters in the hens, but these responses interacted with the ambient temperature. Increasing dietary intakes of lysine gave increasing ($P<0.05$) blood lysine concentrations in the hens kept at 21°C but not at 32°C (temperature x lysine concentration interaction $P=0.044$) (*Figure 3.8*). The blood samples were taken after two hours of first introducing the feed, so birds kept at 21°C would have consumed most of their feed before this time but those kept at 32°C would have eaten only a small proportion. It is therefore logical that birds kept at 21°C would

**Figure 3.7** Cumulative proportion of breeder hens eating their full daily feed allowance kept at 21°C (■) or 32°C (▲) (Experiment 3).



have a blood amino acid profile that be more sensitive to the dietary amino acid supply.

**Figure 3.8** Relationship between variation in dietary lysine concentration and the blood lysine content of broiler breeders kept at 21°C(■) or 32°C(▲) (Experiment 3).




Although there was no evidence of a temperature x lysine interaction in egg mass output, this experiment gives evidence that temperature affects the response of breeder hens to dietary lysine concentration. For example, increased lysine intakes at 21°C resulted in increased body protein levels and decreased body fat contents, whereas at 32°C increased lysine intakes resulted in decreased body protein levels

and little change in body fat contents. The reduction in fat contents associated with increased dietary lysine concentrations had been observed by number of researchers (Griffiths *et al.*, 1977; Cable *et al.*, 1987, 1988; Cabel and Waldroup, 1991; Mendes *et al.*, 1997) when kept at around 21°C. Mendes *et al.*, (1997) observed there was no temperature x lysine interaction in body fatness but these birds were fed *ad libitum* and there tended (P=0.07) to be affects of these different lysine concentrations on the voluntary feed intakes of the birds.

There was a significant temperature x lysine interaction in PCV in the present experiment. This lysine response was evident at 21°C, with a peak PCV at 60-70 g lysine/ kg diet but there was no response 32°C. Braham *et al.*, (1961) also found that lysine deficiency reduced PCV and haemoglobin levels in chickens but did not report their rearing temperatures.

Birds kept at 21°C gained more weight than birds kept at 32°C. Its possible that the lower efficiency of feed utilization of the birds kept at 32°C may have been due to the cost of insensible heat loss.

In conclusion, the present experiment has provided some evidence that the response to dietary lysine supply depends upon ambient temperature. Birds kept at low temperatures (21°C) had lower blood lysine concentrations, lower PCV and deposited more body fat when given lower density lysine intakes. However, these responses were not evidence at 32°C. This experiment used a longer feeding period although still less than one half of the laying period that would be used in commercial broiler breeder hens. No temperature x dietary lysine interactions were observed in the egg production characteristics of the broiler breeders in this

experiment nor in the two previous short-term experiments. However, the physiological data indicate that the deleterious effects of low lysine diets, if fed extended periods during lay may become more profound in birds that are kept at low temperatures rather than at high temperatures.

## 3.4 Experiment 4

*Estimation of abdominal fat in live broiler breeders using cloacal calipers*

### 3.4.1. Introduction

The abdomen is a major site for fat deposition in the bird and the amount of abdominal fat is highly correlated to the total body fat of the whole bird (Sturkie, 1965). Dissection of abdominal fat to measure fat deposition is relatively simple, but it is time-consuming, expensive and potential breeding stock are lost. Therefore in commercial broiler breeding work, research work and on-farm monitoring of flocks there is a need for a quick and inexpensive method of estimating body fat. A caliper fat measurement is a technique that can estimate the amount of abdominal fat within a live bird. A probe is inserted in the cloaca and the distance between the probe and the outer skin of the abdomen is measured. No internal organs or skeleton are present at this site, so the measurement relates predominately to the depth of abdominal fat. Previous studies found significant linear relationships between the caliper measurements and the fatness of broilers (Rose and Abebe, 1984; Mirosh and Becker, 1982) and layers (Rose and Michie, 1983), but there is no direct evidence using adult broiler breeders.

The specific objectives of the present experiment were to identify whether there was a significant relationship between measurements and the proportion of abdominal fat in

broiler breeders. A range of body fatnesses was obtained by sampling the birds from experiment 3 in which two different ambient temperatures (21 °C and 32°C) and six different lysine concentrations were compared (40, 50, 60, 70, 80 and 90 g/kg Crude Protein).

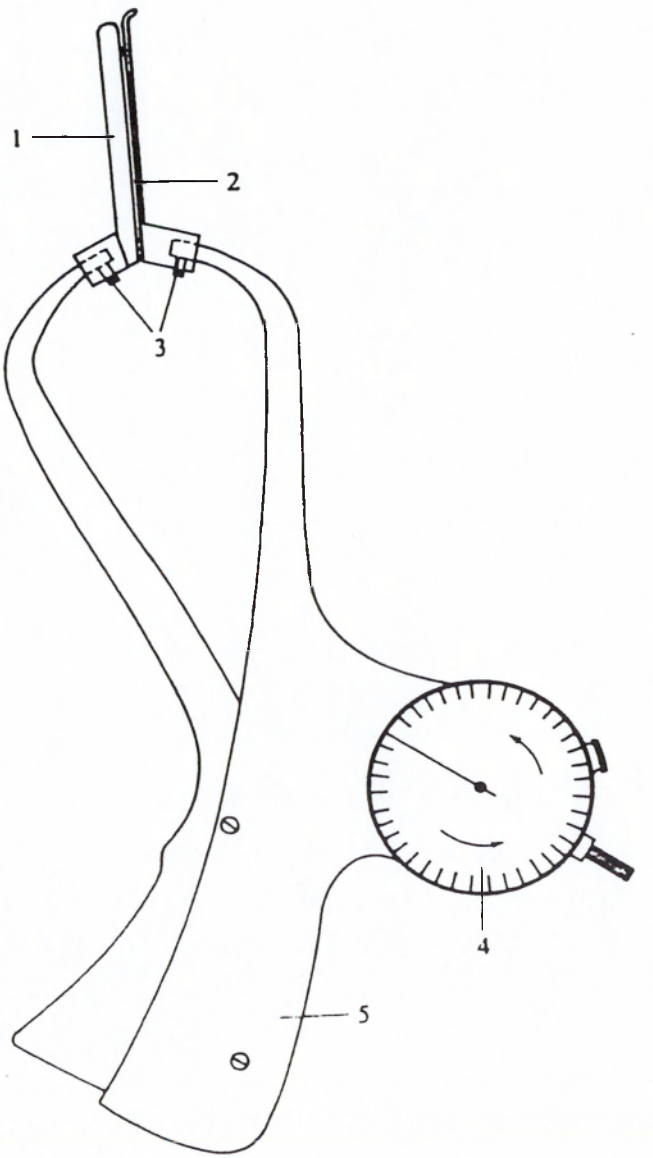
## 3.4.2. Materials and Methods

Ninety-six Ross (308 Broiler Breeder) were individually caged and fed the experimental diets described in experiment 3. At 36 weeks of age the birds were, from experiment 3, individually weighed and abdominal fat caliper measurements taken.

### 3.4.2.1 The calipers and technique of measurement

The instrument was modified from skinfold thickness calipers (supplied by CMS Weighing Equipment, 18 Camden High Street, London NW1, UK.) used for fatness estimation of humans. A cylindrical rod, 7mm in diameter, was inserted into the bird's cloaca, a flattened bar was positioned on its abdomen and a constant pressure was exerted between the two arms of the caliper (*Figure 3.9*). The distance between the two probes was read on a dial gauge. The cylindrical end had been lightly greased with petroleum jelly and disinfected after each used. The measurement techniques were as described by Pym and Thompson (1980), except that the fully fed birds were weighed

**Figure 3. 9** Modified design of broiler breeder abdominal fat calipers used in experiment 4.




1. Cylindrical probe 7 mm diameter
2. Flattened bar
3. Retaining screws
4. Dial gauge
5. Caliper handles containing spring mechanism

and measurements were taken on the left side of the abdomen of each bird. The same operator took all measurements. After abdominal fat calliper measurements were taken, the birds were individually weighed, killed and chilled for 24 hours and then the abdominal fat (defined as the abdominal leaf fat and the fat surrounding the gizzard and the lower intestine) was dissected out and weighed.

### 3.4.2.2 Experimental design

**Statistical analysis**

The relationship between the caliper measurements (independent variable) and body fat variables (dependent variables) were compared by regression analysis. Temperature treatment was included in the regression model as a grouping factor.

### 3.4.3 Results

*Figures 3.10 and 3.11* show a plot of the mean caliper measurement against the proportion of abdominal fat and against the weight of abdominal fat per kilogram of body weight.

There were significant linear ($P<0.001$) relationship between the proportion of abdominal fat and the cloacal caliper measurements. However, there was a large amount of unexplained variation ($r=0.41$).

There were no ($P>0.05$) significant differences in the relationships for the birds kept either at 21°C or 32°C.

**Figure 3.10** Relationship between caliper measurements and the proportion of abdominal fat in broiler breeders (■= birds kept at 21°C and ●= birds kept at 32°C) n = 85.

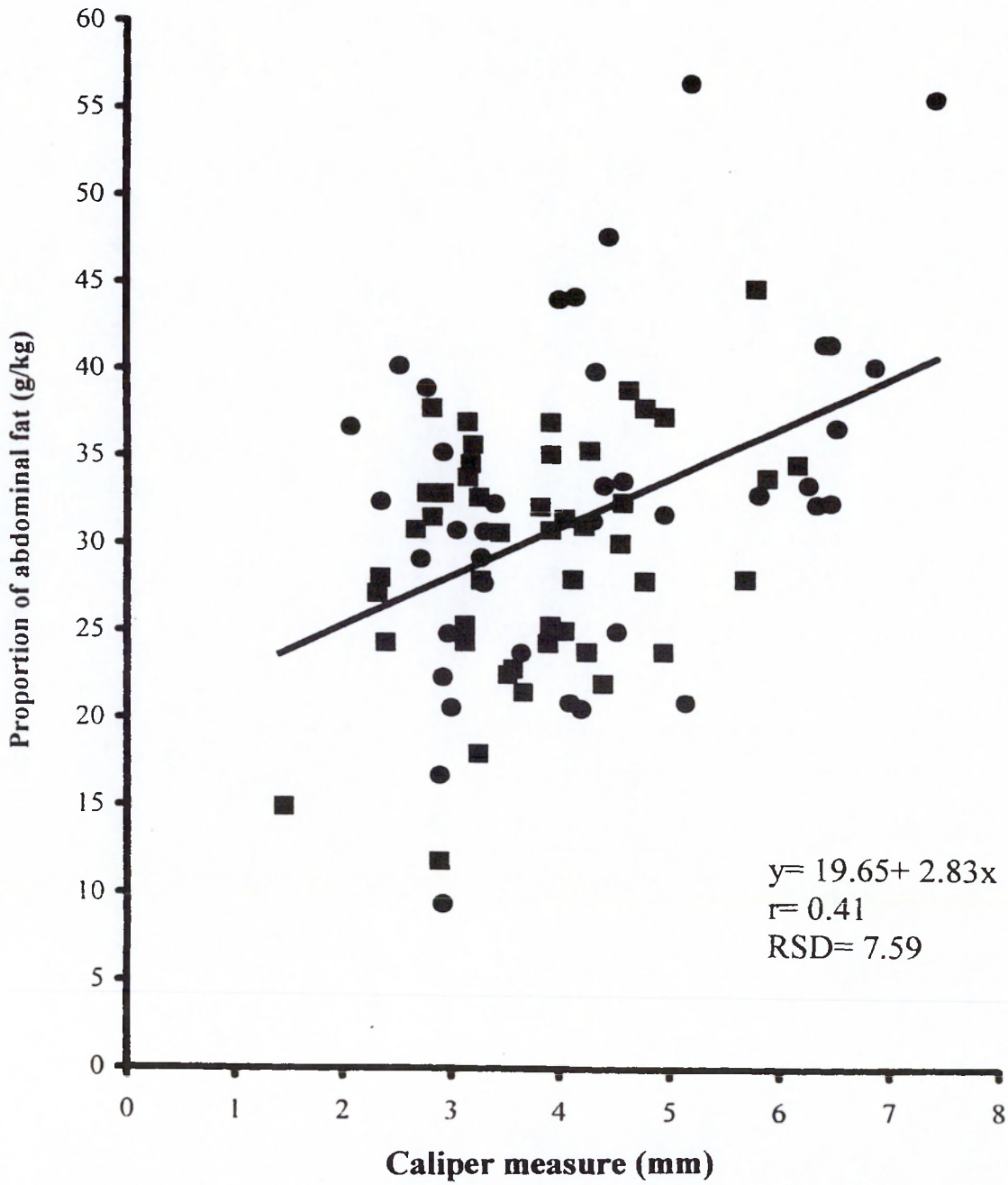
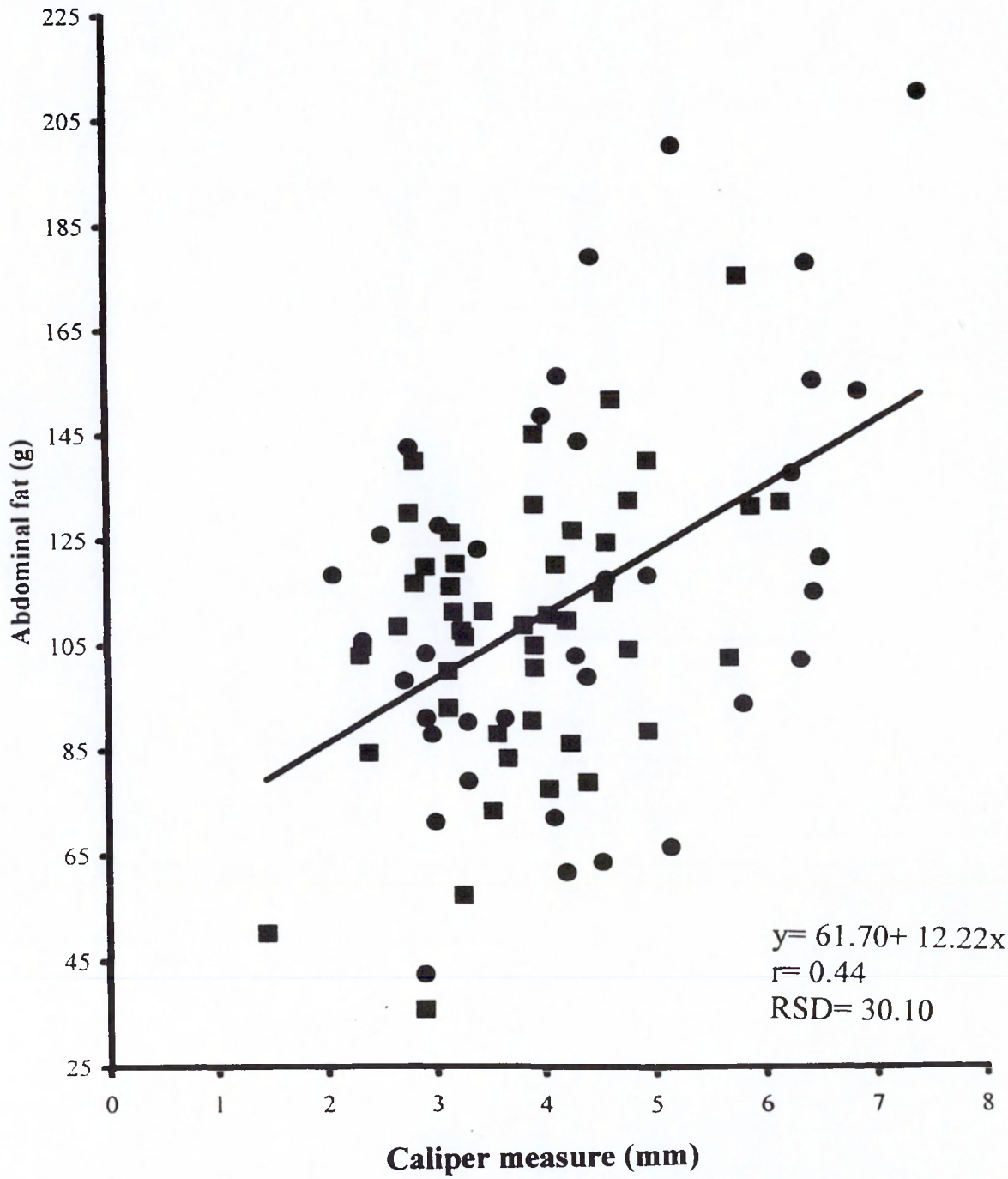


**Figure 3.11** Relationship between caliper measurements and the abdominal fat weight in broiler breeders (■= birds kept at 21°C and ●= birds kept at 32°C) n = 85.



### 3.4.4 Discussion

There was a significant correlation ($P<0.001$) between the cloacal caliper measurements and proportion of abdominal fat and also with weight of abdominal fat. The regression equations were similar to those previously reported (Mirosh and Becker, 1982; Rose and Michie, 1983; Rose and Abebe, 1984).

In the present experiment, the correlation between the caliper measurement and abdominal fat was very low ($r=0.41$). Mirosh and Becker (1982) also found a low correlation coefficient ($r=0.30$) in broiler chickens. Rose and Michie (1983) found a higher correlation in laying hens ($r=0.81$) and turkeys ($r=0.76$), but the correlation was lower in broilers ($r=0.51$) and particularly female broilers ($r=0.44$).

It appears that the calipers do not give a good estimate of fatness on meat-line birds and particularly in females. Therefore, the callipers only gave a relatively imprecise technique for estimating abdominal fat in broiler breeders. To get reliable data, a large number of birds would be needed to be used in the comparison.

## 3.5 Experiment 5

*Effect of constant and cyclic environmental temperatures, dietary protein and lysine levels on the reproductive performance of broiler breeders.*

### 3.5.1. Introduction

The previous experiments have demonstrated clear effects of temperature on broiler breeders that have enabled the effects of constant ambient temperature to be quantified. However, egg production in hot climates frequently results in large diurnal changes in ambient temperatures rather than one constant high temperature. Section 2.2 reviewed the available literature on diurnal cycling temperatures on the egg production of laying hens. However, there is a lack of documented information on the response of broiler breeders to high cycling temperatures. Therefore, there is a need to examine whether the performance responses of broiler breeders are similar to those of commercial egg-laying strains.

Experiment 2 and 3 demonstrated that increasing to dietary lysine gave increase (P=0.017 (exp. 2) and P=0.053 (exp. 3)) in egg weight. Variations in dietary lysine supply usually occur because of differences in the overall crude protein concentration of the diet rather than differences in the lysine supply alone. Morris *et al.* (1999) have indicated that the optimum dietary concentration of a limiting amino acid is dependent upon the dietary concentration of crude protein. There is no published evidence whether laying broiler breeders respond in a similar direction to commercial egg laying strains.

There is a need to examine whether the response of broiler breeders to dietary lysine concentration is dependent upon the total protein supply.

The specific objectives of this experiment were therefore to examine the following factors;

- To compare the effects of two constant ambient temperatures (32°C and 21°C) on the productive performance of laying broiler breeders and to compare with the effects of two cycling temperatures ((35°C (16hrs) - 29°C (8hrs) overall mean temperature = 32°C) and (14°C (16hrs) - 28°C (8hrs) overall mean temperature = 21°C)).
- To examine and explain the effect of four dietary lysine concentrations (40, 52.5, 65 and 90 g/kg crude protein) each given at three different crude protein concentrations (120, 155 or 190 g/kg CP).

## 3.5.2. Materials and Methods

### 3.5.2.1 Diets

Twelve experimental diets were prepared that comprised three protein concentrations each with four different lysine concentrations ( 40, 52.5, 65 and 90 g/kg crude protein). Three diets were prepared with either 120, 155 or 190 g/kg crude protein (*Table 3.24*). The concentration of all amino acids except lysine, and other nutrients met or exceeded the requirements of broiler breeder hens according to the National Research Council (1994) and Ross Breeders Limited (1998). Each diet was deficient only in lysine

concentration. Three further dietary levels of lysine concentration were achieved in each of the three crude protein concentrations by adding L-lysine-HCl to the deficient diet in replacement for maize starch (*Table 3.24*), to give four concentrations of lysine ( 40, 52.5, 65 and 90 g/kg crude protein). All experimental diets were stored in the same environmental conditions.

### 3.5.2.2 Temperature

Four different temperature treatments were compared;

1. A constant 21°C both night and day.
2. A constant 32°C both night and day.
3. A fluctuating temperature regimen {14 °C for 16 hours (5pm to 9am daily) followed by 28 °C for 8 hours (9am to 5pm daily)}. Equivalent to a mean daily temperature of 21°C.
4. A fluctuating temperature regimen {35°C for 16 hours (5pm to 9am daily) followed by 29°C for 8 hours (9am to 5pm daily)}. Equivalent to a mean daily temperature of 32°C.

Each temperature treatment was applied to two replicate rooms in each of two replicate time blocks giving a total of four independent replicates for each treatment. Relative humidity was maintained in the range 70-75% in all environments for a 28 day period. The daily lighting programme hours was kept at 16 hours (5am to 9pm) of artificial illumination to the end of all the experiment with a mean light intensity of 60 lux throughout the experimental period.

**Table 3.24** Composition of the three basal lysine deficient† broiler breeder diets fed in experiment 5 (27-35 weeks of age).

| Ingredients | Crude protein (CP) (g/kg diet) | | |
|---|---|---|---|
| | 120 g/kg CP | 155 g/kg CP | 190 g/kg CP |
| Wheat | 749.0 | 685.5 | 622.0 |
| Maize gluten meal (600g/kg CP) | 2.5 | 71.3 | 140.0 |
| Sunflower seed meal (330 g/kg protein) | 80.0 | 80.0 | 80.0 |
| Full fat soya | 17.0 | 17.0 | 17.0 |
| Maize starch | 20.5 | 19.3 | 18.0 |
| Soya oil | 11.0 | 6.7 | 2.4 |
| Limestone | 80.0 | 80.0 | 80.0 |
| Dicalcium phosphate | 10.0 | 10.0 | 10.0 |
| Sodium bicarbonate | 2.0 | 2.0 | 2.0 |
| Salt | 2.0 | 2.0 | 2.0 |
| DL-methionine | 2.8 | 2.8 | 2.8 |
| L-threonine | 1.6 | 1.6 | 1.6 |
| L-tryptophan | 1.6 | 1.9 | 2.2 |
| Vitamin-Mineral Supplement‡ | 20.0 | 20.0 | 20.0 |
| Total | 1000.0 | 1000.0 | 1000.0 |
| **Nutrient Composition:** | | | |
| Calculated analysis | | | |
| Crude Protein ( CP) (g/kg) | 120.0 | 155.0 | 190.0 |
| Lysine (g\kg CP) | 40.0 | 40.0 | 40.0 |
| Methionine (g\kg CP) | 39.0 | 36.8 | 35.3 |
| Tryptophan (g\kg CP) | 23.4 | 19.0 | 17.0 |
| Methionine+ Cystine (g\kg CP) | 56.8 | 55.7 | 55.2 |
| Threonine (g\kg CP) | 47.5 | 46.5 | 46.0 |
| Isoleucine (g\kg CP) | 40.0 | 40.0 | 40.0 |
| Arginine (g\kg CP) | 65.5 | 65.0 | 64.4 |
| Valine (g\kg CP) | 47.2 | 47.9 | 48.3 |
| Histidine (g\kg CP) | 22.6 | 22.0 | 21.6 |
| Metabolisable Energy (MJ/kg) | 11.5 | 11.6 | 11.7 |
| Calcium (g\kg) | 37.5 | 37.5 | 37.5 |
| Phosphorus (g\kg) | 5.2 | 5.2 | 5.2 |
| Sodium (g\kg) | 1.8 | 1.8 | 1.7 |
| Potassium (g\kg) | 4.4 | 4.3 | 4.2 |
| Linoleic acid (g\kg) | 13.7 | 14.2 | 14.6 |
| Choline (mg\kg ) | 1130 | 1080 | 1041 |

† The other 3 experimental diets included additional (L-lysine HCl) in replacement for maize starch.

‡Supplied per kg of diet: *trans*-retinol(A), 4.8 mg; cholecalciferol(D3), 125μg; α-tocopherol acetate(E), 183.8 mg; thiamine(B1), 3 mg; riboflavin(B2), 10 mg; pyridoxine(B6), 5 mg; vitamin B12, 12 mg; nicotinic acid, 60 mg; pantothenic acid, 15 mg; folic acid, 2.5 mg; biotin, 205 μg; choline chloride, 500 mg; Fe, 20 mg; Co,1 mg; Mn, 100 mg; Cu, 10 mg; Zn, 80 mg; I, 2 mg; Se, 0.2 mg; Mo, 0.5 mg; Ca, 206g/kg; P, 100.5g/kg; Na, 50g/kg; Ash , 882g/kg.

### 3.5.2.3 Bird management

Ninety-six Ross 27-week old hens (308 Broiler Breeder, Ross Breeders Ltd.) were randomly placed in one of 12 identical cages within each of eight environmentally controlled rooms (*Figure 3.7*) at Harper Adams University College, Shropshire in time block 1. In time block 2, ninety-six Ross 32 week old hens (308 Broiler Breeder, Ross Breeders Ltd.) were similarly randomly placed in one of 12 identical cages within each of the same eight rooms. A daily feed restriction programme was followed, 156 g/bird d then a reduction of 2.5 g/ bird / 28 days period (Ross Breeders Limited 1998). Each room was randomly allocated to a temperature at the beginning of the experiment and kept at this temperature until the completion of the experimental time block then re-randomized. Relative humidity was maintained in the range of 70-75%.

### 3.5.2.4 Experimental measurements

The measurements of egg production, composition and shell strength (shell deformation and shell weight *per* unit area) and estimation of abdominal fat were as described in sections 3.1.2.5 and 3.4.2.1. Body temperature and feeding activity were determined in a single day as described in section 3.3.2.2.3.

**A. Blood characteristics**

A series of tests was performed on the collected blood from the birds in time block 2. The blood was collected two hours after the birds had been fed and during the high

ambient temperature of the cycling and constant temperatures. Blood collection procedures were as described in section 3.3.2.2.1.

Haemoglobin was determined photometrically as cyanmethaemoglobin. Haemoglobin values were read directly using a 540 nm wavelength photometer (Veterinary Laboratories Agency, Shrewsbury, SY1 4HD, UK).

The whole blood viscosity was measured using a Brookfield (LVD VII+CP) cone-and-plate viscometer (Brookfield, Stroughton, MA, USA). Plasma was obtained by centrifuging the blood for 5 min at 2000 rpm. Plasma viscosity was measured by the same method as whole blood viscosity. Blood viscosity results were expressed in centipoise units (cp).

Blood pH was determined within 2 min of sample collection using an automated blood pH meter (Model RL 150) with a micro-blood assembly and thermal block unit (set at 25°C). The pH meter was standardized with buffer solutions (pH 7.0, 4.0). The whole and plasma blood was injected into the micro-blood assembly, and the pH value was determined.

**B. Electrolyte composition**

Calcium, phosphorus, sodium and potassium values were determined at the Veterinary Laboratories Agency, Shrewsbury, UK.

The calcium content of the samples was determined using atomic absorption spectrophotometery techniques. Phosphorus concentration was determined colorimetrically by the molybdo-vanadate method (AOAC (2000) method number 965.17). A flame photometry method was used to determine sodium and potassium using 480 nm wavelength absorbency.

### 3.5.2.5 Experimental Design

**Statistical analysis**

A split-plot design was used in which the two time replicates resulted in four replicates of each main plot temperature treatment. Within each room, there were 12 individually caged birds (sub-plots) that were given one of the 12 different diets. Each dietary treatment was therefore replicated 16 times in the whole experiment. Data were compared by analysis of variance of the measured and calculated variables, using a split-plot design that examined the effects of temperature of the rooms and the effect of diet and the diet x temperature interactions in the sub-plots (cages) (*GENSTAT* statistical package, Lawes Agricultural Trust, 1998). The four main temperature treatments were arranged in a 2 x 2 factorial design (mean temperature (21 or 32°C) x temperature regimen ( constant or cycling)).

### 3.5.3 Results

Throughout the experiment the allocated amounts of feed were always eaten. The mean mortality during the experiment was 5.2% (10 birds in total), which was not associated with particular treatments.

Birds kept at 32°C (constant and cycling) had a 22% decrease in egg mass output ($P<0.001$), a decreased shell weight ($P<0.001$), an increase in deformation ($P<0.001$) and an increased ($P<0.001$) body weight loss compared to the 21°C treatments. There were no ($P>0.05$) differences between constant and cycling temperatures. Also, there were no statistically significant ($P>0.05$) mean temperature x temperature regimen (constant or cycling) interactions. However, the decrease in egg mass output was numerically greater ($P>0.05$) in the birds kept at the cycling 32°C temperature and the eggs of these birds tended ($P=0.067$) to have a greater deformation.

Increasing crude protein concentrations tended to give a quadratic increase in egg weight and shell weight *per* unit area (*Table 3.26*, $P=0.002$; *Table 3.28*; $P= 0.093$ respectively) and the highest values were at 155 CP. Also, body weight gain tended to be increased linearly (*Table 3.25,* $P=0.079$) with increasing crude protein concentrations.

Increasing lysine level concentration gave a quadratic increase in body weight gain (*Table 3.25*, $P=0.024$) with the lowest body weight loss being achieved at 65g lysine/kg CP. The effect tended ($P>0.05$) to be larger at 21°C than at 32°C. Similarly, egg mass outputs tended ($P>0.05$) to increase with increasing lysine concentrations at 21°C but not at 32°C.

There were significant (*Table 3.26,* $P<0.05$) crude protein x lysine concentration interactions in egg mass and egg numbers. Increasing dietary lysine concentrations increased egg mass output and egg numbers at 150 and 190 g/kg crude protein, but there was no consistent effect at the lowest crude protein concentration (120 g/kg).

There was a significant difference ($P<0.001$) between different temperatures in the birds' feeding times (*Table 3.33*). The birds kept at 21°C (constant and cycling) ate 100% of their daily feed allowance in the first 10 hours compared with only 61% of the birds kept at 32°C. However, the birds kept at the cycling 32°C tended ($P>0.05$) to eat their daily allocation of feed faster than those kept at the constant 32°C. Increasing crude protein or dietary lysine concentrations gave no significant (*Table 3.33,* $P>0.05$) differences in bird feeding times.

**Table 3.25** Body weight change of broiler breeders (27-35 weeks of age) kept either at a constant 21, a constant 32, a daily fluctuating temperature (mean of 21°C) or a daily fluctuating temperature (mean of 32 °C) and fed on diets with three protein concentrations each with four concentrations of dietary L-lysine (Experiment 5)

| Temperatures ( °C ) Variables | Crude protein (CP) (g/kg diet) Lysine concentrations( g/kg CP ) | | | | | | | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 120 CP | | | | 155 CP | | | | 190 CP | | | | |
| | 40 | 52.5 | 65 | 90 | 40 | 52.5 | 65 | 90 | 40 | 52.5 | 65 | 90 | |
| **21 Constant** | | | | | | | | | | | | | |
| Body weight change (g /d/ hen ) | -8.35 | -2.07 | -4.42 | -0.69 | 2.39 | -6.10 | 5.72 | 1.11 | -5.44 | 3.89 | 3.12 | -0.48 | -0.94 |
| **21 Cycling** | | | | | | | | | | | | | |
| Body weight change (g /d/ hen ) | -1.99 | -9.64 | 6.73 | 0.26 | -8.06 | 3.61 | 0.75 | 5.81 | -7.34 | 2.19 | 10.56 | -0.96 | 0.16 |
| **32 Constant** | | | | | | | | | | | | | |
| Body weight change (g /d/ hen ) | -22.48 | -7.90 | -1.95 | -12.14 | -13.04 | -21.57 | -6.78 | -3.00 | -16.32 | -9.60 | -23.16 | 2.98 | -11.25 |
| **32 Cycling** | | | | | | | | | | | | | |
| Body weight change (g /d/ hen ) | -14.45 | -10.59 | -8.94 | -18.99 | -22.79 | -7.04 | -12.08 | -18.56 | -2.16 | -3.37 | -0.53 | -10.38 | -10.82 |
| Mean dietary effects | | | | | | | | | | | | | |
| Body weight change (g /d/ hen ) | -11.82 | -7.55 | -2.17 | -7.89 | -10.37 | -7.78 | -3.10 | -3.66 | -7.82 | -1.72 | -2.50 | -2.21 | |

**Table 3. 25** *( Continue)*

| | Statistical significance and SEM of treatment means | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Temperature (T) | | Lysine (L) | | CP | | T x L | T x CP | CP x L | T x CP x L | |
| | ( *n*= 4 ) | | ( *n*=48) | | ( *n*=64) | | ( *n*=12) | ( *n*=16) | ( *n*=16) | ( *n*=4) | |
| | P | SEM | P | SEM | P | SEM | P | P | P | P | SEM |
| Body weight change (g /d/ hen ) | P=0.004 | 2.202 | Quadratic (P=0.024) | 1.744 | Linear (P=0.079) | 1.510 | Linear (P=0.075) | P>0.10 | P>0.10 | Quad.Lin (P=0.033) | 6.190 |

**Table 3.26** Egg laying performance; total egg mass output, egg weight and egg number of broiler breeders (27-35 weeks of age) kept either at a constant 21, a constant 32, a daily fluctuating temperature (mean of 21°C ) or a daily fluctuating temperature (mean of 32 °C) and fed on diets with three protein concentrations each with four concentrations of dietary L-lysine (Experiment 5)

| Temperatures (°C) | Variables | Crude protein CP (g/kg diet) | | | | | | | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lysine concentrations( g/kg CP) | | | | | | | | | | | | |
| | | 120 CP | | | | 155 CP | | | | 190 CP | | | | |
| | | 40 | 52.5 | 65 | 90 | 40 | 52.5 | 65 | 90 | 40 | 52.5 | 65 | 90 | |
| **21Constant** | | | | | | | | | | | | | | |
| | Egg mass ( g/hen/d) | 53.54 | 33.66 | 49.23 | 35.16 | 31.41 | 51.69 | 54.61 | 41.92 | 42.85 | 49.20 | 40.81 | 60.52 | 45.38 |
| | Egg weight (g/d) | 58.73 | 57.92 | 58.81 | 55.41 | 56.15 | 60.74 | 61.78 | 62.21 | 60.68 | 61.10 | 56.14 | 63.29 | 59.42 |
| | Egg number (no./hen/d) | 0.91 | 0.58 | 0.85 | 0.63 | 0.57 | 0.85 | 0.89 | 0.67 | 0.71 | 0.81 | 0.72 | 0.96 | 0.76 |
| **21 Cycling** | | | | | | | | | | | | | | |
| | Egg mass ( g/hen/d) | 38.50 | 44.25 | 49.97 | 55.76 | 49.63 | 44.25 | 42.18 | 53.52 | 46.09 | 45.45 | 46.99 | 48.29 | 47.11 |
| | Egg weight (g/d) | 55.87 | 59.50 | 56.00 | 58.26 | 62.76 | 60.24 | 58.48 | 58.79 | 57.86 | 60.54 | 57.63 | 56.95 | 58.57 |
| | Egg number (no./hen/d) | 0.69 | 0.74 | 0.89 | 0.96 | 0.79 | 0.74 | 0.71 | 0.91 | 0.80 | 0.75 | 0.82 | 0.85 | 0.80 |
| **32 Constant** | | | | | | | | | | | | | | |
| | Egg mass ( g/hen/d) | 46.65 | 37.71 | 41.72 | 35.64 | 29.89 | 25.15 | 39.84 | 35.82 | 28.35 | 44.03 | 36.11 | 41.07 | 36.83 |
| | Egg weight (g/d) | 57.32 | 55.65 | 52.74 | 50.71 | 60.14 | 52.32 | 53.98 | 52.57 | 53.09 | 57.08 | 56.25 | 53.79 | 54.64 |
| | Egg number (no./hen/d) | 0.82 | 0.68 | 079 | 0.67 | 0.54 | 0.47 | 0.74 | 0.66 | 0.52 | 0.78 | 0.64 | 0.75 | 0.67 |
| **32 Cycling** | | | | | | | | | | | | | | |
| | Egg mass ( g/hen/d) | 36.41 | 27.64 | 33.35 | 31.16 | 44.09 | 39.54 | 40.66 | 31.82 | 26.71 | 22.93 | 43.53 | 39.63 | 34.79 |
| | Egg weight (g/d) | 53.61 | 50.22 | 50.46 | 55.73 | 60.90 | 55.12 | 58.46 | 56.65 | 54.37 | 48.29 | 54.88 | 52.55 | 54.27 |
| | Egg number (no./hen/d) | 0.68 | 0.54 | 0.65 | 0.56 | 0.72 | 0.72 | 0.69 | 0.55 | 0.49 | 0.48 | 0.79 | 0.75 | 0.63 |

**Table 3.26** *( Continue)*

| | 120 CP | | | | 155 CP | | | | 190 CP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **40** | **52.5** | **65** | **90** | **40** | **52.5** | **65** | **90** | **40** | **52.5** | **65** | **90** |
| Mean dietary effects | | | | | | | | | | | | |
| Egg mass ( g/hen/d) | 43.78 | 35.82 | 43.57 | 39.43 | 38.75 | 40.28 | 44.32 | 40.77 | 36.00 | 40.40 | 41.86 | 47.38 |
| Egg weight (g/d) | 56.38 | 55.82 | 54.51 | 55.03 | 59.99 | 57.11 | 58.17 | 57.56 | 56.50 | 56.75 | 56.22 | 56.65 |
| Egg number (no./hen/d) | 0.77 | 0.63 | 0.79 | 0.71 | 0.66 | 0.69 | 0.76 | 0.70 | 0.63 | 0.70 | 0.74 | 0.83 |

Statistical significance and SEM of treatment means

| | Temperature (T) ( *n*= 4 ) | | Lysine (L) ( *n*=48) | | CP ( *n*=64) | | T x L ( *n*=12) | T x CP ( *n*=16) | CP x L ( *n*=16) | T x CP x L ( *n*=4) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | SEM | P | SEM | P | SEM | P | P | P | P | SEM |
| Egg mass ( g/hen/d) | P<0.001 | 1.783 | Linear (P=0.062) | 1.477 | P>0.10 | 1.279 | P>0.10 | Quadratic (P=0.048) | Lin.Lin (P=0.011) | Lin.Lin (P=0.015) | 5.212 |
| Egg weight (g/d) | P=0.018 | 1.167 | P>0.10 | 0.659 | Quadratic (P=0.002) | 0.571 | Cubic (P=0.061) | Quadratic (P=0.066) | P>0.10 | P>0.10 | 2.477 |
| Egg number (no./hen/d) | P=0.004 | 0.028 | Linear (P=0.031) | 0.024 | P>0.10 | 0.021 | P>0.10 | P>0.10 | Lin.Lin (P=0.009) | Lin.Lin (P=0.025) | 0.085 |

**Table 3. 27** Egg composition (expressed as a proportion of the egg weight); egg shell, egg yolk and egg albumen of broiler breeders (27-35 weeks of age) kept either at a constant 21, a constant 32, a daily fluctuating temperature (mean of 21°C) or a daily fluctuating temperature (mean of 32 °C) and fed on diets with three protein concentrations each with four concentrations of dietary L-lysine (Experiment 5)

| Temperatures ( °C ) | Variables | Crude protein CP (g/kg diet) | | | | | | | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lysine concentrations( g/kg CP ) | | | | | | | | | | | | |
| | | 120 CP | | | | 155 CP | | | | 190 CP | | | | |
| | | 40 | 52.5 | 65 | 90 | 40 | 52.5 | 65 | 90 | 40 | 52.5 | 65 | 90 | |
| **21 Constant** | | | | | | | | | | | | | | |
| | Egg shell | 0.093 | 0.103 | 0.092 | 0.092 | 0.095 | 0.088 | 0.090 | 0.088 | 0.096 | 0.088 | 0.097 | 0.098 | 0.094 |
| | Egg yolk | 0.279 | 0.283 | 0.295 | 0.295 | 0.280 | 0.281 | 0.288 | 0.296 | 0.285 | 0.273 | 0.271 | 0.278 | 0.284 |
| | Egg albumen | 0.628 | 0.614 | 0.612 | 0.609 | 0.625 | 0.631 | 0.623 | 0.616 | 0.619 | 0.639 | 0.633 | 0.624 | 0.623 |
| **21 Cycling** | | | | | | | | | | | | | | |
| | Egg shell | 0.094 | 0.083 | 0.097 | 0.099 | 0.095 | 0.097 | 0.094 | 0.095 | 0.095 | 0.091 | 0.096 | 0.089 | 0.094 |
| | Egg yolk | 0.290 | 0.298 | 0.296 | 0.296 | 0.280 | 0.286 | 0.290 | 0.297 | 0.315 | 0.293 | 0.280 | 0.308 | 0.294 |
| | Egg albumen | 0.616 | 0.619 | 0.607 | 0.605 | 0.625 | 0.617 | 0.617 | 0.609 | 0.590 | 0.617 | 0.624 | 0.604 | 0.612 |
| **32 Constant** | | | | | | | | | | | | | | |
| | Egg shell | 0.088 | 0.066 | 0.080 | 0.085 | 0.084 | 0.080 | 0.086 | 0.082 | 0.079 | 0.086 | 0.071 | 0.091 | 0.082 |
| | Egg yolk | 0.303 | 0.315 | 0.310 | 0.300 | 0.286 | 0.300 | 0.308 | 0.296 | 0.318 | 0.298 | 0.283 | 0.305 | 0.302 |
| | Egg albumen | 0.609 | 0.619 | 0.610 | 0.615 | 0.630 | 0.620 | 0.605 | 0.622 | 0.602 | 0.617 | 0.647 | 0.604 | 0.617 |
| **32 Cycling** | | | | | | | | | | | | | | |
| | Egg shell | 0.076 | 0.082 | 0.074 | 0.073 | 0.087 | 0.075 | 0.086 | 0.088 | 0.061 | 0.082 | 0.081 | 0.085 | 0.079 |
| | Egg yolk | 0.288 | 0.306 | 0.316 | 0.277 | 0.302 | 0.299 | 0.291 | 0.278 | 0.312 | 0.281 | 0.318 | 0.284 | 0.296 |
| | Egg albumen | 0.636 | 0.612 | 0.609 | 0.650 | 0.611 | 0.626 | 0.624 | 0.634 | 0.627 | 0.637 | 0.602 | 0.631 | 0.625 |

**Table 3.27** *( Continue)*

| | 120 CP | | | | 155 CP | | | | 190 CP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **40** | **52.5** | **65** | **90** | **40** | **52.5** | **65** | **90** | **40** | **52.5** | **65** | **90** |
| Mean dietary effects | | | | | | | | | | | | |
| Egg shell | 0.088 | 0.084 | 0.086 | 0.088 | 0.090 | 0.085 | 0.089 | 0.088 | 0.083 | 0.087 | 0.086 | 0.091 |
| Egg yolk | 0.290 | 0.301 | 0.305 | 0.292 | 0.287 | 0.292 | 0.294 | 0.292 | 0.308 | 0.286 | 0.288 | 0.294 |
| Egg albumen | 0.622 | 0.616 | 0.610 | 0.620 | 0.623 | 0.623 | 0.617 | 0.620 | 0.610 | 0.627 | 0.626 | 0.616 |

Statistical significance and SEM of treatment means

| | Temperature (T) ( *n*= 4 ) | | Lysine (L) ( *n*=48) | | CP ( *n*=64) | | T x L ( *n*=12) | T x CP ( *n*=16) | CP x L ( *n*=16) | T x CP x L ( *n*=4) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | SEM | P | SEM | P | SEM | P | P | P | P | SEM |
| Egg shell | P<0.001 | 0.0199 | P>0.10 | 0.0133 | P>0.10 | 0.0116 | P>0.10 | Quad.Lin (P=0.025) | P>0.10 | Lin.Lin (P=0.037) | 0.0486 |
| Egg yolk | P>0.10 | 0.0498 | P>0.10 | 0.0247 | P>0.10 | 0.0214 | Linear (P=0.018) | P>0.10 | Quad.Lin (P<0.001) | P>0.10 | 0.0960 |
| Egg albumen | P>0.10 | 0.0522 | P>0.10 | 0.0269 | P>0.10 | 0.0233 | Quadratic (P=0.020) | P>0.10 | Quad.Lin (P<0.001) | P=0.082 | 0.1034 |

**Table 3.28** Egg shell strength; egg deformation and shell weight per unit area of broiler breeders (27-35 weeks of age) kept either at a constant 21, a constant 32, a daily fluctuating temperature (mean of 21°C) or a daily fluctuating temperature (mean of 32 °C) and fed on diets with three protein concentrations each with four concentrations of dietary L-lysine (Experiment 5)

| Temperatures ( °C ) | Variables | Crude protein CP (g/kg diet) | | | | | | | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lysine concentrations( g/kg CP ) | | | | | | | | | | | | |
| | | 120 CP | | | | 155 CP | | | | 190 CP | | | | |
| | | 40 | 52.5 | 65 | 90 | 40 | 52.5 | 65 | 90 | 40 | 52.5 | 65 | 90 | |
| **21 Constant** | | | | | | | | | | | | | | |
| | Egg deformation(μm) | 26.2 | 22.4 | 26.2 | 23.9 | 23.9 | 23.5 | 26.0 | 27.7 | 24.4 | 28.3 | 24.8 | 24.3 | 25.1 |
| | Shell weight *per* unit area (mg/cm$^2$) | 79.0 | 86.5 | 78.3 | 80.5 | 79.6 | 76.2 | 77.0 | 76.9 | 80.3 | 76.2 | 82.6 | 85.4 | 79.9 |
| **21 Cycling** | | | | | | | | | | | | | | |
| | Egg deformation(μm) | 25.2 | 22.4 | 24.4 | 23.0 | 23.2 | 21.8 | 24.1 | 23.7 | 22.9 | 24.3 | 23.1 | 25.2 | 23.6 |
| | Shell weight *per* unit area (mg/cm$^2$) | 78.4 | 80.0 | 80.7 | 84.8 | 82.3 | 83.1 | 80.1 | 80.3 | 80.3 | 78.6 | 81.9 | 74.7 | 80.4 |
| **32 Constant** | | | | | | | | | | | | | | |
| | Egg deformation(μm) | 24.0 | 35.5 | 35.7 | 24.9 | 28.7 | 31.7 | 29.8 | 34.8 | 36.9 | 27.2 | 32.6 | 29.6 | 31.0 |
| | Shell weight *per* unit area (mg/cm$^2$) | 74.4 | 55.2 | 66.1 | 71.0 | 72.9 | 67.1 | 71.4 | 67.3 | 63.5 | 72.2 | 58.9 | 76.1 | 68.0 |
| **32 Cycling** | | | | | | | | | | | | | | |
| | Egg deformation(μm) | 33.4 | 27.3 | 33.8 | 39.7 | 30.3 | 41.0 | 31.2 | 31.3 | 33.2 | 29.6 | 35.6 | 32.1 | 33.2 |
| | Shell weight *per* unit area (mg/cm$^2$) | 62.1 | 69.2 | 60.0 | 61.5 | 76.6 | 62.4 | 73.6 | 71.5 | 49.4 | 66.2 | 66.7 | 71.5 | 65.9 |

**Table 3.28** *( Continue)*

| | 120 CP | | | | 155 CP | | | | 190 CP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **40** | **52.5** | **65** | **90** | **40** | **52.5** | **65** | **90** | **40** | **52.5** | **65** | **90** |
| Mean dietary effects | | | | | | | | | | | | |
| Egg deformation(μm) | 27.2 | 26.9 | 30.0 | 27.9 | 26.5 | 29.5 | 27.8 | 29.4 | 29.4 | 27.4 | 29.0 | 27.8 |
| Shell weight *per* unit area ($mg/cm^2$) | 73.5 | 72.7 | 71.3 | 74.5 | 77.8 | 72.2 | 75.5 | 74.0 | 68.4 | 73.3 | 72.5 | 76.9 |

Statistical significance and SEM of treatment means

| | Temperature (T) ( $n$= 4 ) | | Lysine (L) ( $n$=48) | | CP ( $n$=64) | | T x L ( $n$=12) | T x CP ( $n$=16) | CP x L ( $n$=16) | T x CP x L ( $n$=4) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | SEM | P | SEM | P | SEM | P | P | P | P | SEM |
| Egg deformation (μm) | P<0.001 | 1.24 | P>0.10 | 0.65 | P>0.10 | 0.56 | P>0.10 | P>0.10 | P>0.10 | Quad.Lin (P<0.001) | 2.48 |
| Shell weight *per* unit area ($mg/cm^2$) | P<0.001 | 1.59 | P>0.10 | 1.12 | Quadratic (P=0.093) | 0.97 | P>0.10 | Quadratic (P=0.012) | Lin.Quad (P=0.044) | Lin.Lin (P=0.020) | 4.03 |

**Table 3.29** Abdominal fatness caliper measurements of broiler breeders (27-35 weeks of age) kept either at a constant 21, a constant 32, a daily fluctuating temperature (mean of 21°C) or a daily fluctuating temperature (mean of 32 °C) and fed on diets with three protein concentrations each with four concentrations of dietary L-lysine (Experiment 5)

| Temperatures ( °C ) Variables | Crude protein CP(g/kg diet) Lysine concentrations( g/kg CP ) | | | | | | | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 120 CP | | | | 155 CP | | | | 190 CP | | | | |
| | 40 | 52.5 | 65 | 90 | 40 | 52.5 | 65 | 90 | 40 | 52.5 | 65 | 90 | |
| **21 Constant** | | | | | | | | | | | | | |
| Caliper measurements (mm) | 4.2 | 5.3 | 2.9 | 5.5 | 3.9 | 4.5 | 4.1 | 4.0 | 4.9 | 3.8 | 3.9 | 4.5 | 4.3 |
| **21 Cycling** | | | | | | | | | | | | | |
| Caliper measurements (mm) | 3.9 | 3.5 | 3.6 | 4.4 | 4.1 | 4.2 | 4.9 | 4.1 | 5.7 | 3.8 | 3.7 | 4.4 | 4.2 |
| **32 Constant** | | | | | | | | | | | | | |
| Caliper measurements (mm) | 4.1 | 5.1 | 4.1 | 4.2 | 4.2 | 4.9 | 4.1 | 3.5 | 4.4 | 4.4 | 4.2 | 4.7 | 4.3 |
| **32 Cycling** | | | | | | | | | | | | | |
| Caliper measurements (mm) | 5.4 | 5.3 | 4.8 | 5.2 | 5.4 | 4.8 | 4.9 | 4.6 | 4.7 | 5.3 | 5.0 | 4.5 | 4.9 |
| Mean dietary effects | | | | | | | | | | | | | |
| Caliper measurements (mm) | 4.4 | 4.8 | 3.9 | 4.8 | 4.4 | 4.6 | 4.5 | 4.1 | 4.9 | 4.3 | 4.2 | 4.5 | |

**Table 3.29** *( Continue)*

| | Statistical significance and SEM of treatment means | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Temperature (T) | | Lysine (L) | | CP | | T x L | T x CP | CP x L | T x CP x L | |
| | ( *n*= 4 ) | | ( *n*=48) | | ( *n*=64) | | ( *n*=12) | ( *n*=16) | ( *n*=16) | ( *n*=4) | |
| | P | SEM | P | SEM | P | SEM | P | P | P | P | SEM |
| Caliper measurements (mm) | P>0.10 | 0.24 | P>0.10 | 0.18 | P>0.10 | 0.16 | P>0.10 | Quadratic (P=0.075) | P>0.10 | P>0.10 | 0.65 |

**Table 3.30** Blood components of broiler breeders (27-35 weeks of age) kept either at a constant 21, a constant 32, a daily fluctuating temperature (mean of 21°C) or a daily fluctuating temperature (mean of 32 °C) and fed on diets with three protein concentrations supplied with 65 g/kg dietary L-lysine concentrations (Experiment 5)

| Temperatures (°C) | Variables | Crude protein (g/kg diet) | | | Mean temperature effects |
|---|---|---|---|---|---|
| | | **120** | **155** | **190** | |
| **21 Constant** | | | | | |
| | Whole blood pH | 6.95 | 7.02 | 7.01 | 6.99 |
| | Plasma blood pH | 7.18 | 7.20 | 7.18 | 7.19 |
| | Whole blood viscosity (cP) | 3.36 | 3.21 | 3.30 | 3.29 |
| | Plasma blood viscosity (cP) | 1.93 | 1.87 | 1.65 | 1.82 |
| | Haemoglobin (g/dL) | 8.60 | 8.95 | 8.50 | 8.68 |
| **21 Cycling** | | | | | |
| | Whole blood pH | 7.01 | 7.08 | 7.01 | 7.03 |
| | Plasma blood pH | 7.17 | 7.23 | 7.15 | 7.18 |
| | Whole blood viscosity (cP) | 3.07 | 2.65 | 3.63 | 3.12 |
| | Plasma blood viscosity (cP) | 1.85 | 1.51 | 1.64 | 1.67 |
| | Haemoglobin (g/dL) | 7.90 | 7.35 | 8.70 | 7.98 |
| **32 Constant** | | | | | |
| | Whole blood pH | 6.88 | 6.96 | 6.98 | 6.94 |
| | Plasma blood pH | 7.15 | 7.20 | 7.20 | 7.18 |
| | Whole blood viscosity (cP) | 2.96 | 3.16 | 3.08 | 3.07 |
| | Plasma blood viscosity (cP) | 1.59 | 1.53 | 1.75 | 1.62 |
| | Haemoglobin (g/dL) | 6.95 | 7.60 | 10.50 | 8.35 |
| **32 Cycling** | | | | | |
| | Whole blood pH | 7.00 | 6.97 | 6.89 | 6.95 |
| | Plasma blood pH | 7.25 | 7.21 | 7.15 | 7.18 |
| | Whole blood viscosity (cP) | 3.15 | 3.43 | 3.06 | 3.22 |
| | Plasma blood viscosity (cP) | 1.92 | 1.74 | 1.50 | 1.72 |
| | Haemoglobin (g/dL) | 8.65 | 6.75 | 6.70 | 7.37 |

**Table 3.30** *( Continue)*

| | Mean dietary effects | | |
|---|---|---|---|
| Whole blood pH | 6.96 | 7.01 | 6.97 |
| Plasma blood pH | 7.19 | 7.21 | 7.17 |
| Whole blood viscosity (cP) | 3.14 | 3.11 | 3.27 |
| Plasma blood viscosity (cP) | 1.82 | 1.66 | 1.64 |
| Haemoglobin (g/dL) | 8.03 | 7.66 | 8.60 |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=2) | | Diet ($n$=8) | | Temperature x Diet interaction ($n$=2) | |
| | P | SEM | P | SEM | P | SEM |
| Whole blood pH | P>0.10 | 0.037 | P>0.10 | 0.031 | P>0.10 | 0.062 |
| Plasma blood pH | P>0.10 | 0.034 | P>0.10 | 0.028 | P>0.10 | 0.058 |
| Whole blood viscosity (cP) | P>0.10 | 0.128 | P>0.10 | 0.181 | P>0.10 | 0.322 |
| Plasma blood viscosity (cP) | P>0.10 | 0.072 | P>0.10 | 0.086 | P>0.10 | 0.158 |
| Haemoglobin (g/dL) | P>0.10 | 0.885 | P>0.10 | 0.631 | P>0.10 | 1.358 |

**Table 3.31** Blood electrolyte contents of broiler breeders (27-35 weeks of age) kept either at a constant 21, a constant 32, a daily fluctuating temperature (mean of 21°C) or a daily fluctuating temperature (mean of 32 °C) and fed on diets with three protein concentrations supplied with 65 g/kg dietary L-lysine concentrations (Experiment 5)

| Temperatures (°C) | Variables | Crude protein (g/kg diet) | | | Mean temperature effects |
|---|---|---|---|---|---|
| | | **120** | **155** | **190** | |
| **21 Constant** | | | | | |
| | Ca (mMol/L) | 6.5 | 6.6 | 7.04 | 6.7 |
| | P (mMol/L) | 2.4 | 1.9 | 2.57 | 2.3 |
| | K (mMol/L) | 9.8 | 9.0 | 9.1 | 9.3 |
| | Na (mMol/L) | 148.0 | 155.0 | 156.5 | 153.2 |
| **21 Cycling** | | | | | |
| | Ca (mMol/L) | 6.1 | 5.7 | 6.8 | 6.0 |
| | P (mMol/L) | 1.5 | 1.6 | 2.1 | 1.7 |
| | K (mMol/L) | 11.0 | 10.5 | 10.2 | 10.6 |
| | Na (mMol/L) | 144.0 | 151.0 | 140.0 | 145.0 |
| **32 Constant** | | | | | |
| | Ca (mMol/L) | 6.1 | 6.1 | 5.7 | 6.0 |
| | P (mMol/L) | 2.1 | 1.8 | 1.7 | 1.9 |
| | K (mMol/L) | 9.2 | 7.9 | 8.6 | 8.6 |
| | Na (mMol/L) | 152.5 | 160.0 | 159.5 | 157.3 |
| **32 Cycling** | | | | | |
| | Ca (mMol/L) | 5.7 | 5.7 | 4.9 | 5.4 |
| | P (mMol/L) | 1.5 | 1.3 | 1.1 | 1.3 |
| | K (mMol/L) | 6.6 | 9.4 | 8.5 | 8.2 |
| | Na (mMol/L) | 149.5 | 145.5 | 149.5 | 148.2 |

**Table 3.31** *( Continue)*

| | Mean dietary effects | | |
|---|---|---|---|
| Ca (mMol/L) | 6.1 | 6.0 | 6.1 |
| P (mMol/L) | 1.9 | 1.7 | 1.9 |
| K (mMol/L) | 9.1 | 9.2 | 9.1 |
| Na (mMol/L) | 149.5 | 152.9 | 151.4 |

| | Statistical significance and SEM of treatment means | | | | | |
|---|---|---|---|---|---|---|
| | Temperature ($n$=2) | | Diet ($n$=8) | | Temperature x Diet interaction ($n$=2) | |
| | P | SEM | P | SEM | P | SEM |
| Ca (mMol/L) | P>0.10 | 0.36 | P>0.10 | 0.26 | P>0.10 | 0.56 |
| P (mMol/L) | P>0.10 | 0.23 | P>0.10 | 0.10 | Linear (P=0.081) | 0.29 |
| K (mMol/L) | P>0.10 | 0.58 | P>0.10 | 0.55 | P>0.10 | 1.07 |
| Na (mMol/L) | P>0.10 | 10.84 | P>0.10 | 12.00 | P>0.10 | 22.40 |

**Table 3.32** Body temperatures and range (highest minus lowest) of the diurnal change in body temperature of broiler breeders (27-35 weeks of age) kept either at a constant 21, a constant 32, a daily fluctuating temperature (mean of 21°C ) or a daily fluctuating temperature (mean of 32 °C) and fed on diets with three protein concentrations each with four concentrations of dietary L-lysine (Experiment 5)

| Temperatures (°C) | Variables | Crude protein CP (g/kg diet) | | | | | | | | | | | | Mean temperature effects |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lysine concentrations( g/kg CP) | | | | | | | | | | | | |
| | | 120 CP | | | | 155 CP | | | | 190 CP | | | | |
| | | 40 | 52.5 | 65 | 90 | 40 | 52.5 | 65 | 90 | 40 | 52.5 | 65 | 90 | |
| **21 Constant** | | | | | | | | | | | | | | |
| Daily mean body temperature (°C) | | 41.2 | 41.3 | 41.2 | 41.3 | 41.3 | 41.3 | 41.3 | 41.4 | 41.5 | 41.3 | 41.2 | 41.5 | 41.3 |
| Range of body temperature (°C) | | 0.08 | 0.12 | 0.25 | 0.18 | 0.29 | 0.10 | 0.00 | 0.13 | 0.30 | 0.35 | 0.35 | 0.22 | 0.20 |
| **21 Cycling** | | | | | | | | | | | | | | |
| Daily mean body temperature (°C) | | 41.3 | 41.3 | 41.2 | 41.2 | 41.3 | 41.0 | 41.3 | 41.3 | 41.3 | 41.2 | 41.4 | 41.3 | 41.3 |
| Range of body temperature (°C) | | 0.60 | 0.70 | 0.45 | 0.53 | 0.63 | 0.43 | 0.63 | 0.50 | 0.43 | 0.47 | 0.93 | 0.75 | 0.59 |
| **32 Constant** | | | | | | | | | | | | | | |
| Daily mean body temperature (°C) | | 42.8 | 42.1 | 41.9 | 42.6 | 42.2 | 42.3 | 42.6 | 42.4 | 42.6 | 42.0 | 42.6 | 42.2 | 42.4 |
| Range of body temperature (°C) | | 0.95 | 0.35 | 0.15 | 0.55 | 0.07 | 0.22 | 0.33 | 0.47 | 0.88 | 0.17 | 0.25 | 1.27 | 0.47 |
| **32 Cycling** | | | | | | | | | | | | | | |
| Daily mean body temperature (°C) | | 41.8 | 41.9 | 42.2 | 42.8 | 41.8 | 41.8 | 42.1 | 42.4 | 42.4 | 42.4 | 41.9 | 42.6 | 42.2 |
| Range of body temperature (°C) | | 0.52 | 0.50 | 0.70 | 0.55 | 0.41 | 0.53 | 0.58 | 0.51 | 0.91 | 0.28 | 0.43 | 0.48 | 0.53 |

**Table 3.32** *( Continue)*

| | 120 CP | | | | 155 CP | | | | 190 CP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **40** | **52.5** | **65** | **90** | **40** | **52.5** | **65** | **90** | **40** | **52.5** | **65** | **90** |
| Mean dietary effects | | | | | | | | | | | | |
| Daily mean body temperature (°C) | 41.7 | 41.7 | 41.6 | 42.0 | 41.7 | 41.6 | 41.8 | 41.9 | 41.9 | 41.7 | 41.8 | 41.9 |
| Range of body temperature (°C) | 0.54 | 0.42 | 0.39 | 0.45 | 0.35 | 0.32 | 0.38 | 0.40 | 0.63 | 0.32 | 0.49 | 0.68 |

Statistical significance and SEM of treatment means

| | Temperature (T) ( *n*= 4 ) | | Lysine (L) ( *n*=48) | | CP ( *n*=64) | | T x L ( *n*=12) | T x CP ( *n*=16) | CP x L ( *n*=16) | T x CP x L ( *n*=4) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | SEM | P | SEM | P | SEM | P | P | P | P | SEM |
| Daily mean body temperature (°C) | P<0.001 | 0.06 | Quadratic (P=0.009) | 0.05 | P>0.10 | 0.04 | Linear (P=0.002) | P>0.10 | Quad.Quad (P=0.078) | Quad.Lin (P=0.019) | 0.17 |
| Range of body temperature (°C) | P<0.001 | 0.048 | Quadratic (P=0.005) | 0.037 | Quadratic (P=0.002) | 0.032 | Quadratic (P<0.001) | Quadratic (P=0.081) | Quad.Quad (P=0.064) | Lin.Lin (P<0.001) | 0.131 |

**Table 3.33** Feeding time (expressed as number of hens to eat all their daily allocation of feed within 3 time periods) of broiler breeders (27-35 weeks of age) kept either at a constant 21°C, a constant 32°C, a daily fluctuating temperature (mean of 21°C) or a daily fluctuating temperature (mean of 32°C) and fed on diets with three protein concentrations each with four concentrations of dietary L-lysine (Experiment 5).

| Treatments | Number of hens to eat all their daily allocation of feed | | | $\chi^2$ | *P* |
|---|---|---|---|---|---|
| | **<2 hrs.** | **2.1-10 hrs.** | **>10.1 hrs.** | | |
| Temperatures (°C) | | | | | |
| **21 Constant** | 7 | 13 | 0 | | |
| **21 Cycling** | 12 | 12 | 0 | | |
| **32 Constant** | 0 | 9 | 9 | | |
| **32 Cycling** | 3 | 13 | 7 | | |
| | | | | 31.94 | P<0.001 |
| Crude protein (CP) (g/kg diet) | | | | | |
| **120 CP** | 8 | 14 | 4 | | |
| **155 CP** | 10 | 12 | 7 | | |
| **190 CP** | 5 | 20 | 5 | | |
| | | | | 4.34 | NS (P>0.05) |
| Lysine concentrations ( g/kg CP) | | | | | |
| **40** | 3 | 11 | 5 | | |
| **52.5** | 7 | 11 | 4 | | |
| **65** | 6 | 13 | 4 | | |
| **90** | 7 | 11 | 3 | | |
| | | | | 2.37 | NS (P>0.05) |

### 3.5.4 Discussion

The major objectives of this experiment were to examine the effects of 12 diets that differed in their lysine concentrations and crude protein concentrations fed to broiler breeders when kept at four ambient temperatures (two constant temperatures and two cycling regimens).

As in the previous experiments, increasing temperature gave a decrease ($P<0.001$) in egg mass output. There was 18% decrease at 32°C compared to the 21°C group and a decrease of this order was expected (Section 2.2). There was an unexpected difference in rectal temperature in the birds kept at 32°C (constant) compared to 21°C (constant). The difference was 1.0°C compared to 0.34°C in experiment 3. This indicates that there may have been a higher heat load on the birds than was experienced in the previous experiment although environmental factors such as temperature and relative humidity were apparently similar between the two experiments.

There were no significant ($P>0.05$) differences in egg mass output between constant and cycling temperature regimens in the present experiment. At 21°C, a reduction of 6% in egg mass output would have been expected. A difference of this relatively small magnitude would be difficult to detect in this experiment because of the high unexpected variation relative to the amount of treatment replication that was available. However, there was also no observed difference between 32°C constant and the mean 32°C cycling regimen. The review in section 2.2 indicated that the egg mass output of birds kept in cycling regimens was generally greater than the mean temperature response. The data set examined in section 2.2 did not extend to 35°C, one of the temperatures used in the present experiment, so it was not possible to directly predict

the response to this cycling temperature regimen. However, if extrapolate the response curve described in section 2.2 it indicates that egg production would have ceased at a constant 35°C. If the assumption was made that birds at 35°C have no ability for egg mass production, the predicted response (using the equation derived in section 2.2) of the birds give the cycling temperature regimen in the present experiment would be close to the observed results.

The increasing dietary crude protein concentrations in the present experiment gave an increase in egg weight ($P=0.002$), no increase in egg mass output ($P>0.05$) and a tendency for a decrease in body weight loss ($P=0.079$). This agrees with the work of Spratt and Leeson (1987) and Joseph *et al.* (2000) who also examined these dietary responses in young laying broiler breeders. The data suggest that the lower protein intakes failed to meet the birds requirements for protein accretion.

There was a significant quadratic ($P<0.05$) crude protein x temperature interaction in egg mass output, although this was due to some differences in response between the constant and cycling 32°C treatments that were not consistent with increasing temperature. Cowan and Michie (1980) examined the response of commercial egg laying birds to dietary protein concentration at various ambient temperatures. They found that, although increasing dietary protein increased ($P<0.001$) egg mass output, there were no ($P>0.05$) temperature x protein interactions. Evidence from published information concluded that there was no temperature x protein interactions (Section 2.2.3).

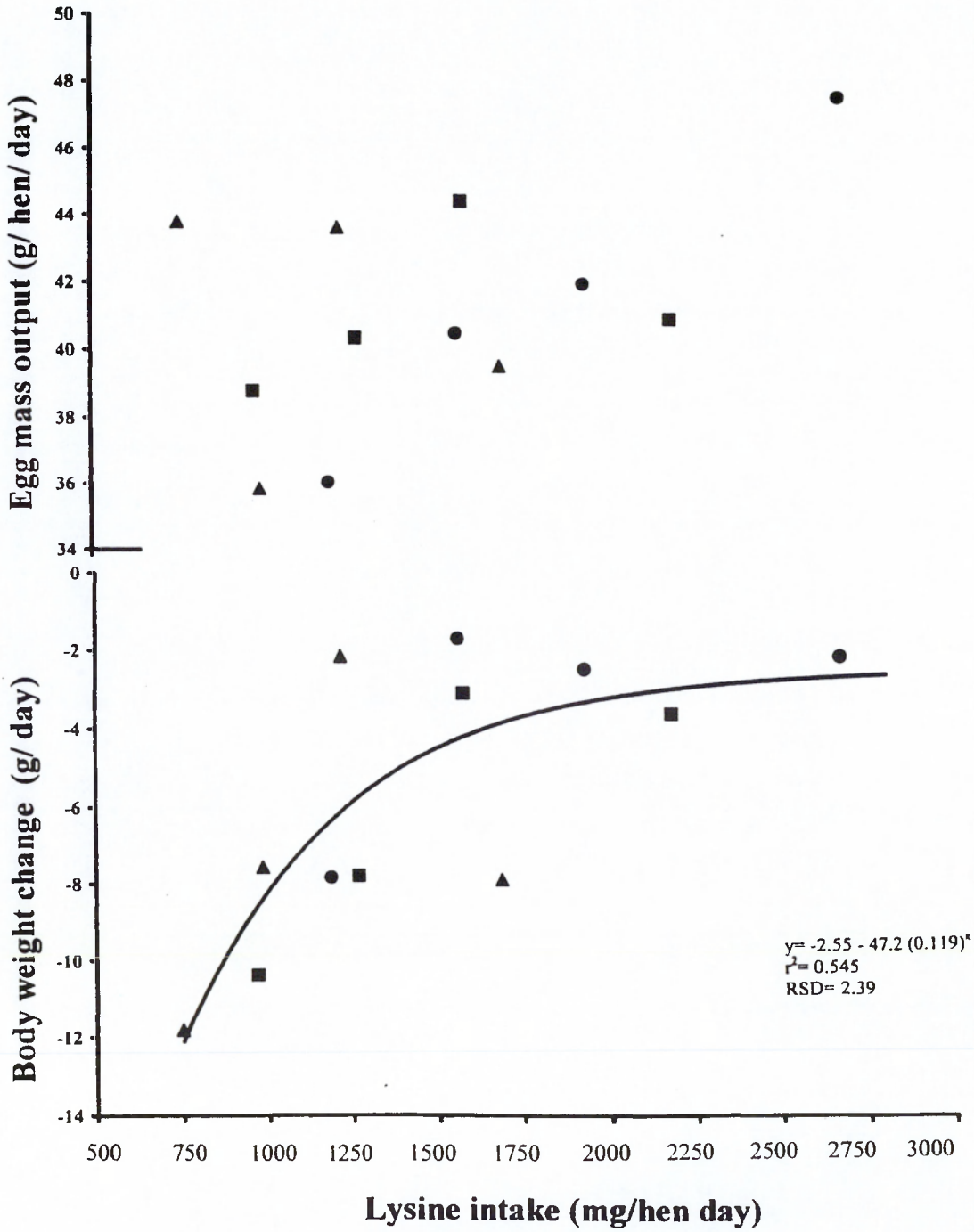
Increasing dietary intakes of lysine gave a quadratic reduction ($P<0.05$) in body weight loss with an optimum of 65g lysine/kg CP and a tendency ($P=0.062$) for a similar response in egg mass output. The reason for this difference in response is not

clear. The only observed differences between the two experiments were the high body temperatures in the birds at 32°C in the present experiment. In this situation, it is possible that broiler breeders were more sensitive to the amino acid balance and so an optimum amino acid concentration could be demonstrated.

Although a statistically significant ($P<0.05$) crude protein x lysine interaction in egg mass output was indicated in this experiment, there were no consistent differences in the responses to increasing lysine concentration at different crude protein concentrations. The data thus appear to support the proposal by Morris *et al.*, (1999) that describing limiting amino acid response as a proportion of the crude protein supply is a good means of detecting responses. An alternative method would be to describe requirements as a daily intake of each amino acid. An examination of this approach with the present data was undertaken in a regression analysis (*Figure 3.12*). There was no significant relationship between daily intake of lysine and egg mass output. There was however, an asymptotic relationship with body weight change (*Figure 3.12*) that indicated that the major reduction of the body weight loss was achieved with an intake of approximately 2000 mg of lysine per hen per day. It is thus difficult to decide from these data which method of describing the response to lysine supply (dietary intake versus proportion of the crude protein) is preferable.

In conclusion this experiment has give expected differences in hatching egg production of broiler breeders kept at different temperatures. Low protein diets appeared to fail to meet the birds requirements for protein accretion The experiment gave evidence that expressing amino acid concentrations as a proportion of the crude protein supply gave a satisfactory fit to die data.

**Figure 3.12** Relationships between dietary lysine intake and egg mass output; body weight change of broiler breeders fed 12 diets that differed in their lysine concentration and crude protein concentrations (▲= 120 CP, ■ = 155 CP, ● = 190 CP) in experiment 5



# Chapter 4

# 4. General Discussion

## 4.1 Responses to ambient temperature

A main objective at the start of this project was to describe and quantify the effect of ambient temperature on the egg production performance and hatching egg quality of broiler breeders. Although there were large numbers of published experiments that have examined the effects of the temperature on commercial egg laying birds, there were a number of areas that required further examination. First, there was a lack of a recent summary of the experimental results that have examined all previous research evidence. Second, there are substantial differences in body composition and management methods between commercial egg laying birds and broiler breeders, and it has not been established whether the two strains of chicken respond similarly to different temperatures. Third, although it has been established that high temperatures reduce shell strength, there was a lack of evidence on their effects on hatching egg quality and subsequent chick quality.

A quantitative evaluation of the effect of ambient temperature (constant or cycling) was shown in section 2.2 and described biologically logical effects. At constant temperatures, an exponential curve with the addition of linear trend gave the best ($P<0.001$) description of egg production variables. The information provided by this model of the responses of laying hens to constant temperatures has a number of uses: The results can be used by commercial egg producers to evaluate the most economically

efficient temperatures to run their egg production units. Second, it allows researchers to compare their data with the mean response of all other published papers.

The quantitative literature evaluation gave a second important finding: The egg mass outputs of birds given cycling regimens at high temperatures were greater than would be predicted from the egg outputs of the mean temperatures. The model that was developed indicated that a number of characteristics of the cycling temperature regimen need to be known to be able to predict the responses of the birds. Practical egg production in high temperature environments almost invariably involves operating with high amplitude daily cycling temperatures. Understanding the factors that affect the responses of the birds will help egg producers predict the changes in egg outputs of their birds.

Experiments 1, 2, 3, and 5 in the present study compared the egg mass outputs of broiler breeders kept at constant high temperatures with hens kept at a constant 21°C. In all four experiments, the differences in responses of the birds to the high temperatures were similar to those predicted from the quantitative review of the responses of egg-laying strains. The project therefore has demonstrated a similarity in the responses between egg-laying strains and meat-line strains of laying hens. Experiment 5 included treatments in which cycling temperatures regimens were compared and responses were obtained that, although were not different to the equivalent constant temperatures, were consistent with the expected response of egg-laying strains.

The broiler breeders were restrictedly fed throughout all experiments and they ate their daily allocation of feed at all ambient temperatures. However, high temperatures gave some evidence of insensible heat loss (panting) due to high body temperatures. There is evidence that the efficiency of feed utilization decreases rapidly after the point of heat stress is reached in poultry (Howlider and Rose, 1987) due to the increased

energy requirements of panting and other forms of insensible heat loss. The poor feed utilization of weight loss of the breeders kept at high temperatures in the present experiment was probably also a consequence of some amount of insensible heat loss.

The effects of different ambient temperatures on hatching egg quality and chick quality has produced useful new information. The breeder hens kept at high ambient temperatures produced eggs with less shell and a weaker shell. However, it did not appear that these differences affected shell porosity or affected the hatchability or viability of the hatched chicks. One experiment indicated that flocks kept at high temperatures had a lower (P=0.007) fertility. A reduced mating activity of the birds in these flocks may have given this effect. In general, different ambient temperatures had no effects on hatching egg quality and chick quality. The major effects of temperature that would affect the profitability of a broiler breeder unit were the effect on egg mass outputs and egg weights.

## 4.2 Responses to dietary lysine supply

The quantitative literature review indicated that there was a curvilinear response in the egg mass outputs of laying hens given increasing dietary amounts of different limiting amino acids. The statistical analysis indicated that the greatest precision in predicting the response was obtained by describing limiting amino acid concentrations as a proportion of the crude protein supply. This supported the proposal of Morris *et al.*(1999) and for these reasons, the dietary lysine concentrations used in the present experiments were described in these units.

The responses of the breeder hens to dietary lysine supply in the present study were relatively smaller than observed for the effect of temperature. There was, however, a trend ($P<0.1$) for a curvilinear response in egg mass output in most (three out of four) of the experiments in this project. The lack of response to dietary lysine in experiment 1 was probably due to the low egg mass outputs obtained from this flock. Prochaska *et al.* (1996) observed that the treatment responses of hens given different dietary lysine concentrations were reduced in older birds with lower egg mass outputs compared to younger, higher-producing birds. McDonald (1979) also observed that laying hen strains with high egg mass outputs had a greater magnitude of response in egg numbers and egg weights to dietary lysine supplementation compared to lower-producing strains. The flock of birds in experiment 1 of the present study was moved to Harper Adams at point of lay in comparison to being reared on site for the subsequent experiments. This disturbance was probably the major cause of their subsequent poor performance. The shape of the response curve was unexpected: The quantitative review of egg-laying strains to dietary lysine supply indicated an exponential response curve. Egg mass outputs increased towards an asymptote and increasing dietary lysine concentrations past this point did not decrease egg mass outputs. The response curve in the present broiler breeder experiments indicated that egg mass outputs may decrease at high lysine concentrations. A similar response curve has been found in the growth of broiler chickens to dietary lysine concentrations (Abebe and Morris, 1990a). In two of the present experiments, there were similar trends ($P<0.1$) for quadratic responses to dietary lysine concentration in the body weight changes of the breeder hens. These data therefore indicate that there was a poorer utilization of the feeds that contained high lysine concentrations. The breeder hens were fed restrictedly, so a poorer efficiency of

feed utilization would be expected to result in a decreased egg mass output and increased body weight loss. Most egg-laying strains of hens are fed *ad-libitum*. These birds would be able to increase their feed intakes to compensate for the poorer efficiency of utilization of the high lysine feeds and so avoid a decrease in their egg mass outputs.

Little previous work has examined the effect of dietary lysine supply on hatching egg quality and chick viability. The results of this project indicate diet not affect these quality variables.

## 4.3 Temperature x dietary lysine interactions

A third main objective of this project was to examine whether there were interactions between dietary lysine supply and temperature. Hatching egg producers in countries of the Arabian Gulf not only have to deal with high ambient temperatures but also have high unit costs for limiting amino acids. It is important to understand whether their flocks are more sensitive to dietary amino acid supply.

The results of these experiments gave some evidence that there were temperature effects on the response to dietary lysine. Experiment 3 included measurements on the body pool of lysine in the breeder hens. Increasing dietary lysine gave an increase in blood lysine concentration in birds kept at 21°C but no consistent differences in the birds kept at 32°C. The egg mass outputs of the birds kept at 21°C were significantly higher than those at 32°C, so these birds would have a greater metabolic demand for this amino acid. The imbalance in the dietary lysine pool at 21°C would be expected to ultimately affect the efficiency of the utilization of the feed. The birds kept at 32°C and

21°C had the same daily feed allocation and measurements indicated that all feed was eaten. A considerable amount of panting occurred in the birds kept at 32°C, especially in the periods after feeding. This requirement for insensible heat loss would increase the energy requirements for maintenance in these birds (Van Kampen, 1981). Consequently, given a fixed energy intake, the dietary amino acids would have been deaminated and used as an energy source. This would have effectively reduced the amino acids available to support egg mass output.

No temperature x lysine interactions were demonstrated in these relatively short-term experiments in egg production variables. However, there was a similar interaction ($P<0.05$) in body protein contents in experiment 3 and tendencies ($P<0.1$) for similar interactions in body fatness in experiment 3 and body weight change in experiment 5. The data indicate a greater potential effect of dietary lysine imbalance at low temperatures than at high temperatures. Rose and Salah Uddin (1997) observed a similar temperature x dietary lysine interaction in the growth response of broiler chickens.

In summary, the egg production characteristics and hatching egg quality responses of broiler breeder hens to dietary lysine supply were demonstrated to be approximately independent of ambient temperature. Hatching egg producers in hot climates can therefore adopt the dietary recommendations for lysine obtained from experiments conducted in cooler conditions. The project has, however, provided evidence that temperature x lysine interactions occur in the physiological pool of lysine and body weight and compositional changes of breeder hens. There is a risk that, in extended feeding periods, dietary imbalances of lysine could affect the efficiency of hatching egg production to a greater extent in cooler environments than in high ambient temperatures.

# Chapter 5

# 5. General Conclusions

- A quantitative literature review indicated that there was a curvilinear ($P<0.001$) response in most egg production variables for egg-laying strains of hen to different ambient temperatures. Egg mass outputs and egg weight decreased with increasing temperature but the decrease accelerated at temperatures above 27°C. There was a positive linear relationship ($P<0.001$) between egg shell strength and temperature.

- Cycling temperature regimens gave greater egg mass outputs by the egg-laying hen strains than would be predicted from the mean of the daily temperatures.

- A quantitative evaluation showed that the egg-laying responses of hens to a single limiting amino acids were curvilinear. Increases in dietary amino acid concentration gave small increases in egg number, egg weight and egg mass outputs until an asymptote was reached.

- Expressing dietary amino acid concentrations as a proportion of the crude protein supply reduced the unexplained variation in the published laying hen response data compared to expressing as a proportion of the total diet. However, there was no further advantage to expressing the amino acid concentration as a proportion of the ideal protein supply.

- The egg production (egg mass output and egg weight) response of broiler breeder hens to different ambient temperatures was shown to be similar to that demonstrated previously for egg-laying strain birds.

- Although increasing ambient temperatures for breeder hens decreased ($P=0.049$) the proportion of shell in their hatching eggs, there were no effects ($P>0.05$) on the hatchability or chick quality. One experiment indicated that high temperature decreased ($P<0.05$) the fertility of the hatching eggs, probably due to a reduced bird mating activity.

- There was no difference ($P>0.05$) between broiler breeder hens kept at 21°C or 32°C in the digestibility of amino acids or the metabolisability of the gross energy.

- Different dietary lysine concentrations ($P>0.05$) did not affect hatchability or chick quality.

- Increasing dietary lysine increased ($P=0.044$) blood lysine concentration and increased ($P=0.026$) blood packed cell volumes in the birds kept at 21°C but not in those kept at 32°C.

- Increasing dietary lysine concentrations in two experiments tended to give a curvilinear response ($P<0.1$) in body weight change of the hens with a similar

shape to that observed in the egg mass output response curve. A similar temperature x dietary lysine interaction ($P=0.031$) carcass protein content was observed.

- There was a positive ($P<0.001$) linear correlation between the cloacal calliper measurements and the proportion of abdominal fat. However, there remained a high proportion of unexplained variation ($r^2=0.171$), so the calliper technique did not give sufficient accuracy in the estimate of fatness in individual female meat-line birds.

# Chapter 6

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# Chapter 7

# 7. Appendices

7. 1A Composition of the standard broiler breeder diet fed in experiment 1

| Ingredients | Amount (kg) |
|---|---|
| Wheat | 720.0 |
| Maize gluten meal (600g/kg CP) | 10.0 |
| Dehulled soya bean meal | 110.0 |
| Fish meal | 20.0 |
| Maize germ meal | 40.0 |
| Limestone | 60.0 |
| Dicalcium phosphate | 14.0 |
| Sodium bicarbonate | 2.0 |
| Salt | 2.0 |
| L-lysine HCl | 1.0 |
| DL-methionine | 1.0 |
| Vitamin-Mineral Supplement[†] | 20.0 |
| Total | 1000.0 |
| **Nutrient composition:** | |
| Determined chemical analysis | |
| Crude Protein ( CP) (g/kg) | 157.0 |
| Lysine (g\kg CP) | 52.0 |
| Methionine (g\kg CP) | 30.0 |
| Tryptophan (g\kg CP) | 16.2 |
| Methionine+ Cystine (g\kg CP) | 39.5 |
| Threonine (g\kg CP) | 40.5 |
| Isoleucine (g\kg CP) | 43.0 |
| Arginine (g\kg CP) | 65.0 |
| Valine (g\kg CP) | 51.0 |
| Histidine (g\kg CP) | 24.5 |
| Calculated analysis | |
| Metabolisable Energy (MJ/kg) | 11.6 |
| Calcium (g\kg) | 31.7 |
| Phosphorus (g\kg) | 6.5 |
| Sodium (g\kg) | 1.9 |
| Potassium (g\kg) | 5.8 |
| Linoleic acid (g\kg) | 9.0 |
| Choline (mg\kg ) | 1154 |

[†]Supplied per kg of diet: *trans*-retinol(A), 4.8 mg; cholecalciferol(D3), 125µg; α-tocopherol acetate(E), 36.8 mg; thiamine(B1), 3 mg; riboflavin(B2), 10 mg; pyridoxine(B6), 5 mg; vitamin B12, 12 mg; nicotinic acid, 60 mg; pantothenic acid, 15 mg; folic acid, 2.5 mg; biotin, 205 µg; choline chloride, 500 mg; Fe, 20 mg; Co,1 mg; Mn, 100 mg; Cu, 10 mg; Zn, 80 mg; I, 2 mg; Se, 0.2 mg; Mo, 0.5 mg; Ca, 206g/kg; P, 100.5g/kg; Na, 50g/kg; Met., 50g/kg; Ash , 882g/kg.