

THE EFFECTS OF EXCESS DIETARY CRUDE PROTEIN ON THE
EFFICIENCY OF UTILIZATION OF PROTEIN BY BROILER CHICKENS

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This thesis is dedicated to
my wife Mercy and
my daughter Aba

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DECLARATION

I Harry Kofi Swatson hereby certify that the research reported in this thesis, has not been in its entirety or in part been previously submitted to any university or institution for the purpose of the award of a degree.

Signature *Harry Swatson* Date *31/3/2003*

I, Professor Rob Gous, chairperson of the supervisory committee, approve the release of this thesis for examination

Rob Gous
Signature Date *31/3/2003*

PREFACE

The investigations described in this thesis were conducted at the Ukulinga Research Farm, University of Natal, Pietermaritzburg in South Africa, under the supervision of Prof Rob Gous. The thesis consists of a series of investigations conducted over a period of four years and reported in six chapters, each of which was prepared as an individual manuscript with the aim of publishing them in scientific journals. Some Chapters are at various levels of the review process, whilst others are being modified for submission as a series on the effects of excess dietary protein on broiler performance and the efficiency of protein utilisation. Author citation and referencing are found at the end of the thesis.

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CHAPTER 1:

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

It has long been recognised that a broiler chicken can achieve its potential if given the correct nutrient supply (Fraps 1943; Combs 1962). This implies that an optimum balance between dietary energy and protein must be maintained to enable the broiler chicken to achieve its desired food intake and thereby, meet its requirement for protein and lipid deposition. What is not so obvious is why, when high levels of protein (and hence amino acids, AA's) are fed, the bird does not achieve its potential. Harper *et al.* (1970) has provided a well-documented review showing that diets containing excesses of AA's in diets of chicks and rats depresses growth rate. Several authors (D'Mello and Lewis, 1970; Wethli *et al.*, 1975; Morris *et al.*, 1987; Morris *et al.*, 1999) have also reported suboptimal performance when diets containing excess protein or AA's are fed to chickens.

The general theme of this thesis is how the efficiency of protein utilization in chickens is influenced by excess dietary protein. In investigating the effects of excess dietary protein certain questions need to be answered, firstly, should only the dietary crude protein content be taken into account or is there sufficient energy in the feed to process this excess protein? If energy is limiting, can enough information be assembled to describe more precisely the mechanisms involved in the lowering of the efficiency of protein utilisation? Also to be considered is whether these effects are accounted for by varying energy (E: P) protein ratios, daily food allocation (DFA) and protein quality (PQ). A consideration of the different growth periods is also taken into account since the critical concentration of essential nutrients required to maximise the efficiency of protein utilisation might be different at different ages. It is possible that the efficiency of nutrient utilization could also be increased by nutrient manipulations that increase gastrointestinal tract (GIT) development and enzyme activity. Even though not in the original plan of the research work, it was possible to make use of birds (10 to 24d) on two experiments to determine whether changes in enzyme activity, GIT and mucosal structure of the small intestine occurred at varying energy (E: P) protein ratios, daily

food allocation (DFA) and protein quality (PQ). This data is important to the understanding of GIT adaptation to feeding status and the efficiency of protein utilisation.

There are many fruitful perspectives from which to view animal efficiency. In practical terms, feed efficiency is most often defined as weight gained per unit feed intake or as feed conversion ratio, relating to feed consumed per unit of body weight gained. Our perspective is to determine how efficiently protein is partitioned between the requirements for protein (feather and tissue) growth above maintenance. The net efficiency of protein utilization is described conventionally in terms of body protein deposition per unit of dietary protein intake. The net efficiency of protein utilization is thus a complex measure integrating effects of biological parameters such as food intake, protein quality (i.e. AA digestibility, availability and balance), protein growth rate and maintenance requirements. These appropriate considerations are aimed at eliminating bias in the determined results. They are essential in evaluating differences in e_p amongst different or the same chicken genotype(s) in response to nutritional and environmental factors. It is speculated that an improvement in the digestibility or metabolizability of nutrients, the reduction in maintenance energy and changes in body composition that enhance energy and protein gain could lead to an increase in e_p . These factors are not independent; as a consequence several approaches can be used to determine the biological efficiency of conversion of dietary protein into tissue protein from aspects of the bird, nutrition and the environment.

Kyriazakis and Emmans (1992 a, b) proposed an important step forward in relating tissue deposition to nutritional input in pigs. These authors saw the efficiency of protein utilization, e_p , as dependant on the E: P ratio of the feed on offer. The reduction in e_p with decreasing E: P ratio is of great importance in poultry nutrition. However, this relationship has not been studied with any degree of precision using individually or group fed broiler chickens on diets of determined E: P ratios. It also appears that insufficient precise evidence has been established using adequate replications of individually fed broiler chickens or with diets where dietary protein is surplus to that needed for protein deposition. In this thesis, attention is focused on the broiler chicken, for which much quantitative investigations on the effect of excess dietary protein on e_p are scarce.

1.2 OUTLINE OF THE THESIS

The main aim of the work described in this thesis was to discover why it is that when protein is fed in excess, birds do more poorly than when just adequate amounts of protein are offered. The hypothesis that dietary E: P ratio influences the efficiency of protein utilization in broiler chickens was also tested. This will provide a useful tool for modelling the protein growth of the bird. This was achieved by quantifying the effects of dietary energy to protein (E:P) ratios; daily feed allocation (DFA) and protein quality (PQ) on the biological performance (food intake and growth rate), carcass compositional changes and e_p . It must be noted that diets fed in the series of experiments were fed at very high dietary crude protein levels ranging from 250 to 500 g CP/kg diet. It was thus assumed that almost all amino acids (AA's) were supplied in amounts more than adequate to support maximum lean tissue growth rate, which the birds are capable of achieving (Morris *et al.*, 1999).

The first chapter relates to the review of literature. Literature pertaining to some factors influencing e_p is reviewed followed by a general introduction to the experiments. Chapters 2 to 7 relate to a series of investigations in which the dietary protein fed to the experimental birds was surplus to that required for maximum protein deposition. The assumption made in these investigations is that other nutrients i.e. minerals, trace elements and vitamins, are non-limiting. A number of dietary factors (AA balance or PQ, E: P ratios and DFA) were combined and some traits measured, both on a whole animal level (i.e. biological performance, carcass compositional changes and protein efficiency) as well as organ level (some digestive characteristics). Both sets of data are discussed in relation to the factors involved, and an attempt is made to relate digestive characteristics to bird performance. The growth periods considered in these investigations were for birds 10 to 24 and 23 to 37 days of age. In the general discussion, attention is not only paid to a combination of the results of the series of investigations reported in this thesis but also to gaps in knowledge pertaining to e_p and the gastrointestinal response of chickens to dietary treatments. Finally, the main conclusions drawn on the basis of the results of the investigations are summarized and some important practical implications of the results of this study are suggested.

1.3 FACTORS INFLUENCING THE EFFICIENCY OF PROTEIN UTILISATION

1.3.1 Introduction

For an animal to carry out its functions efficiently, it requires resources provided by the feed on offer and the environment. At any given time it seeks to consume an amount of food in order to achieve its potential growth rate (Emmans, 1981). Its degree of success will depend on the feed on offer and the physical environment in which it is found. The environment, the dietary composition, the genotype and its physiological state are therefore important determinants of the efficiency with which resources are used for growth. The provision of adequate levels of each of these resources, in addition to a good balance of these is a prerequisite to improving the efficiency with which these resources are used for growth. While by no means complete, a number of important factors influencing the efficiency with which protein resources are used for growth are discussed below.

1.3.2 The effect of feed protein (AA) balance and excess dietary protein

When birds are offered diets in which protein in general and an AA in particular is limiting, responses to increasing amounts of the limiting AA can be obtained up to a point where the second limiting AA becomes limiting. As the protein level is increased further, and protein is no longer limiting, further responses can only be obtained with the supplementation of the limiting AA (Morris *et al.*, 1987, 1999). The extent of utilization of one AA is limited if the diet is deficient in another AA, for example -for arginine in the presence of excess lysine (D'Mello, 1975); isoleucine in the presence of excess leucine (Burnham *et al.*, 1992); and valine in the presence of leucine (D'Mello, 1975). In case of lysine this effect is independent of arginine (Classen and Stevens, 1995). Morris *et al.* (1999) indicated that an impairment of utilization of the first limiting AA observed with high protein diets could be attributed to a change in net utilization for protein deposition (a change in slope) or a change in the maintenance cost (a change in intercept).

A major effect of AA imbalance is a decreased food intake (Harper *et al.* 1970; Boorman, 1979 and Boorman and Ellis, 1996) resulting in a decreased growth rate. Morris *et al.* (1999) showed depression in the growth rate of broilers when poor quality proteins were offered in

amounts that raise the intake of all AA's except the limiting AA by 15 to 25%. A similar growth depression, but of correspondingly smaller magnitude, was reported by Wethli *et al.* (1975) in their investigations with poor quality proteins. Wethli *et al.* (1975) conducted investigations into the growth responses of broiler chicks to increasing amounts of comparatively poor-quality proteins, supplemented with their limiting AA's. The trial results showed that there was a gradual improvement in growth rate on the unsupplemented groundnut meal diets up to 360 g/kg but was comparatively lower than chicks receiving the low CP herring containing diets. There was however a progressive and significant improvement in live weight gains for chickens offered the methionine plus lysine supplemented groundnut meal diets of CP contents between 120-to 270-g/kg diet.

In a second experiment a series of diets based on soybean meal-maize mixture was fed supplemented and unsupplemented with methionine. The trial results showed that maximum response of chicks to the unsupplemented soybean meal diets was markedly lower than the supplemented soybean meal diets even at higher CP levels.

It can be deduced from these trials that growth could not be maximized when soybean meal or groundnut meal is utilized as a supplement to cereal-based diets even though very high levels (i.e. low E: P ratios) were used. Furthermore, the ratio of AA's in poorer quality oil-seed cakes occur in disproportion to the ideal balance required by the chick such that the utilization of the first limiting AA is reduced. However, it has been shown that some of the improvement in growth was due to AA imbalances being corrected with the use of pure supplements of the limiting AA's. Imbalances in AA's can explain the apparent differences in the utilization of limiting AA's in chicks offered excess dietary protein. Morris *et al.* (1999) reported that in some instances imbalances result in a decline in the efficiency of utilization of the first limiting AA. However, the growth depression that was observed with diets of high dietary protein content, which were limiting in an EAA, was as a consequence of lowered food consumption.

In their extensive review of literature pertaining to AA requirements of chicks, Morris *et al.* (1999) considered two scenarios. Firstly, comparisons were made with diets of low crude protein contents (i.e. <220g CP/kg) in which the response to adding the first limiting AA (LAA) is constrained by the supply of the second LAA. In general, growth rate was noted to be dependent on the intake of the first LAA with the response to dietary supplementation of this AA being dependent upon the supply of the second LAA. Comparisons were carried out

at high dietary crude protein contents based on the assumption that all AA, except the one to be tested, were provided in amounts that exceeded the need for the animal's maximum lean tissue growth. In these investigations birds offered the high protein diets (i.e. 280 to 300 g/kg or low E: P ratios) did not or could not take in enough energy to support the maximum rate of protein deposition associated with these genotypes in these environments. The food intakes for birds fed diets in the lower range (220 to 240 g CP/kg) were similar to those birds fed 280 to 300 g CP/kg. It was concluded that protein utilization was reduced at very high protein levels or very low E: P ratios.

In explaining the reasons for poor performance on high intakes of poor-quality proteins, Boorman and Ellis (1996) argued that, apart from a small increase in net energy yield due to large intakes of poor-quality protein feed, maximum response may not be achieved because of the depletion of the limiting AA's from tissue protein. Fisher and Morris (1970) indicated that the primary effect of AA imbalance was on food intake, which subsequently affects the weight gain. The same conclusion was made previously by Leung and Rogers (1969) and Harper *et al.* (1970).

There is experimental evidence to show that, diets unbalanced in AA composition in comparison to the AA requirement are fed, the metabolism of some AA's may be disrupted by the competition for similar transport systems (Simon, 1989). Furthermore, the excessive dietary supply of some AA's may produce metabolic disorders of a toxic nature characterized by the increased concentration of the AA's in the tissues and in plasma and/ or the accumulation of degradation products. Most AA's are degraded mainly or exclusively in the liver, but degradation of branched chain AA's is mainly initiated in the muscles by the action of a common aminotransferase (Moundras *et al.*, 1993). Simon (1989) noted that this transamination in muscle leads to alanine formation and release into the blood. The control of AA oxidation is a necessity to animals, because it prevents accumulations of excess AA's from taking place (Moundras *et al.*, 1993).

Antagonistic effects that result in reductions in the efficiency of AA utilization have been observed for arginine in the presence of excess lysine (D'Mello and Lewis, 1970), valine in the presence of excess leucine in turkey diets (D'Mello, 1975) isoleucine in the presence of excess leucine (Burnham *et al.*, 1992). The earlier work of Morris *et al.* (1987) was reviewed by Morris and Abebe (1990). They indicated that the excess arginine as supplied by the high protein diets was not the cause of the depression of lysine utilization. According to Morris *et*

al. (1999) reductions in the efficiency of AA utilization may not be as a consequence of antagonisms between structurally related AA's. This is because when lysine, methionine or tryptophan is made limiting and arginine is added; the effect of loading the diet with protein did not exert a deleterious effect under lysine-limiting conditions. These authors also underlined the fact that the impaired utilization of the first limiting amino acid was an occurrence observed when diets are loaded with high levels of poor quality protein.

The addition of the limiting AA to the imbalancing mixture allowed the restoration of biological performance. It was therefore assumed that imbalancing mixtures impaired the utilization of the limiting AA. Fisher and Shapiro (1961) and Fisher *et al.* (1964) conducted experiments in which the food intakes of groups of chicks fed diets with imbalances were equalized with those of controls. Fisher and Shapiro (1961) undertook these investigations to compare the efficiency of utilization of the limiting AA and total protein under conditions of imbalance using 7-day old crossbred chickens. In one experiment, diets containing three levels of energy were designed to ensure equal consumption of balanced and imbalanced rations. The objective of this experiment was to determine the efficiency with which the nitrogen portion of an imbalanced ration is utilized for growth. There was a typical growth depression and decrease in food intake response in birds fed the imbalanced diet. The imbalanced group receiving the low-energy diet consumed basically the same quantity of dietary lysine and had similar growth rates as did the balanced group offered the medium-energy ration. The high-energy group without the addition of the imbalancing mixture showed a similar trend. This group's food intake or lysine intake and growth rate was similar to the imbalanced group on a medium energy regime. The carcass nitrogen content of the imbalanced groups was higher than that of the corresponding balanced group whose food intake and growth was similar (Table 1.1).

According to Boorman (1980) a more efficient utilization of dietary nitrogen for nitrogen deposition will depend on the amount of the increase in nitrogen retention and the amount of the imbalancing mixture. An increased efficiency suggests the utilisation of some of the imbalancing mixture for protein deposition. It was speculated that the higher carcass nitrogen content might be due to the lower energy intake. Thus in spite of the lower energy intake chicks offered the imbalanced diets utilized protein and the limiting AA to the same extent as chicks consuming a higher level of energy in the absence of the imbalancing AA mixture.

Table 1.1 The carcass composition of birds fed balanced or imbalanced AA mixtures (Fisher and Shapiro, 1961)

Dietary Energy	² Imbalancing AA mixture	¹ Composition		
		Carcass Moisture	Carcass Fat	Carcass Nitrogen
	%	%	Dry weight	% Dry weight
Medium	-	68.8	35.0	8.70
Low	4	69.8	31.7	9.66
High	-	68.5	36.6	8.40
Medium	4	68.8	31.9	9.29

¹ Determinations were carried out on duplicate or triplicate samples of the pooled carcasses of duplicate/treatment.

² Lysine content of the ration was suboptimal at 4% of protein level.

In addition, the widened ratio between calories and protein in the high energy rations could have led to a reduction in growth as compared to the corresponding rations at lower energy levels. Furthermore, the imbalances did not affect the efficiency of utilization of the following AA's: lysine, arginine, tryptophan, and methionine.

Fisher *et al.* (1964) also initiated their study to compare the efficiency of utilization of "normal" compared with excess protein intakes (i.e. intakes beyond that required to meet the AA requirement of a broiler) under dietary stress in the form of diets imbalanced with respect to an essential AA. Crossbred male chicks were fed either a 22 or 28% protein diet for 2 to 4 weeks before being fed a low protein diet deficient in an EAA or a second diet, which was further imbalanced through the addition of an AA mixture devoid of the same limiting AA. A summary of the effect of previous protein intake on the response of chickens to low protein diets deficient in sulphur AA's and lysine are depicted in Tables 1.2 and 1.3. In both experiments, it was consistently observed that, the biological performance of the chicks (i.e. weight gain) that had been pre-fed the 28% protein diet were better than those offered the 22% protein diet irrespective if they were imbalanced or not. There was a slight difference in the response between lysine and the SAA's.

Table 1.2 *Effect of previous dietary protein level on the response of chickens to low protein (LP) diets deficient in sulphur AA's (SAA's) (Fisher et al., 1964)*

Measurement	Preliminary protein level 22%	Preliminary protein level 28%
Experiment 1		
Starting weight, 4-weeks, g	429 ± 6	423 ± 2 ¹
Carcass N, 4 weeks, %	9.26 ± 0.29	9.61 ± 0.09
7-day gain with:		
(A) LP, Low SAA diet, ² g	111 ± 8	112 ± 7
(B) As (A) + SAA-deficient AA mix, ³	99 ± 8	114 ± 7
Experiment 2		
Starting weight, 2-weeks, g	187 ± 3	184 ± 4
Carcass N, 2 weeks, %	8.64 ± 0.33	8.94 ± 0.31
21-day gain with:		
(A) LP, Low SAA diet, ² g	141 ± 12	141 ± 16
(B) As (A) + SAA-deficient AA mix, ³	119 ± 14	158 ± 12

¹Mean ± SE. ²13% isolated soy-peanut protein diet; Methionine content 2% of protein.

³ A complete AA mixture for growing chicks from which the SAA's were omitted and at 4% of the diet.

Table 1.3 *Effect of previous dietary protein level on the response of chickens to low protein (LP) diets deficient in Lysine. (Fisher et al., 1964)*

Measurement	Preliminary protein level 22%	protein level 28%
Experiment 1		
Starting weight, 3-weeks, g	326 ± 2	325 ± 2 ¹
Carcass N, 3 weeks, %	9.71 ± 0.11	10.10 ± 0.16
14-day gain with:		
(A) LP, Low SAA diet, ² g	136 ± 16	164 ± 20
(B) As (A) + SAA-deficient AA mix, ³	96 ± 20	129 ± 20
Experiment 2		
Starting weight, 3-weeks, g	363 ± 5	359 ± 4
Carcass N, 2 weeks, %	10.35 ± 0.27	10.93 ± 0.10
N consumed (g/ b/ 3 weeks)	19.1	22.5
21-day gain with:		
(A) LP, Low SAA diet, ² g	303 ± 10	358 ± 13
(B) As (A) + SAA-deficient AA mix, ³	219 ± 9	252 ± 10

¹Mean ± SE.

²13% sesame protein diet; Lysine content 4% of protein.

³ A complete AA mixture for growing chicks with Lysine omitted and added at 4% of the diet.

Lysine seemed to be relatively more limiting than the SAA's. This is substantiated by the observation of a better growth with the SAA's-limiting diets than with the deficient diets, in addition to the greater growth depression with the lysine deficient diets as a result of the addition of the AA mixture. The observation of similar magnitudes in carcass nitrogen was significant ($P < 0.05$) only for the lysine experiment. Birds fed the higher dietary protein level had a higher deposition of nitrogen in the carcass. It may be possible that, excesses of some AA's can be utilized to the advantage of animals subjected to certain stresses such as AA imbalances. Similar inferences that imbalances do not affect the efficiency of utilization of AA's were made from experiments in which the food intakes of animals fed control and imbalanced diets were equalized by insulin injections (Kumata and Harper, 1962), by force feeding (Leung *et al.*, 1968), or by exposing experimental animals to a cold environment (Klain *et al.*, 1962; Leung *et al.*, 1968). These findings are in agreement with the results of Boorman and Ellis (1996) who fed diets containing moderately and severely imbalanced AA mixtures of concentrations up to 547 g CP/kg in their investigations and observed no reduction in the utilization of the first limiting AA in comparison to the well-balanced control.

The improved utilization of the limiting AA may be due to a stimulation of the uptake of the limiting AA into some proteins, especially in the liver (Harper *et al.*, 1970) when a diet of an imbalanced AA supply is fed. In addition, the provision of the limiting AA for the ongoing synthesis is supported from the reutilization of AA's derived from the continuous muscle tissue protein degradation via the plasma free AA pool. Harper *et al.* (1970) indicated that there would be no increase in the overall catabolic rate of the limiting AA in the muscles. Deviations in diet composition from a balanced AA profile result in a poorer growth rate as a result of the decreased food consumption but this does not necessarily lead to a poorer utilization of the limiting AA (Boorman, 1980).

Based on the contradictory observations, Harper *et al.* (1970) tried to reconcile the conflict between the former view of an impaired utilization of the limiting AA and the, subsequent view of an improved utilization. These researchers indicated that, on equalizing the food intake of the control and that of the group fed the imbalanced diet, growth rates were equalized and therefore the efficiency of utilization of the limiting nutrient (AA) remained constant.

Some experimental evidence indicates that the food intake of rats fed imbalanced diets is stimulated when the imbalance is corrected by the supplementation of the limiting AA, and

the efficiency of utilization of the limiting AA by rats fed the corrected diet may be lower than that of rats fed the control diet (Kumata and Harper, 1962; Munaver and Harper, 1959). Harper *et al.* (1970), speculated that, the extra amount of the limiting AA's seems to stimulate food intake and thereafter is degraded. It is of interest to note that there is some inconsistency in the observation of such an effect and its occurrence depends on (i) the particular experimental conditions; (ii) how the measurement of the complicated variable (efficiency of nutrient utilization) is carried out (Harper *et al.*, 1970). Furthermore, without corrections being made for maintenance, comparisons will be valid only between groups growing at equal rates. Thus determined values will be dependent on the rate of growth. It is clear from these experiments that an AA supplement may stimulate growth without necessarily stimulating food intake, depending on its dietary deficiency. Such an occurrence may be accompanied by a change in body composition that could mask a change in the efficiency of utilization of the AA. If such changes are temporary, their detection will be dependent on whether measurements of appreciable accuracy can be made over a short period of experimentation.

When dietary protein content is increased beyond that required to meet the amino acid requirement of the broiler, growth rate is impaired (Morris *et al.*, 1987; Gous *et al.*, 1990, Morris and Njuru, 1990; Morris *et al.*, 1999). But what metabolic explanations can be advanced to explain the effects of feeding protein in excess of the birds' requirements? The effects of feeding an excess of well-balanced protein containing 300 and 600 g casein /kg to rats was investigated by Moundras *et al.* (1993). These authors reported lower concentrations of threonine, serine, glycine and glutamine in arterial plasma and muscle. This was accompanied by a large increase in threonine-serine dehydrase activity in the liver. This underlined the fact the occurrence of high rates of gluconeogenesis in the liver exerted a deleterious effect on the AA made available to the peripheral tissues. A significantly lower growth rate was reported for rats fed the 600 g/kg casein diet in comparison to those fed diets of 300 g/kg, which showed normal growth rates. Regardless of dietary crude protein content (*viz.* 300 or 600 g/kg), plasma concentrations of EAA's with the exception of threonine did not differ from that determined in rats fed 150 g/kg casein. The high rates of gluconeogenesis in the liver resulting in the under-supply of threonine to the tissues of the body possibly accounted for the lower growth rates of rats fed diets containing very high concentration of balanced protein. Similar pathways for the lowered utilization of methionine, lysine and tryptophan have not been fully investigated (Morris *et al.*, 1999). It is also possible that a large number of mechanisms may be involved in determining the efficiency of utilizing

dietary protein fed in excess of requirement. Morris *et al.* (1999) indicated the need for the liver to cope with surpluses of AA's from the digestion of high protein diets, increases the activity of numerous catabolic enzymes. This occurs only if there is a big difference between the first limiting AA and the other AA's. Consequently some of the limiting AA's derived from the diet are lost and could possibly result in the decline in the efficiency of utilization of the FLAA (Morris *et al.*, 1987; Morris and Abebe, 1990; Morris *et al.* 1992). This only occurs if there is a big difference between the first limiting AA and other AA's. This seems not to be supported by Boorman and Ellis (1996), whose data showed no loss of efficiency when birds were fed diets containing very high concentration of poor quality proteins.

1.3.3 The effect of daily food allocation or restriction

Feed restriction can be obtained by (i) restricting the amount of the feed fed or (ii) diluting a diet with poorly digestible feedstuffs which will prevent the bird from eating larger amounts of the feed on offer. A severe reduction in the amount of food fed may reduce the development of the gastrointestinal tract, whilst diluting the diet could lead to substitution and interaction effects between feedstuffs. Faulkner (1993) conducted experiments in which a range of diets, varying in E: P ratios from 40.83 to 72.5 MJ ME/kg protein were produced by diluting a basal diet with skim milk powder, starch, sucrose and oil. The birds aged seven days were then fed three allocations of the different diets: *ad libitum*, 0.8 and 0.6 of the amount consumed when fed *ad libitum*.

As the dietary E: P ratio increased from 47.37 to 72.5 MJ ME/kg protein (Table 1.4) there was a continuous decrease in protein intake for male chicks offered *ad libitum* access to dietary treatments. A similar declining trend was observed for female chicks fed E: P ratios of 47.37 to 72.5 MJ ME/kg protein. An increase in feed intake of the *ad libitum* fed birds ensured that they met their energy demands for the accretion of whole body protein on diets high in protein content. The gross efficiency of protein utilization (protein gain/protein intake) increased slightly with an increasing feed intake due to a dilution of the protein requirements for maintenance. With further increases in feed intake, a decreasing amount of the protein that was retained was deposited in the lean tissue. A further increase in dietary E: P ratio above the optimum (54.15 MJ ME/kg protein) results in the over consumption of energy. Birds may thus be limited by the 'heat production barrier' and will not be able to lose enough heat. It is expected that net protein efficiency will decrease if feed intake is severely depressed.

Table 1.4 The effect of dietary E: P ratios on protein intake (g/d), protein gain (g/d) and the efficiency of protein utilization in male and female broiler chickens (Faulkner, 1993).

Daily Food Allocation	E: P ratio (MJME/kg protein)	Protein intake (g/d)		Protein gain (g/d)		Net Efficiency	
		Male	Female	Male	Female	Male	Female
<i>ad lib.</i>	40.83	7.53	7.76	4.80	4.30	0.639	0.561
	47.37	8.20	8.85	5.79	5.75	0.707	0.657
	53.80	7.84	6.74	5.85	5.13	0.751	0.760
	59.40	6.85	6.70	5.19	5.05	0.757	0.752
	65.60	6.61	5.97	5.16	4.41	0.781	0.740
	72.50	5.71	5.28	4.54	4.17	0.794	0.790
<i>0.8 ad lib</i>	40.83	5.25	5.24	3.62	3.14	0.690	0.599
	47.36	5.63	6.09	4.56	4.44	0.810	0.729
	53.80	4.71	4.77	4.77	3.67	0.880	0.780
	59.40	4.68	4.09	4.09	3.58	0.901	0.765
	65.60	4.32	4.14	4.14	3.24	0.875	0.749
	72.50	3.81	3.65	3.65	3.14	0.921	0.826
<i>0.6 ad lib.</i>	40.83	4.01	4.05	2.80	2.35	0.699	0.579
	47.36	4.25	4.61	3.34	2.95	0.786	0.640
	53.80	4.07	3.54	3.42	2.87	0.841	0.810
	59.40	3.43	3.53	2.94	2.97	0.858	0.842
	65.60	3.67	3.25	3.03	2.61	0.824	0.802
	72.50	2.87	2.56	2.56	2.47	0.587	0.859

The net protein efficiency was in general better for the restrictively fed broilers than for the *ad libitum* fed broilers. This is in line with observations by Bikker (1994) that the efficiency of nutrient conversion into lean tissue is optimised at a low feeding level, whilst the efficiency of nutrient conversion into total body mass is optimised at a high feeding level. It could be speculated that the higher protein efficiency could be partly accounted for by a better digestibility of protein and a more efficient nitrogen metabolism in restrictively fed broilers. As E:P ratio increased from 40.83 to 65.60 MJ ME/kg protein, birds fed restricted amounts of feed showed an improved e_p over those offered *ad libitum* access to the feeds.

1.3.4 The effect of metabolizable energy to digestible crude protein (ME:DCP) ratio

The efficiency with which protein is utilized for growth is known to depend on the amount of dietary energy supplied, up to some limit (Edwards and Campbell, 1991). The ME:DCP ratio is used in the discussion, as it is only the digestible protein that is incorporated into body

protein tissue. Kyriazakis and Emmans (1992a, b) described the relationship between e_p and ME:DCP in pigs with a linear-plateau model, with a maximum e_p value of 0.81 (Figure.1.1).

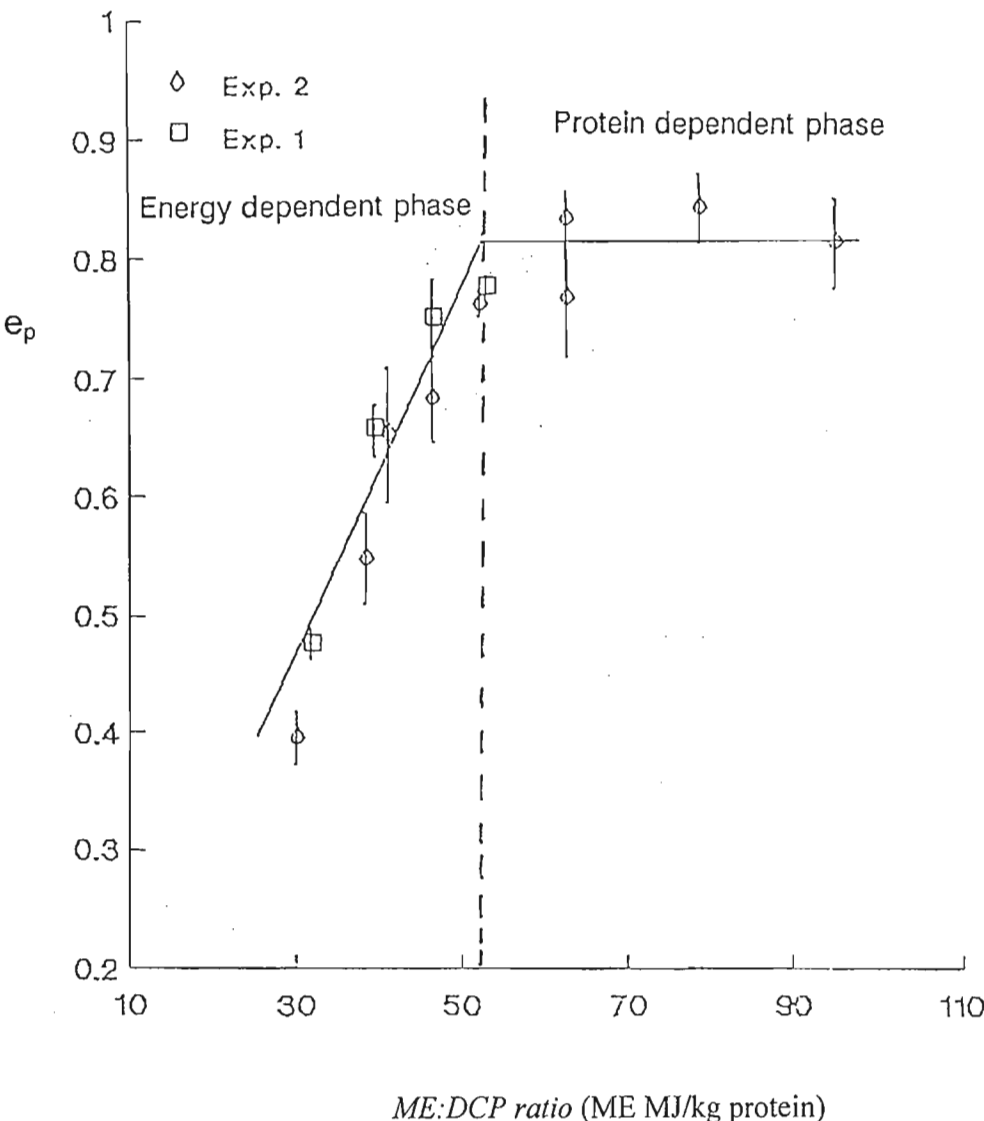


Figure 1.1 The efficiency of protein utilization (e_p) as a function of the ME:DCP ratio of the feed (Kyriazakis and Emmans, 1992b)

The slope of the ascending part of the curve was 0.0112 and the inflection point occurred at an E: P ratio of 52.42 MJ ME/kg protein. The overall estimate of e_p of 0.85 at 102 MJ ME/kg DCP of Ferguson (1996) is higher than that reported by Kyriazakis and Emmans (1992a, b) of 0.83 at 73 MJ ME/kg DCP. The difference is attributed to the significant temperature*protein interaction which resulted in an improved e_p that was associated with pigs being fed protein deficient diets at high temperatures (Ferguson, 1996). This enabled the animals to reduce their heat increment of protein retention. A decrease in the ME:DCP ratio beyond the critical value for e_p results in a decrease in protein deposition. In the protein dependent phase, an

animal offered increasing amounts of protein of a consistent quality, together with a given quantity of dietary energy, deposits protein linearly until the highest numerical value is attained at a specific level of protein intake, above which there is no more protein deposition. Edwards and Campbell (1991) indicated that this linear increase in protein intake is independent of energy intake, sex or genotype, when protein is limiting. Furthermore, in the energy dependent phase, additional protein is deposited only when there is an increase in energy intake with dietary protein supply being equal to or above requirement (Edwards and Campbell, 1991).

Kyriazakis and Emmans (1992a, b) indicated that the critical point beyond $e_p = 0.81$, at which increases in the amount of energy relative to protein result in no further improvements, occurs at a ratio of ME:DCP of 72.55 MJ ME/g DCP for pigs. The extra ME consumed, after meeting protein growth and maintenance requirements, is deposited as fat. When the ratio falls below 72.55 MJ ME /g DCP there will be insufficient energy to make use of all protein supplied, leading to the catabolism of excess protein and a reduction in e_p . In birds, amino acid nitrogen that is produced as a result of deamination of excess AA's is excreted mainly as uric acid and related compounds. The energetic costs of forming these nitrogenous waste products and the disposal of the energy in them reduces the efficiency with which these protein resources are used for accretion of whole body protein. Increasingly, hypotheses are being advanced suggesting that e_p has a maximum value when protein intake is limiting and energy supply is adequate (Bikker 1994; Ferguson, 1996).

As the dietary protein content at constant energy level is decreased so will the E: P ratio increase. The implications are that at very high E: P ratios, the broiler will be limited in its ability to consume adequate amounts of feed to meet its protein requirement, because the extra energy ingested increases the heat production of the bird above a threshold level. This will prevent it from being able to dissipate this extra heat, thus e_p will be lowered.

1.3.5 The effect of sex and genotype

There is disagreement about the effect of sex and genotype on e_p in animals. Certain authors, ARC (1981), and Campbell and Taverner (1988) reported that the sex and genotype of an animal influence the efficiency with which protein resources are used for protein accretion. Differences in genotype can have a large effect on rate of protein deposition and therefore the e_p . This will also affect E: P ratios, with higher ratios being needed by those genotypes with a

higher capacity for protein deposition. Animals bred for leanness would require higher concentrations of protein (amino acids) in their diets than those not possessing a high lean: fat ratio (Batterham *et al.* 1985; Baker, 1993). It must be borne in mind that, in calculating the e_p , it is the protein intake and protein retention that are used- not the protein concentration or body weight (protein and lipid). Kyriazakis and Emmans (1992a) investigated e_p of male and female pigs offered increasing amounts of a starch mixture at constant protein intake, for 4 to 8 weeks from 12 kg liveweight. The effect of sex on the e_p values was significant ($P<0.05$). Male pigs utilised protein more efficiently than females. These researchers indicated that because females have lower rates of maximum protein retention than males, protein intake becomes non-limiting at lower levels than for males. Consequently the decline in e_p values when protein intake is non-limiting occurs earlier in the females than males.

The conclusion that the sex and genotype of an animal influences e_p conflicts with the findings of Campbell *et al.* (1983), Dunkin and Black (1987) and Kyriazakis and Emmans (1995) whose investigations indicated that the sex and genotype of an animal does not influence e_p . In an experiment with Large white vs. Chinese Mieshan pigs fed similar limiting amounts of protein, Kyriazakis and Emmans (1995) reported no difference in e_p between the two very different pig genotypes. This finding could indicate that e_p has not altered by selection for lean tissue growth. In other investigations, no differences in e_p were reported between young male and female pigs up to 40kg liveweight (Kyriazakis and Emmans (1992b).

Experimental evidence for the effect of sex on e_p in broiler chickens is largely lacking. Perusal of preliminary investigations by Faulkner (1993) indicates that male broiler chickens exhibited a higher maximum e_p (0.84) than females (0.79), but the slope and inflection points were very similar (Figure 1.2). Female broilers had a slightly steeper slope in e_p (0.0152 vs. 0.0128) than males. Females were therefore more efficient up to a maximum point, so they had a lower maximum e_p .

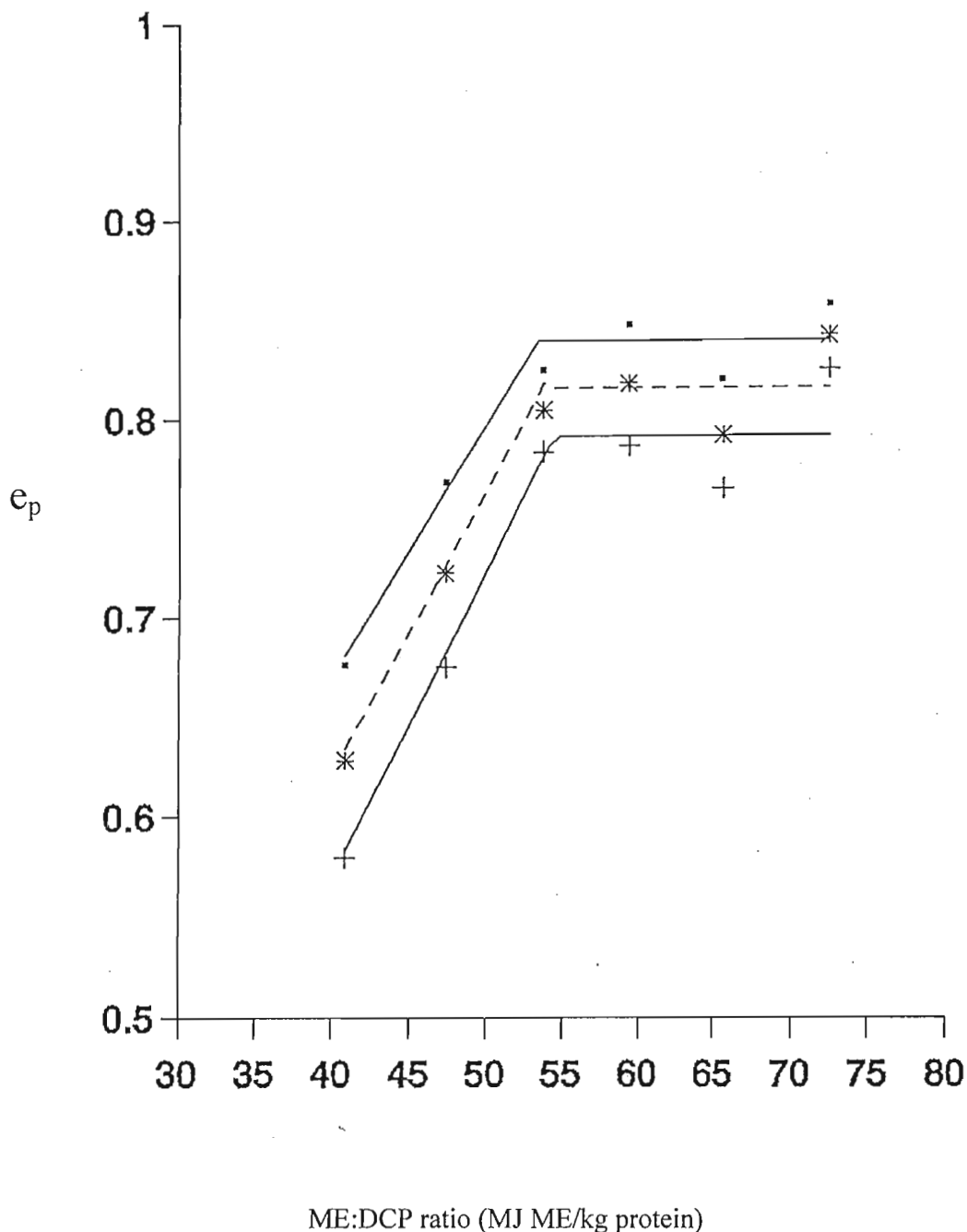


Figure 1.2 The effect of dietary ME:DCP ratios on the efficiency of protein utilisation (e_p) in male (●) and female (+) broilers, and with the combination of both sexes (*) (Faulkner, 1993).

When the results were pooled, the slope of the ascending part of the response curve was 0.0141 with an inflection point occurring at an E: P ratio of 54.15 MJ/kg protein. These values agree closely with those estimated by Kyriazakis and Emmans (1992b) (viz: 0.0112 and 54.42 MJ/kg) for pigs.

It is interesting to speculate why e_p is affected by the sex of the animal in some investigations. Ferguson (1996) suggested that with pigs, the apparent contradictions as regards the effect of sex and genotype on e_p might be ascribed to the differences in experimental design and implementation, the stage of maturity of the experimental animals as well as the level of protein intake.

The maintenance protein (amino acid) requirements for broilers make up a minute proportion of its total metabolism. However, its maintenance requirements are a function of the degree of protein maturity. Females tend to mature faster than males due to the greater gain in mature protein. Assuming a mature protein weight (P_m) of 1.2 and 0.9 for male and female broilers, the rate of protein maturity (B) at a given time is 0.036 and 0.038 for male and female broilers respectively. The rate of maturing for feather protein (B_{FP}) is 0.057 and 0.060 for male and female broilers respectively; this is considerably higher than for tissue protein gain. Feather growth takes place at different rates in male and female chickens and requires dietary intakes of cystine. Since females are fast feathering it is possible that at the onset of growth, a comparatively larger proportion of the total ingested protein will be used for plumage growth. Thus an improvement in protein utilization could be obtained by modifying the dietary cystine concentration to meet such changes in the growth of the female chicken. The lowered utilization of dietary protein for protein accretion in females is thus partly accounted for by this fact.

Males on the other hand make greater use of dietary protein (amino acid) for tissue protein synthesis since their rate of feather growth is not as rapid as in females in feather sexable strains. Feathers make up about four percent of the body weight of a chicken at three weeks of age. This increases to about seven percent at four weeks of age and remains constant thereafter (Scott *et al.*, 1976).

1.3.6 The effect of the bioavailability of amino acids

Correct investigative procedure dictates that a precise assessment of the efficiency of protein utilization will be inadequate if careful attention is not paid to the bioavailability of nutrients (Baker, 1986). This factor takes into account the nutritional value of the protein itself. Reference has to be made to the percentage of nitrogen that is used for whole body protein synthesis in assessing the nutritional value of the protein. Forbes (1995) noted that a reduced bioavailability might be due to processing methods and long periods of storage of the protein

sources. These factors will result in the estimated percentage of the limiting amino acid utilized for protein synthesis to differ from that estimated from measurements. Available evidence indicates that most ingredient-carrying nutrients are bound in one form or another. For instance amino acids in dietary protein may be bound in a polymeric peptide linkage and will therefore not be 100% available (Baker, 1986). Clearly since the feed or protein (and hence AA's) is not completely digested (digestible) it will be impossible for e_p to be 100%. Thus a characterization of the basal diets as to their bioavailability is necessary before e_p studies are conducted. To use any measure other than digestible protein (DCP) to describe E:P ratio is not adequate because the digestibility of the protein can be very different for different proteins and foodstuffs, and this will obviously influence e_p .

Peisker (1996) summarized and pointed out some problems associated with the bioavailability and efficiency of utilization of limiting amino acids. The author indicated that, unlike crystalline amino acids that are readily absorbed and are completely available for metabolism, most AA's from feed proteins are less than completely available. Thus the formulation of diets on a gross amino acid basis will give rise to differences in terms of the efficiency of a single amino acid to support protein synthesis at the tissue level - between the bound and free synthetic amino acids. Furthermore, the efficiency of utilization of the same limiting amino acid from different foodstuffs might be different because of the effects of antinutritive factors (i.e. trypsin-inhibitors, tannins, glucosinolates, gossypol or alkaloids). These are known to have a pronounced effect on protein digestibility. It is also known that about 40% of the limiting amino acids are catabolised in metabolism, others are damaged during processing but can be absorbed but not utilized (Peisker, 1996). Therefore, the efficiency of utilization of the limiting AA from various foodstuffs is expected to be different. Morris *et al.* (1999) indicated that diet formulation that is based on total AA content of raw materials or tabulated means give rise to some uncertainty about the availability of the limiting AA. These values are also of limited applicability to particular batches of raw ingredients used in experiments.

During prolonged storage and heat treatment of raw feed ingredients, several complex series of reactions (i.e. Maillard reaction) are initiated by the condensation between the carbonyl group of a reducing sugar and the free amino group of an AA or protein (McDonald *et al.*, 1991). The amino acid, lysine, has been noted to be particularly susceptible to this type of reaction. The amino acids in protein feeds that have taken part in these reactions are not normally released by enzyme hydrolysis in the digestive tract (Forbes, 1995). This can result

in as much as a 50% reduction in their availability for protein synthesis, and ultimately reduce the efficiency of protein utilization.

1.3.7 The effect of the metabolic fate of amino acids

From a nutritional point of view, the metabolic fate of amino acids appears to be one of the major limitations for the efficient utilization of these AA's (Gerrits, 1996). A low efficiency (0.64 for broiler chickens and 0.55 for White Leghorns) of absorbed dietary protein for whole body protein accretion by broiler chickens has been reported by Scott *et al.* (1976). It is questionable why such a large proportion of the absorbed amino acids are not used for whole body accretion. This can be partly explained by considering briefly an overview of the metabolic fate of amino acids.

1.3.7.1 Oxidation of amino acids to provide energy for maintenance.

Whitemore and Fawcett (1976) and Edwards and Campbell (1991) indicated that for pig's protein deposition tends to increase linearly with increasing protein intake, up to the point where energy intake limits the deposition of protein. At low energy intakes, AA's are available in excess of the bird's requirements. Amino acids are preferentially oxidized in animals to provide a source of energy because the maintenance of energy flow within the body is thought to be physiologically more important for survival than for growth (Gerrits, 1996). The use of some of the AA's for energy may limit the efficient utilization of dietary protein. It is also assumed that at extremely low protein-free energy intakes, the oxidation of AA's from the body becomes a necessity for survival. A consequence of AA catabolism is the production of the highly toxic ammonia, which must be excreted from the body of the bird. The elimination of ammonia as uric acid results in a net loss of four moles of ATP (McDonald *et al.*, 1991). This loss will result in a lowering of the efficiency with which dietary protein will be utilised for protein accretion. The efficiency of AA's as sources of energy is also low. For example glucose produces 21.2 moles ATP/100g nutrient in comparison to 12.2 from aspartic acid (McDonald *et al.* 1991). The use of AA's as a source of energy is therefore a wasteful process reducing the efficiency of the ingested protein for the accretion of whole body protein.

1.3.7.2 Gluconeogenesis from amino acids

Under conditions where a bird is restricted in its food intake, fasted or offered diets containing very low carbohydrate content, the body's stores of liver and muscle glycogen will be inadequate to supply the required amounts of glucose. However, certain tissues and processes specifically require glucose as an energy source. These include the red blood cells, kidney medulla, fatty acid synthesis and to a large extent the central nervous system and the brain (Scott *et al.*, 1976). In the absence of a dietary source of glucose, there is a specific requirement to use AA's for gluconeogenesis. Under these circumstances, some amino acids are degraded to provide carbon skeletons that can be converted to glucose. The clearance of glucose from the blood also occurs at a relatively fast rate, but the storage capacity of glucose is limited (Gerrits, 1996). It is speculated that gluconeogenesis from amino acids may be required between feeding times. The chicken, being a "nibbler" rather than a meal eater, may be expected to differ in mechanisms controlling the emptying of the proventriculus and the gizzard in comparison to those of other simple stomached animals. The feed consumed by the chicken first passes to the crop and is periodically ejected into the proventriculus (Scott *et al.* 1976), thus these mechanisms may allow gluconeogenesis to occur between the periodic ejection intervals which may result in the improvement in the efficiency of nutrient utilisation. Gluconeogenesis thus ensures that circulating glucose does not fall to dangerously low levels. During starvation there is a breakdown of fat and muscle tissue to provide oxidizable tissue to replace the liver glycogen that has been exhausted. Fatty acids cannot provide the required glucose (i.e. fats cannot be converted to carbohydrate). The gluconeogenic pathway from AA's thus provides the required glucose necessary to maintain essential body processes (i.e. for brain function) when AA's are available in excess of the animal's requirements or if it is forced to catabolise body tissues (McDonald *et al.*, 1991).

1.3.7.3 The utilisation of amino acids for non-protein deposition pathways

The low efficiency of utilization of digestible protein may also partly be attributed to the use of AA's in non-protein deposition pathways. As a consequence there will be no recycling back into protein deposition of the products resulting from these pathways. According to McDonald *et al.* (1991) unless all AA's that are required for protein synthesis are present at the right time, no synthesis will occur. Furthermore, excess AA's will be removed and may be catabolised, when the composition of the absorbed protein (AA's) is out of balance with that required for whole body accretion. It seems quite clear that when protein retention is low this

will lead to a reduction in the net efficiency of protein utilisation. A better efficiency may be due in part to the better digestibility of protein and probably a more efficient AA metabolism with little being used in non-protein deposition pathways.

Certain metabolites (Table 1.5) that result from the metabolism of AA's may be required by the body to maintain other physiological processes. When these metabolites are provided in the diet, it is expected that this will spare the need for the AA from which they are derived and thus improve the efficiency of utilization of that amino acid. Thus Scott *et al.* (1976) reported a growth improvement from the addition of creatine to chick diets that were deficient in arginine

Table 1.5 Some *metabolites derived from amino acid metabolism* (Scott *et al.*, 1976).

Precursor amino acid	Metabolite
Cysteine	Glutathione, taurine and sulphates present in chondroitin sulfate
Arginine	Ornithine, creatine and urea
Methionine	Cysteine, cystine and methyl group metabolism (i.e. creatine, choline)
Histidine	Histamine
Lysine	Carnitine, desmosine
Phenylalanine and tyrosine	Thyroxine, adrenaline, melanine pigments

1.3.8 e_p as a function of maintenance requirements.

Maintenance refers to some low level of activity in a thermally neutral environment. The additional energy required for heat production in a cold environment and the function of activity are regarded as separate functions. Thus maintenance is independent of the environment. When considering factors that affect the efficiency of protein utilisation, it is worth taking into account the effects of meeting or deviating from the maintenance requirements. In broiler production, protein growth is presumably additive to maintenance costs. There are indications that at AA intakes below the maintenance requirements, dietary AA's are utilized for the replacement of obligatory losses. Obligatory losses may result from the dietary AA being absorbed in a form unavailable for protein synthesis, or losses in physiological processes (Bikker, 1994).

Numerous nutritional studies (ARC, 1981; Fuller *et al.*, 1989) suggest that the efficiency of utilisation of the limiting AA for the purpose of replacing obligatory losses is constant. At low and moderate protein intakes that exceed the requirements for maintenance, the marginal efficiency of AA utilization for protein accretion has been found to be largely constant and independent of the level of protein intake in pigs (Batterham *et al.*, 1990, Fuller *et al.*, 1989) and rats (Bolton and Miller, 1985). However the gross efficiency of protein utilization has been reported to increase slightly with an increase in feed intake due to a dilution of the protein requirements for maintenance. With an increase in feed intake, a decreasing proportion of the retained protein is deposited in the lean tissue.

1.3.9 The effect of environmental temperature

Current attempts to obtain accurate information on the utilisation of excess protein resources for protein accretion requires adequate information on both nutritional and non-nutritional factors that could interact with the bird to affect its efficiency of protein utilisation. Some quantitative results on the effect of different levels of excess AA nitrogen and energy in broiler chicks during different times of the year have been presented by Waldroup *et al.* (1976). Overall, their comparative research at different temperatures suggest that broilers could be fed diets containing minimum levels of essential AA's regardless of the crude protein levels. The authors underlined that the minimum dietary protein level is advantageous for broilers kept under heat stressed conditions. In addition, under these conditions the efficiency of protein utilisation was significantly improved.

In investigations with pigs, Ferguson (1996) noted that the environmental temperature has a marked effect on e_p but only when an excess of dietary protein is supplied in the feed. Furthermore, the gross efficiency of protein utilization has been reported to increase slightly with an increase in feed intake due to a dilution of the protein requirements for maintenance. With an increase in feed intake, a decreasing proportion of the digested protein is deposited in the lean tissue. The implication is that protein intake above maintenance is retained with a decreasing efficiency in lean tissue. Ferguson (1996) indicated that irrespective of the ambient temperature, at low protein intakes, where protein is the limiting nutrient, e_p is constant and independent of protein supply, thus confirming the observations of Batterham (1990) and Bikker (1994).

1.4 GENERAL INTRODUCTION TO EXPERIMENTS

There is some evidence suggesting that any surplus of protein in the chicks diet has an adverse effect on biological performance (Wethli *et al.*, 1975; Morris *et al.*, 1987; Abebe and Morris, 1990b; Morris *et al.*, 1992; Morris *et al.*, 1999). This is in spite of the fact that the dietary protein (and hence AA's) content is increased beyond that required to meet the amino acid requirement of a broiler chicken. In these studies maximum growth was slightly depressed and feeding extra protein reduced the efficiency of protein utilisation. When broilers are fed practical commercial feeds in either the starter, grower or finisher stages of growth they also do not utilise the food on offer efficiently. It is known that a broiler can achieve its potential when offered diets with a balanced energy to nutrient supply under suitable environmental and managerial conditions. What is not so obvious is why, when **high** levels of protein are fed, the broiler chickens' performance does not meet expectation.

In spite of the fact that many studies have been published on the effect of dietary energy to protein contents on broiler performance, little is known about the influence of excess protein, as influenced by protein quality (PQ), energy to protein (E: P) ratio and daily food allocation (DFA) on the net efficiency of protein utilisation (e_p) in the period 10 to 24 d and 23 to 37 days of age. These effects will be examined in a series of experiments reported in Chapters 2 to 7 of this thesis. This series of experiments are unique in that very few experiments have been reported in the literature to date, in which the protein (and hence AA's) is not limiting, but in excess. Broiler chickens in these experiments were fed protein mixtures either balanced (BPS) or unbalanced (UPS) at 13 MJ ME/kg and the BPS at varying ME contents (i.e. 11, 12 13 and 14 MJ ME/kg) giving rise to a wide range of E: P ratios.

The objective of the series of experiments reported in this thesis were:

- to test the hypothesis that the net efficiency with which dietary protein is used for growth above maintenance (e_p) is affected by the protein quality (PQ), energy to protein (E: P) ratio and the daily food allocation (*ad libitum*. vs. restricted)
- to provide additional information on the relationship between e_p and E:P ratio for the biological performance (i.e. weight gain and food conversion efficiency) and net the efficiency of protein utilisation (e_p).

- to study the effects of protein quality (PQ), energy to protein (E: P) ratio and daily food allocation (DFA) on the development of visceral organs associated with digestion and nutrient absorption.
- to examine the capacity of the broiler chick to utilize diets containing excess protein but varying in ME content and to determine the response of the GIT to these dietary treatments.

CHAPTER 2:
THE INFLUENCE OF PROTEIN QUALITY (PQ), ENERGY: PROTEIN
(E: P) RATIO AND DAILY FOOD ALLOCATION (DFA) IN THE
PERIOD 10 TO 24 D.

ABSTRACT

The hypothesis that the net efficiency which dietary protein is used for growth above maintenance (e_p) is affected by the protein quality (PQ), energy to protein (E: P) ratio and the daily food allocation (*ad libitum*. vs. restricted) was tested. Eight hundred and ten chicks (10 to 24 days old), in groups of ten, were randomly assigned either to an initial slaughter group or to one of the twenty dietary treatments with four replications.

Two series of feeds differing in PQ, i.e. balanced (BPS) and unbalanced amino acid mixture (UPS), and covering a range of five E: P ratios (viz.: 32.5 to 65 MJ ME/kg protein) were offered at two levels of feed allocation (*ad lib.* and 0.75 of *ad lib.* intake) to chickens. The lysine concentrations of the BPS and the UPS ranged from 12.5 to 24.9 and from 6.0 to 12.4 g/kg respectively. Of all amino acids, only the lysine content was reduced in the UPS.

The BPS significantly ($P < 0.01$) enhanced performance in comparison to the UPS, this confirming the principle that diets supplying an array of nutrients that closely parallel the bird's nutrient needs give rise to an improved biological performance.

The poorer performance observed for birds fed the BPS in the lower E: P ratios suggests that when the dietary protein content is increased beyond that required to meet the amino acid requirements of a broiler, the energy content should also be increased to ensure that sufficient energy is available for the efficient utilisation of dietary protein.

The slopes obtained from fitting a linear regression model to the e_p 's for the BPS and the UPS at the *ad lib.* and 0.75 of the previous *ad lib.* intakes were 0.0132 (± 0.00044), 0.0109 (± 0.00062), 0.0122 (± 0.00056) and 0.0112 (± 0.00069) respectively. Of greater importance was the finding that there were no significant differences between the slopes of e_p vs. E: P ratio for birds fed the BPS and the UPS. The equation describing the overall relationship between E: P and e_p was 0.012009 (± 0.00029) E: P.

Keywords: excess protein, daily food allocation, efficiency, and broiler

INTRODUCTION

The effects of consuming diets containing protein (and hence AA's.) in excess of the needs of the broiler chicken have been shown to reduce growth and protein (AA) utilisation (Morris *et al.*, 1987; Gous *et al.*, 1990, Morris and Njuru, 1990). It is generally accepted that growth rate and the efficiency of food utilisation in birds from day old to 21 d of age improves with increasing dietary protein content, up to about 230 g CP/kg (Morris *et al.*, 1987). Little growth response to dietary protein was observed for diets containing 240 to 280 g CP/kg. This lack of response in growth may be due to a reduced food intake by birds fed diets containing excess protein, due in turn to the constraining effects of environmental temperature. If this were not the case, the birds would tend to eat more of a poorly balanced feed to meet this requirement.

In practice least-cost diets are formulated for broiler chickens by specifying minimum levels of dietary crude protein (CP) or AA's, with very little attention being paid to the surpluses of AA's. These surpluses of AA's do arise unless a maximum protein concentration has been specified. An increase in dietary AA concentration occurs above the minimum requirement when poor quality protein sources such as cassava (*manihot esculenta*) protein leaf meal, *leucaena leucocephala*, copra or palm kernel oil cake and groundnut meal are included. It is only when good quality protein and synthetic AA's are unavailable or the cost of these is prohibitive that large amounts of poor quality proteins are used in broiler feeds. This is done in order to meet the minimum requirements of the limiting essential AA's in the diet. The effects of feeding a diet with an imbalance of AA's, has been demonstrated using free AA's (Harper *et al.*, 1970) and with the feeding of large amounts of poor quality protein (i.e. groundnut-wheat-barley, soybean-maize meal) mixtures (Wethli *et al.*, 1975). It has been suggested that when higher-than-normal protein contents are economically desirable, (i.e. when poor quality proteins are fed at levels beyond that recommended in order to meet the AA requirement of a broiler) an upward adjustment of the dietary lysine level is a prerequisite to the formulation of efficient diets (Lewis, 1965; Morris *et al.* 1987; Morris *et al.* 1999). A criterion that can be used to determine how efficiently birds are converting protein feeding-stuffs into edible products of high quality is the net efficiency of protein tissue gain. In order to optimise the efficiency of protein utilisation, knowledge of the response relationships between nutrient intake (protein and energy) and animal performance (protein retention) is required.

The efficiency of utilisation of protein is defined as the ratio of protein retained and the digested protein consumed. It is well documented for pigs that the net efficiency of protein utilisation above maintenance, e_p , is related to the energy to protein (E: P) ratio in the feed on offer (Campbell *et al.*, 1985a; Kyriazakis and Emmans.1992a, b). However, there is a disagreement between these two authors about the underlying reason for the interaction between energy and protein in the feed. Campbell *et al.* (1985a) saw protein retention as being divided into a protein dependent phase and an energy dependent phase, whereas Kyriazakis and Emmans (1992a, b) saw the efficiency of protein utilisation, e_p , as a function of the metabolizable energy to digestible crude protein (ME:DCP) ratio in the feed on offer. Kyriazakis and Emmans (1992a, b) indicated that the critical point beyond $e_p = 0.81$, at which increases in the amount of energy relative to protein result in no further improvements, occurs at a ratio of 52.42 MJ ME/kg CP (the inflection point) for pigs. When the ratio falls below 52.42 MJ ME/kg CP (which accounts for the apparent 'energy dependent phase) there will be insufficient energy to make use of all protein supplied, leading to the catabolism of excess protein and a reduction in e_p . At these ratios, protein retention is a linear function of daily energy intake. The relationship between E: P and e_p , which is given by the slope of the ascending part of the model, was:

$$e_p = 0.0112 \text{ (s.e. 0.00022) } \times \text{E: P} \quad (R^2 = 71.6\%).$$

Kyriazakis and Emmans (1992 b) by incorporating their values into an algorithm were able to replicate precisely the results of Campbell *et al.* (1985 a). They indicated that, although the theory proposed by Campbell *et al.* (1985 a) was fundamentally correct, it is practically inadequate for modelling the protein growth in animals.

There is a paucity of information on the relationship between e_p and the E: P content in the feed for broiler chickens. Some preliminary work on this relationship was carried out by Faulkner (1993), who demonstrated that, the E: P ratio at which e_p is at a maximum decreased when the bird was fed an unbalanced diet. When offered a balanced AA mixture, e_p will be numerically greater than that on an unbalanced AA mixture. According to Morris *et al.* (1999), the concept of E: P ratio is very important because under nonlimiting conditions, the ME content of the diet determines feed intake and consequently protein intake when all nutrients are in adequate supply in the feed. They noted that protein utilisation would be lowered if the E: P ratios were reduced below a critical value. The dietary E: P ratio is of special importance, not at high E: P ratios, but when this is low i.e. when excess protein is

fed. This is because of a general overload of AA's derived from the hydrolysis of AA's that stimulate the activity of various catabolic enzymes (Scott *et al.*, 1976; Morris *et al.*, 1999). Metabolic explanations for the poor utilisation of high intakes of excesses of dietary protein have been advanced by Moundras *et al.* (1993) and Morris *et al.* (1999). It was reported by Moundras *et al.* (1993) that dietary protein levels of 150 to 300 g CP/kg in diets fed to rats had little effect on their biological performance. However, high protein diets significantly depressed daily weight gain and final body weight when fed at 600 g CP/kg. Moundras *et al.* (1993) attributed the poor performance from increasing the dietary protein level beyond the needs of the rat to the depletion of some glucogenic AA's. This consequence is critical for threonine, whilst the decreased availability of some AA's was regarded to have some physiological relevance in the control of protein intake. Data provided by Boorman and Ellis (1996) showed no loss of efficiency in the utilisation of the limiting amino acid in diets containing high concentrations of poor quality proteins. This is because the birds were fed *ad lib.* and therefore consumed sufficient energy. It is clear that the broiler chicken can achieve its potential if given the correct nutrient supply. What is not so obvious is why, when high levels of protein are fed beyond that required to meet its AA requirement, it does not achieve its potential.

This experiment deals with diets in which the protein (and hence AA's) is not limiting, but in excess. Although, many studies have been published on the effect of dietary energy to protein contents on broiler performance, little is known about the influence of excess protein, as influenced by protein quality (PQ); E: P ratio and daily food allocation (DFA) on e_p in the period 10 to 24 d. These effects will be examined in this study.

MATERIALS AND METHODS.

Two series of feeds differing in protein quality, and composed of five E: P ratios, were offered at two levels of daily food allocation (*ad lib.* and 0.75 of *ad lib.* intake) to sexed (male or female) broiler chickens between 10 and 24 days of age. Only 80 cages were available for this experiment, so each feeding treatment was replicated four times. The measures of response were feed intake (g/d), body weight gain (g/d), feed conversion efficiency (FCE)(g gain/kg food intake), rates of lipid (LR) and protein retention (PR) (g/d), and the efficiency of protein utilisation (e_p).

Birds and housing.

Five hundred male and five hundred female day-old Ross broiler chickens were obtained from a commercial source. They were reared from day-old to nine days of age in single-tier electrically heated battery brooders. The birds were housed in groups of 10 and under a 23L: 1D lighting schedule using standard brooding practices. During the pre-test period (0-9 days post hatching) the chicks received a standard commercial starter feed (220 g CP/kg) on an *ad lib.* basis. At ten days of age, 800 chicks (400 male and 400 female) of similar weight were randomly placed in-groups of ten in cages in the experimental unit, to accustom them to the pens, after being weighed to the nearest gram. The chicks were assigned either to an initial slaughter group (10 birds per sex) or to one of the 20 dietary treatments with four replications, such that the average starting weight 0.182 ± 0.017 were similar for each treatment. Two replicates of ten male or female chicks were assigned to each of the 20 dietary treatments. The house temperature was monitored daily throughout the duration of the trial.

Experimental feeds and design.

Two series of diets designed to contain excess protein (generally in the range 200 to 400 g CP/kg) mostly beyond that required to meet the AA requirements of a broiler (7 to 28d) were used in this study (Table 2.1 and 2.2), one being balanced and the other being an unbalanced (UPS) AA mixture. The balanced protein series (BPS) consisted mainly of soybean and fishmeal, whereas the unbalanced protein series (UPS) was based on maize gluten and lupines. The basal diets were formulated to contain 13 MJ AME/kg and 400 g CP (nitrogen x 6.25:CP)/kg formulated feed.

Diets were formulated to meet or exceed the requirements for all essential nutrients (Table 2.3). In the formulation of the BPS, total essential amino acids (TEAA) were minimised. These high protein basal diets were then blended in appropriate proportions with a protein-free diet of equal energy content to obtain different protein contents but similar AA proportions. The resulting five E: P ratios (viz.: 32.50, 37.14, 43.33, 52.0 and 65 MJ ME/kg protein) within each protein series had a constant energy content, of 13 MJ AME/kg and crude protein contents of 400, 350, 300, 250 and 200 g CP/kg respectively.

Table 2.1. *The raw ingredient composition and nutrient contents of the balanced (BPS), unbalanced (UPS) protein series and the protein free feeds used in the experiment*

Raw ingredient (g/ kg)	BPS	UPS	Protein free
Maize	173.92	109.80	-
Fish meal 65	83.28	-	-
Maize gluten	50.00	360.32	-
Soya protein isolate (66%)	50.00		
Soybean oil cake (50%)	529.53	-	-
Lupin		451.50	
Sunflower Oil Cake (37%)	14.88		-
Sunflower oil	75.62	40.14	60.00
Starch	-	-	333.90
Sugar	-	-	333.90
MCP ¹	9.33	16.11	20.00
Vitamins + Minerals	2.50	2.50	2.50
Salt	-	2.21	3.00
Sodium bicarbonate	1.61	1.31	1.30
Limestone	9.33	16.11	18.33
Filler	-	-	227.07
Calculated Nutrients:			
Dry matter	904.95	905.90	840.60
Crude protein	400.00	400.00	
AMEn (MJ/kg)	13.00	13.00	13.00
Fat	100.00	103.20	200.00
Crude fibre	26.73	58.90	
Ash	51.54	26.10	1.3.00
Calcium	10.00	10.00	10.00
Phosphorus	5.00	5.00	5.00
Sodium	1.50	1.50	1.50
Chloride	1.80	1.80	1.80

MCP¹ Monocalcium phosphate

The formulations were based on the requirement for essential amino acids (EAA's) as suggested by the EFG (1998) growth model (Table 2.3) for broilers aged 10 to 24 d, the mean over this period being 12.5 g lysine/kg feed. The lysine concentrations in g/kg for the BPS and UPS had a range of 12.5 to 24.9 and 6.0 to 12.1 respectively. The experiment reported here had varying dietary protein concentration either in the UPS or BPS (in isoenergetic diets). It is however expected that the conclusions derived from this study would be equally applicable to AA's present beyond that required to meet the AA requirement of a broiler. Birds were provided *ad lib.* or restricted access to mash mixtures for the 14-day experimental period.

Table.2.2. *The analysed amino acid composition (g/kg) of the balanced (BPS) and unbalanced (UPS) protein sources used in the experiment.*

Amino acid	BPS	UPS
Lysine	24.49	12.48
Methionine	15.59	6.73
Methionine+cystine	10.65	13.20
Threonine	15.28	13.98
Tryptophan	4.29	2.52
Arginine	26.71	26.27
Histidine	10.44	8.61
Isoleucine	18.92	17.82
Leucine	32.29	50.00
Phenylalanine	19.15	20.97
Phenylalanine + tyrosine	30.31	40.59
Valine	20.25	18.38

Table 2.3. *The essential amino acid requirements (g/kg diet) of broiler chickens between 7 to 21 days of age according to various authors*

Amino acid	Leclercq <i>et al.</i> , (1987)	NRC (1994) ¹	EFG (1998) ²
Lysine	12.40	11.00	12.00
Methionine	5.20	5.00	4.00
Threonine	7.40	8.00	7.60
Tryptophan	2.30	2.00	1.90
Arginine	13.40	12.50	12.00
Histidine	5.00	3.50	4.20
Isoleucine	9.60	8.00	7.20
Leucine	17.30	12.00	12.60
Phenylalanine	-	7.20	7.20
Valine	10.80	9.00	8.30
Proline		6.00	6.00
TSAA ³	9.30	9.00	7.40

¹NRC National Research Council (1994)

²EFG Broiler simulation model

³TSAA, Total Sulphur Amino Acids.

(i) *Ad libitum* treatments

Birds allocated to these treatments were given free and continuous access to one of the five feeds varying in E: P ratio and either of the two (BPS and UPS) protein series. Feed consumption was measured daily by weighing the food and trough at the start and end of each 24-hour period.

(ii) Restricted treatments

Birds designated to these treatments were allocated food at 0.75 of the average consumption of the respective *ad lib.* treatments of the previous day. The same levels of restriction were applied for each of the two protein series.

Management and slaughter procedure

At the beginning of the trial period, eight birds were selected at random, killed by cervical dislocation and the entire carcass frozen until processed. The remaining birds were fed twice daily and weighed once weekly. Feed remaining in the trough at the end of 24 hours was collected and weighed daily, the average of these intakes over equivalent treatments being used to calculate the amounts to be offered to the birds on the restricted treatments. At the conclusion of the experiment, four experimental birds/pen were weighed and killed by carbon dioxide asphyxiation. The carcasses were then frozen in gas impermeable plastic bags. At a later stage, all carcasses were ground in a meat mincer, after they have been allowed to thaw in sequence. These ground samples of birds from the same pen were then thoroughly mixed and sub-sampled for chemical analyses (dry matter, protein, ash, gross energy contents of the dry matter (DM)). Crude protein ($N \times 6.25$) was analysed in a LECO FP 2000 nitrogen analyser using the Dumas Combustion method (AOAC 1990). Gross energy, moisture, DM were also analysed using the AOAC (1990) methods of analysis. Gross energy (GE) was determined with a DDS isothermal CP500 bomb calorimeter (Digital Data Systems (Pty) Ltd., Randburg, Johannesburg, RSA). Moisture was determined by placing the sample in a hot air oven at 90°C for 72 hours (AOAC, 1990).

All carcass composition values were from selected chicks from each group. The dry matter (DM) percentage for each group was defined as the weight of the freeze-dried material from the selected chick sample divided by its weight times 100.

Heat production (H), protein retention (PR) and Lipid retention (LR).

It was assumed that the heat storage in the broiler chicken is negligible, thus the heat production (H) during the experimental period was calculated from the equation (Emmans and Fisher, 1986):

$$H = \text{ME intake} - \text{Energy Retained}$$

$$H = (\text{AME}_{\text{n feed}} \times \text{feed intake}) - [(23.8 - 5.63) \times \text{Protein retention} + 39.6 \times \text{Lipid retention}]$$

which assumes that the energy contents of body protein and lipid are 23.8 and 39.6 MJ/kg respectively. The value 5.63 MJ/kg is a constant nitrogen correction factor applicable when a nitrogen correction has been applied to the ME (Emmans, 1994).

Protein retention was calculated as follows:

Protein retention (g/d) = (group mean weight (GMW) x protein content – initial protein content)/n. Where n is the number of samples

Carcass lipid content was determined from the gross energy (GE) values of the carcass using the following equation developed in the department of Animal and Poultry Science, University of Natal, Pietermaritzburg.

$$\text{Carcass lipid (g/g) (dry matter basis)} = 0.8756 + 0.04754 \times \text{Carcass GE (MJ/kg DM)}$$

The net efficiency of protein utilisation above maintenance (e_p)

The net efficiency of protein utilisation (e_p) above maintenance was calculated from the equation proposed by Kyriazakis and Emmans (1992b).

$$e_p = \text{PR} / [(\text{Fi} \times \text{FCP} \times d_{cp} \times v) - \text{MP}] \text{ (kg/kg)}.$$

Where

- Fi = Feed intake (kg/d),
- FCP = Food crude protein content (kg/kg),
- d_{cp} = Digestibility of crude protein,
- v = Biological value (value of digested protein in relation to ideal protein),
- MP = Protein requirements for maintenance (kg/d) and
- PR = Protein retained (kg/d).

d_{cp} is the amount of total crude protein available to the animal as a result of the chemical nature of the raw ingredients of the particular feed. Protein digestibility values for the various raw materials used were obtained from the European Table of Energy Values for Poultry Feedstuffs (1986).

The v and d_{cp} values were used because only the digested protein is incorporated into body protein. The d_{cp} value for the mixed feed was calculated by determining the proportion of digestible crude protein to total crude protein of the various raw materials in the proportion they occurred in the diet. The d_{cp} for the BPS and UPS series were 0.39 to 0.79 (Feeds BPS1...BPS5) and 0.42 to 0.83 (Feeds UPS1...UPS5).

The proportion of total protein digested, which can be incorporated into body protein 'v' was calculated by considering the determined available amino acid concentration of each protein mixture fed to the broiler chickens and the ideal AA balance required for the broiler chicken for the experimental period between 10 to 24 days of age (Table 2.4). The proportion of total protein digested, which can be incorporated into body protein 'v' for the BPS was 1.00 (Feeds BPS1...BPS5). The value 'v' for the UPS was determined as 1 and 0.52 for the basal diets of crude protein contents of 400 and 200 g CP/kg respectively. The ideal amino acid balance for a bird of between 10 and 24 days of age was obtained from the EFG broiler simulation model (1998). This model makes use of a factorial approach to calculating the AA required for the growth of body and feather protein at the rates appropriate for a given genotype at a given degree of maturity.

Table 2.4 *The proportion of total protein digested, which can be incorporated into body protein 'v' for basal diets in the unbalanced protein series.*

Amino Acid	Available AA concentration of basal feed in UPS		Ideal AA balance required	¹ Proportion of AA utilised	
	Dietary crude protein content (g CP/kg)			Dietary crude protein content (g CP/kg)	
	400	200		400	200
Lysine	12.49	6.25	12.0	1.04	0.52
Methionine	6.73	3.37	4.00	1.68	0.84
Isoleucine	17.82	8.91	7.2	2.48	1.24
Histidine	8.61	4.31	4.2	2.05	1.03
Arginine	26.27	13.14	12.00	2.19	1.09
Threonine	13.98	6.99	7.6	1.84	0.92
Leucine	50.00	25.00	12.6	3.97	1.98
Valine	18.38	9.19	7.2	2.55	1.28

¹Proportion utilised = available AA concentration of basal feeds ÷ amount of AA required to achieve an ideal balance

The calculated values of 'v' for the basal diets in the UPS have been highlighted in bold.

The value for MP was calculated from the equation given by Emmans and Fisher (1986):

$$MP = 0.008 \times P_m^{-0.27} \times P \text{ (g/d), where:}$$

P_m = Protein weight at maturity (kg) was estimated as 1.17 and 0.75 for the male and female broiler chickens used in this trial, respectively.

P = Geometric mean weight of body protein (g).

Statistical design and analysis

It will be difficult to interpret the responses to all 20 dietary treatments without making use of some organizing system. The main purpose of this research is how e_p is influenced by the protein quality (PQ), daily food allocation (DFA) and Energy to protein (E:P) ratio. Multiple linear regression analysis involving the variables PQ, DFA and E: P ratio was used to predict the biological performance and the efficiency of protein utilisation. Linear regression equations were also determined for the protein series at each feeding level for: e_p vs. E: P ratio using SAS (1990). At zero dietary energy to protein content, e_p must be zero, thus simple linear equations derived from the experiments were forced through the origin as giving the best estimates of the relationship between e_p vs. E: P ratio. Each group of ten-penned birds was considered as an experimental unit.

Interaction plots of means for each level of a factor with the level of a second factor held constant were used to judge for the presence of interactions. Where parallel lines were obtained in an interaction plot this indicated that there was no interaction. Where there is a greater departure of the lines from the parallel state, a higher degree of interaction occurs. The interaction plots were used only as an exploratory tool to determine the presence of any interaction between factors. The interactions were best judged in conjunction with a General Linear Model (GLM) fitting procedure in which regressions were fitted for each response variable. In order for the model to be "full rank", this required eliminating some of the high order interactions in the model, based on the assumption that they are not important.

RESULTS

Food intake (FI), average daily liveweight gain (ADG), and food conversion efficiency (FCE).

The response in food intake (FI), average daily live-weight gain (ADG) and food conversion efficiency (FCE) of birds to diets containing excess protein and varying in protein quality, DFA and dietary E: P ratios are shown in Table 2.5. BPS significantly ($P < 0.0001$) enhanced biological performance in comparison to the UPS. E: P ratios significantly influenced the FI ($P < 0.001$), ADG ($P < 0.01$) and FCE ($P < 0.001$). Sex of the birds did not have an influence on the biological performance. There were significant ($P < 0.001$) interactions of E: P and PQ for ADG, FI and FCE (Table 2.5).

Table 2.5. The effect of dietary protein quality (PQ), E: P ratios and daily food allocation (DFA) on the biological performance and the efficiency of protein utilisation (e_p), of broiler chickens from 10 to 24 days of age.

Dietary treatment ¹	E: P ratio	DFA ²	Feed Intake (g/d)	Weight gain (g/d)	FCE (g gain/kg food intake)	Protein intake (g/d)	PR (g/d)	e_p
Balanced protein series (BPS)								
1	32.5	1.0	50.9	23.8	468.0	20.4	4.8	0.30
2	37.1	1.0	55.3	26.9	487.4	19.4	5.3	0.41
3	43.3	1.0	58.1	27.9	479.2	17.4	5.4	0.55
4	52.0	1.0	61.8	29.0	468.9	15.5	5.7	0.78
5	65.0	1.0	64.9	24.4	376.5	13.0	4.6	0.94
		Mean	58.2	21.6	456.0	17.1	5.2	0.60
6	32.5	0.75	38.2	12.0	313.4	15.3	2.4	0.21
7	37.1	0.75	41.5	13.6	328.4	14.5	2.9	0.30
8	43.3	0.75	43.6	15.2	349.6	13.1	3.2	0.43
9	52.0	0.75	46.5	17.0	366.5	11.6	3.4	0.65
10	65.0	0.75	48.7	15.7	321.5	9.7	3.0	0.88
		Mean	43.7	14.7	335.9	12.8	3.0	0.49
Unbalanced protein series (UPS)								
16	32.5	1.0	47.4	19.6	413.7	19.0	3.8	0.25
17	37.1	1.0	50.0	20.3	406.8	17.5	3.8	0.33
18	43.3	1.0	53.4	19.6	367.6	16.0	3.6	0.48
19	52.0	1.0	52.8	17.6	333.2	13.2	3.1	0.76
20	65.0	1.0	49.6	11.8	238.5	9.9	1.8	0.88
		Mean	50.64	17.8	352.0	15.1	3.2	0.54
21	32.5	0.75	35.6	10.9	306.9	14.2	2.3	0.20
22	37.1	0.75	37.5	11.4	304.1	13.1	2.2	0.26
23	43.3	0.75	40.1	11.0	274.0	12.0	2.3	0.42
24	52.0	0.75	39.6	10.2	257.0	9.9	1.9	0.64
25	65.0	0.75	37.1	8.5	229.5	7.4	1.5	0.86
		Mean	38.0	10.4	274.3	11.3	2.1	0.48
Source of variation								
PQ			***	***	**	NS	***	NS
DFA			***	***	***	*	***	**
E:P			***	***	***	NS	***	***
Sex			NS	NS	NS	NS	NS	NS
DFA x PQ			***	*	**	*	***	NS
E:P x PQ			***	***	***	***	***	NS
E:P x DFA			***	NS	***	NS	***	NS
Adjusted R ²			94.5	94.7	86.5	97.0	95.0	96.1
SE Mean			0.95	0.71	9.22	0.38	0.13	0.78

The **mean** of each response due to feeding level are highlighted in bold. ¹Each treatment was replicated four times. ² Daily food allocation (proportion of *ad lib.* intake), The protein contents within each feeding level were 400, 350, 300, 250 and 200 g CP/kg. *P<0.05, **P<0.01, ***P<0.001

The FI and FCE response to E: P was dependent on PQ and DFA. There were also significant interactions between DFA x PQ (P<0.1) and E:P x PQ (P<0.0001) for ADG.

Feed intake increased as dietary protein content decreased or E: P ratio increased for birds fed diets in the BPS. However for birds fed diets in the UPS there was a drop in food intake after an E: P ratio of 42 MJ ME/kg protein but to a level higher than that for birds fed the lowest ratio of 32.5 MJ ME/kg protein feed. The lowest feed intake was reported amongst the birds fed the UPS *ad lib.* and containing 400 g CP/kg. Feed intakes, and therefore protein intakes were greater for birds fed the BPS than those fed the UPS. But this was not statistically significant.

As the dietary protein intake increased so did the ADG increase linearly ($P < 0.0001$) until a maximum was attained at 52 MJ ME/kg protein for birds fed diets in the BPS. The ADG for birds fed diets in the UPS increased initially from 19.6 to 20.3 g/d as E: P ratio increased to 37.1 MJ ME/kg protein, thereafter average daily weight gain (ADG) showed a decreasing trend for birds fed the UPS. The effect dietary treatments on ADG at the two feeding levels (i.e. *ad lib.* and 0.75 of *ad lib.* intake) resulted in the BPS treatment having a higher growth rate of 14.9 and 15.1% respectively, compared with birds fed the UPS. Body weight was reduced by feed restriction, resulting in a significant ($P < 0.01$) difference between the bodyweights of birds fed *ad lib.* and those that were fed 0.75 of their *ad lib.* intake.

FCE for birds offered unrestricted access to BPS diets increased initially but declined after an E: P ratio of 37.1 MJ ME/kg protein. As E: P ratio increased from 32.5 to 65 MJ ME/kg protein, FCE declined progressively from 413 to 232 g gain/kg food intake for birds fed diets in the UPS. Birds on the BPS offered at the two DFA's (i.e. *ad lib.* and 0.75 of *ad lib.* intake) improved by 29.6 and 22.4% higher respectively, over that for birds fed the UPS. Feeding diets in the UPS with excess dietary protein negatively affected biological performance.

Protein (PR), lipid (LR) and energy (ER) retention.

The mean composition of the initial slaughter group at 171 ± 1.4 g live weight was 720 ± 2.16 g/kg moisture, 158.7 g/kg protein, 23.98 ± 1.38 MJ ME/kg GE. The daily rate of change in PR, LR and ER are shown in Table 2.5 and 2.6. The main effect of PR at the two levels of daily food allocation for the BPS was improved by 60.2 and 46.1% respectively in comparison to birds fed the UPS. The interaction between dietary PQ and DFA or E:P was significant ($P < 0.001$) for PR. The PR response to E:P was thus dependent on PQ and DFA. .

Table 2.6. *The effect of dietary protein quality, E: P ratios daily food allocation on the lipid gain, energy retention and heat production of male broiler chickens from 10 to 24 days of age.*

Dietary treatment	E: P ratio MJ ME/kg protein	DFA ²	Lipid gain (g/d)	Energy retained (kJ/d)	Heat production (kJ/d)
Balanced protein series					
1	32.5	1.0	0.86	40.6	621.4
2	37.1	1.0	1.30	59.0	659.9
3	43.3	1.0	1.68	74.2	681.0
4	52.0	1.0	1.82	79.8	723.8
5	65.0	1.0	1.91	82.0	761.7
		Mean	1.51	67.1	689.6
6	32.5	0.75	0.31	15.7	480.8
7	37.1	0.75	0.31	16.4	522.7
8	43.3	0.75	0.31	16.6	549.8
9	52.0	0.75	0.38	20.1	583.7
10	65.0	0.75	0.49	24.0	608.8
		Mean	0.36	18.6	549.2
Unbalanced protein series					
11	32.5	1.0	1.01	45.4	570.9
12	37.1	1.0	1.26	55.4	594.8
13	43.3	1.0	1.24	54.0	640.6
14	52.0	1.0	1.32	56.8	629.9
15	65.0	1.0	1.01	42.6	601.9
		Mean	1.17	50.8	607.6
16	32.5	0.75	0.47	21.9	440.3
17	37.1	0.75	0.47	21.7	466.0
18	43.3	0.75	0.44	20.9	500.1
19	52.0	0.75	0.44	20.1	494.9
20	65.0	0.75	0.67	28.89	454.4
		Mean	0.50	22.7	471.1
Source of variation					
PQ			***	***	*
DFA			***	***	***
E:P			NS	*	***
Sex			**	**	NS
DFA x PQ			***	***	NS
E:P x PQ			***	***	***
E:P x DFA			**	NS	NS
SE Mean			0.062	2.60	10.20

The **mean** of each response due to feeding level are highlighted in bold. ¹Each treatment was replicated four times. ² Daily food allocation (proportion of *ad lib* intake). The protein contents within each feeding level were 400, 350, 300, 250 and 200 g CP/kg. *P<0.05, **P<0.01, ***P<0.001

The protein intake (PI) between birds offered *ad lib.* access to diets in the BPS and UPS were 17.14 and 15.12 g/d, but the rate of PR differed significantly ($P<0.05$). PR was highest for birds fed *ad lib.* and decreased as DFA decreased. As the E: P ratio increased from 32.5 MJ ME/kg protein, birds fed the BPS continued to achieve higher protein gains than birds offered the UPS. The lowest protein gain of 1.5 g/d was recorded at the highest E: P ratio of 65 MJ ME/kg protein for birds fed diets in the UPS. There was no statistically significant effect of sex on PR. Lipid retention responses were not significantly different ($P=0.069$) over the different E:P ratios. However, Table 2.6 suggests that LR response to E:P was dependent on PQ and DFA. The ER response to E:P was also dependent on PQ.

Heat production (H)

The calculated amount of H by the birds is shown in Table 2.6. Protein quality and E: P ratio and DFA but not sex had a significant ($P<0.0001$) effect on H. As the dietary E: P ratios increased from 32.5 to 65 MJ ME/kg protein, H for birds offered the BPS at *ad lib.* and 0.75 of *ad lib.* intake increased to a maximum of 761 and 608 kJ/d respectively. There was a decrease in heat production, due to the lower energy intake, as the DFA decreased. The interaction between E:P and PQ was significant ($P<0.001$) for H.

The net efficiency of protein utilisation above maintenance, e_p

The full response surface for e_p was analysed to be:

$$- 2.931 + 1.959 \text{ PQ} + 30.781 \text{ DFA} + 0.065 \text{ E: P} - 1.9720 \text{ DFA PQ} - 0.043 \text{ E:P PQ} - 0.255 \text{ E:P DFA}$$

(Adjusted $R^2 = 0.98$).

The efficiency of protein utilisation was significantly affected by DFA ($P<0.01$), E: P ratio ($P<0.0001$) but not the sex of the birds. A plot of the data (Figure 2.1) also indicated that the straight line fit was satisfactory for the relationship between e_p and the E: P ratio. There was also no inflection point. With the intercept set to zero (i.e. assuming at zero dietary energy to protein contents the e_p is zero) for each level of food allocation linear regression equations were fitted to the data. The lowest e_p occurred in birds fed UPS diets containing 32.5 MJ ME/kg protein. Irrespective of E: P series, e_p values were higher for birds fed *ad lib.*

A linear regression of e_p on E: P ratio in the feed is given in Figure.2.1. The slopes for birds fed diets in the BPS and UPS at *ad lib.* or 0.75 of the previous *ad lib.* intake were 0.0132 ± 0.00044 , 0.0109 ± 0.00062 and 0.0122 ± 0.00056 , 0.0112 ± 0.00069 respectively.

A t-test was performed for the different ranges of data for the slopes to be compared. It was assumed that the variances of both ranges of data for the slopes are unequal. The confidence level for the test (alpha) was set to 0.05. A hypothesized mean difference was set for each variable range. This analysis is referred to as the heteroscedastic t-test (Minitab, 1998). It was used to determine whether the slopes of any two distinct lines are equal or not. No statistically significant differences were observed between the slopes for the treatments in the BPS and the UPS. A linear regression of e_p on dietary E: P ratio using individual data points is given by:

$$= 0.012009 (\pm 0.00029) \text{ E: P}$$

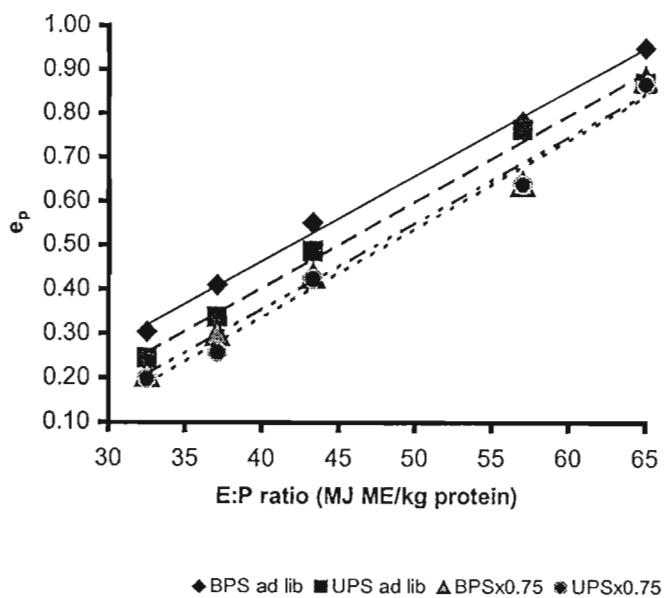


Figure. 2.1. The effect of dietary protein quality, daily food allocation and E:P ratios on the efficiency of protein utilisation (e_p).

DISCUSSION

The experiment examined the effects of sex on the variables measured. There was no significant effect of sex on the biological performance and the results from the two sexes were therefore pooled.

Amino acid balance: effects on the biological performance

Food intake, weight gain and FCE of the birds fed unrestricted amounts of the UPS were 87.0, 84.6 and 77.1% respectively of the values for birds fed BPS diets. At the highest dietary protein content (400 g CP/kg), growth rate was depressed regardless of PQ. The effects measured are in agreement with reports by Boorman and Ellis (1996) and Morris *et al.* (1999). These authors reported a curtailment of maximum response associated with the feeding of surplus protein exceeding the requirement for maximum growth. Diets that supply an array of nutrients that closely parallel the bird's nutrient (energy to protein) needs give rise to a better biological performance. The overall superior growth performance of birds on the BPS confirmed this principle. The suboptimal performances as observed in the current study have been demonstrated using free AA's (Harper *et al.*, 1970) and high amounts of poor quality proteins (Wethli *et al.*, 1975). The effect of excesses of dietary protein is important because the excess load of absorbed AA's do exert an imbalancing effect. This is due to the catabolism of excess AA's (through gluconeogenesis). This gives rise to an altered availability of AA's such as threonine, and other glucogenic AA's as reported for rats (Moundras *et al.*, 1993) and may account for the depressed biological performance of birds fed excess protein. The fall in the concentration of threonine, and other glucogenic AA's, together with an increased availability of branched chain AA's, produces an imbalanced supply of AA's. Lewis (1965) suggested that such surpluses of AA's have to be balanced with increases in the specified minimum concentrations for AA's that may be present in an inadequate ratio in the diet. Morris *et al.* (1999) reviewed data from experiments in which chicks were fed diets containing surplus protein in the range 220 to 300 g CP/kg diet, and energy contents within the range 12 –13 MJ ME/kg. Based on this data, Morris *et al.* (1999) provided the following as the best estimates for use in feed formulation in order to maximise growth and FCE:

Lysine requirement ≥ 0.0587 CP

Tryptophan requirement ≥ 0.012 CP

Methionine requirement ≥ 0.025 CP

where CP is the dietary CP concentration (g/kg)

Specifying these requirements as a proportion of the protein and not as a proportion of the diet, ensures that if it is economically desirable to formulate diets with higher-than-normal

protein contents, an upward adjustment is made to the minimum dietary AA (i.e. lysine) concentration (Morris *et al.*, 1987). The lysine to CP ratio of diets in the BPS and the UPS were 0.061 to 0.062 and 0.015 to 0.031 respectively. The minimum dietary lysine to CP ratio for birds fed the BPS was therefore greater than the minimum recommended by Morris *et al.* (1999), whereas the ratios for diets in the UPS were lower than the minimum recommended, accounting for the depression in growth rate of birds fed these diets.

If birds fed the UPS had increased their food intake, they would have consumed more energy, and also overconsumed AA's that were already in excess. Food intakes of birds fed the UPS at similar E: P ratios to those fed the BPS were lower. There was a decline in food intake by birds fed the UPS at dietary E: P ratios between 54.2 and 65 MJ ME/kg protein.

It is not necessarily heat increment that explains the impaired feed intake. The heat outputs for birds fed the BPS and the UPS at 400 g CP/kg were 621 and 570 kJ/d respectively and these values were lower than for birds within the same series but fed lower CP contents. Morris *et al.* (1999) supplemented diets containing excess protein with the first limiting amino acid and observed an increase in protein deposition and growth rate. Since chicks on the supplemented diets grew normally, these researchers concluded that heat disposal was not the problem limiting growth rate on diets loaded with excesses of poorly balanced high protein diets. In the experiment reported in this thesis diets were not supplemented with AA's.

Amino acid balance: effects on protein retention (PR), lipid retention (LR) and heat production (H).

Dietary treatments significantly affected PR as the E: P ratios increased from 32.5 to 65.0 MJ ME/kg protein. For birds fed diets in the BPS, PR increased to a maximum at 52 MJ ME/kg protein. At E: P ratios below this value, there was insufficient energy available to deposit the protein resulting in lower PR as E: P ratios were decreased further to 32.5 MJ ME/kg protein. Thus each unit increase in energy relative to protein intake from this low E: P ratio resulted in a proportional unit increase in the amount of protein that was deposited. This continued until a point was reached where the E: P ratio was perfectly balanced. At E: P ratios above this value, the bird would be consuming more energy than is required for protein deposition resulting in excess lipid deposition (Gous *et al.*, 1990).

The fact that PR was highest at the second lowest protein intake (250 g CP/kg, 52 MJ ME/kg protein, and 15.31g lysine /kg diet) suggests that sufficient energy was available at this E: P

ratio for conducting the various functions related to protein digestion and deposition. Furthermore the requirement for lysine was met and this improved growth and protein deposition. At the highest dietary CP content (400g CP/kg, 32.5 MJ ME/kg protein, and 24.9 g/kg lysine) the lack of energy appeared to have an adverse effect on protein deposition. At high levels of protein intake, the birds reached a maximum rate of protein growth, which was constrained by the daily food (energy) intake. Consequently when protein intakes were excessive, energy intake limited protein deposition. These findings are in agreement with reports by Harper *et al.* (1970), Holsheimer and Veerkamp (1992) and Morris *et al.* (1999) in which negative effects on performance were observed when diets containing extremely high protein levels were fed. Increasing dietary protein content (i.e. reducing the E: P ratio) reduced LR (Morris and Njuru, 1990). Campbell and Dunkin (1983), Morris and Njuru (1990) and Gous *et al.* (1990) indicated that birds deposit fat, above a certain minimum level, only when faced with a nutrient deficiency.

Several factors including excesses of protein, toxic effects of AA's after absorption and environmental hotness or the lack of energy for conducting the various functions related to protein digestion and deposition may account for the poor performance of birds fed diets in the UPS. Protein deposition for birds fed the UPS was highest for broilers that were fed diets containing dietary lysine at 10.93 to 12.49g/kg, which is within the range suggested by NRC (1994) but the content of 6.0 g/kg lysine at 65 MJ ME/kg protein is below the requirement for maximum protein gain. The lysine: CP ratios in the UPS with a range of 0.015 to 0.031 g lysine /kg CP were below that recommended for maximum growth by Morris *et al.* (1999). The proportion of total protein digested which is able to be incorporated into body protein 'v' for the UPS ranged from 1.00 to 0.52 for diets varying in E: P ratios between 32.5 to 65 MJ ME/kg protein respectively. Thus comparatively less of the protein consumed by birds fed the UPS was incorporated into protein tissue growth as the E: P ratios were increased from 32.5 to 65 MJ ME/kg protein. This is due to a decrease in the dietary lysine content in comparison with birds fed similar E: P ratios in the BPS. These findings are in agreement with the results of Morris *et al.* (1987) and Holsheimer and Veerkamp (1992) in which an increase in dietary lysine content increased PR. At similar lysine contents for broilers fed the BPS (12.25 g lysine/kg) and the UPS (12.49 g lysine/kg), those fed the normal practical CP content (i.e. 200 g CP/kg) had a higher gain of 24.4 g/d in comparison to birds on the highest CP diets in the UPS of 400 g CP/kg that had weight gains of 19.6 g/d. This confirms suggestions by Morris *et al.* (1987) and Holsheimer and Veerkamp (1992) that any surplus

dietary protein exerts a deleterious effect on the utilisation of protein. At equal dietary E: P ratios broilers fed diets with higher lysine content had a comparatively higher protein gain.

Because protein gain of the birds fed the BPS was higher than those fed diets in the UPS, there is more dietary energy that has to be eliminated-so this can either be lost as heat, or deposited as lipid. Thus heat production was associated with the deposition of protein or fat. Heat production of birds fed diets in the BPS was 68.6 kJ/d in comparison to 60.76 kJ/d of the UPS. The findings are in agreement with reports of MacLeod (1997) that heat production is a function of protein deposition rate. A greater amount of heat is produced per MJ of energy stored as protein than for each MJ stored as lipid. In explaining the reasons for the poor performance on high intakes of poor-quality proteins, Boorman and Ellis (1996) argued that apart from a small decrease in net energy yield, maximum response might not be achieved because of the depletion of the first limiting amino acid from tissue proteins.

Daily food allocation: effect on biological performance

Feed restriction was obtained by allocating 0.75 of the previous days *ad lib.* intake to birds. Restricting the amount of a diet prevented the birds from consuming larger amounts of energy. A reduction of the amount of food consumed can have effects on the development of the digestive tract and the rate of passage. Van Soest (1982) indicated that the low passage rate at restricted feeding had a greater effect with diets of lower nutritive value. This is because a prolonged stay of such diets in the digestive tract affords the digestive enzymes a greater opportunity to act on the substrates. However with diets that are easily digested and of a high nutritive value, passage rate does not seem to be very important. Birds offered *ad lib.* access to diets in the BPS and UPS had food intakes, weight gains and FCE that were 24.91, 32.07, 26.34 and 25.0, 41.51, 22.07% greater than broilers allocated diets at 0.75 of the *ad lib.* intake.

The negative effects of restricted feed intake on body weight gain or growth has been extensively reported by previous research (Khantaprab *et al.*, 1997; Gonzales *et al.*, 1998; Tan *et al.*, 1999). This reduction was partly attributed to reductions in thyroxin, insulin-like growth factors (IGF) and growth hormone (Gonzales *et al.*, 1998) when birds are restricted. At restricted feeding it is possible that the dietary protein to energy ratio was low in comparison to that required for soft tissue growth with a similar composition as the *ad lib.* fed birds.

Biological performance (i.e. ADG, FI and FCE) was not determined simply by the AA makeup and digestibility of the dietary protein but by the amount and composition (energy to protein ratio) of the diet in which the protein is supplied. This implies that in the absence of mineral and vitamin deficiencies, PQ, E:P ratio, DFA jointly determined the biological performance of the broiler chickens. The significant ($P<0.001$) interaction between PQ and E:P ratio for FI, ADG and FCE may be explained on the basis of a divergence in responses of birds fed the different protein mixtures to increasing E:P ratios. The weight gain response to DFA was dependent on PQ and E:P ratio. In order to maximise the biological performance of broiler chickens, diets on offer must (a) be balanced in the AA content such that the AA profile closely matches that of the body protein, (b) contain adequate supplies of dietary energy (increase in the E:P ratio) to maintain vital physiological processes related to the growth or deposition of body protein. Feed intake responded differently on account of PQ as well as DFA. Thus, feed intake was affected by PQ but only at low dietary protein content. The response to E:P ratio was only observed on the balanced diets fed *ad libitum*.

DFA, PQ and E:P ratio: effects on PR, LR and H.

Protein retention, LR and ER responded differently on account of PQ, E:P as well as DFA. These significant interactions explain the differences in responses to the dietary treatments. Some of these factors act together either directly or indirectly to regulate PR, LR and H. For instance, PR for birds fed UPS diets declined with increasing E: P ratio. Conversely, PR for birds fed BPS diets increased with increasing E: P ratio and decreasing DFA. Protein retention for chicks fed BPS and UPS diets at an intake of 0.75 of *ad lib.* were 74.9 and 77.9% respectively of the values for birds given *ad lib.* access to diets. In the present studies, the birds restrictively fed BPS and UPS diets retained less lipid (23.78 and 42.64%), less energy (27.7 and 44.7%) and produced less heat (79.6 and 77.5%) than birds offered *ad lib* access to diets in the respective protein series. Heat production increased with an increase in daily feed allocation; consequently birds fed diets loaded with excess protein in the BPS at *ad lib.* intake produced more heat than those fed at 0.75 of the previous *ad lib.* intake and those birds fed diets in the UPS. When birds were offered *ad lib.* access to the dietary treatments they retained protein and lipid at a rate according to their nutritive status, daily feed allocation, and the E:P ratio of the feed. Birds restrictively fed used all the energy available to synthesize protein, while very little energy was retained as lipid.

The results of this experiment point to the fact that PR, LR, ER and growth may approach a maximum determined by a combination of factors such as the PQ and DFA when a large amount of non-protein energy is available. Conversely PR and growth decrease with a decrease in food (or total energy) intake even though excess protein is available. The beneficial effect of an increase in E:P ratio or energy supply can be regulated by inadequate protein intake (i.e. protein of an ideal AA balance and quantity). Conversely, an increased protein intake may not be utilised effectively for PR and growth because of insufficient energy intake.

Efficiency of protein utilisation above maintenance (e_p).

The efficiency of protein utilisation was not determined simply by the quality of the dietary protein or the AA composition, but by the DFA and the E:P ratio. Thus the amount and the composition of the diet in which the protein is supplied, determines e_p . This is line with observations by Fuller and Crofts (1997). These authors indicated that, in the absence of vitamin and mineral deficiencies, the quality of the dietary protein on offer, the amount of protein given and the non-protein energy given with it jointly determine e_p .

The efficiency of protein utilisation above maintenance, e_p , was 10% lower for birds offered *ad lib.* access to diets in the UPS than in the BPS. At a food allocation of 0.75 of *ad lib.* intake, e_p for birds fed diets in the BPS was 2.1% greater than for birds fed the UPS. The efficiency of protein utilisation was in general better for *ad lib.* fed birds than for those restrictively fed. The better protein efficiency of the birds fed the BPS may be due to the increased amount of available dietary energy. Protein retention for birds offered *ad lib.* access to diets in the BPS and UPS were 25.1 and 22.13% greater than those fed at an intake of 0.75 of *ad lib.*

The ascending slopes for the BPS and UPS were not significantly different from those determined by Kyriazakis and Emmans (1992a, b) and Faulkner (1993) of 0.0112 and 0.0128, respectively. The results showed no inflection points for the range of E:P ratios used in this experiment. Faulkner (1993) fed diets with E:P ratios greater than the maximum of 65 MJ ME/kg protein, up to a maximum of 72.50 MJ ME/kg protein and obtained an inflection point at 54.15 MJ ME/kg.

Assuming that, in commercial practice a 230-g/kg protein and a 13.4 MJ ME/kg protein diet is fed *ad lib.* to broilers in the starter period (0 to 3wks of age) according to the NRC (1994)

specifications, this would yield an E: P ratio of 58.17 MJ ME/kg protein, which is similar to the value of 54.17 MJ ME/kg protein reported by Faulkner (1993). These E: P ratios suggest that broilers fed *ad lib.* in the starter stage are being fed close to their optimum for lean tissue accretion. Turkeys in the starter period (0 to 3 wks) are fed a 280 g CP/kg diet at 11 MJ ME/kg protein (NRC, 1994) giving rise to an E: P ratio of 39.29 MJ ME/kg protein. This value is considerably lower than the for broilers, which may indicate that in turkey starter diets, there is not enough energy available to deposit the protein consumed, resulting in poor protein utilisation. It may thus be advisable to increase the energy content of turkey starter diets to obtain an increase in protein utilisation and therefore growth. Ducks and geese are fed diets similar to those of chickens. The inflection point of 61.5 MJ ME/kg protein calculated from the NRC (1994) requirements starter diets for ducks and geese is an indication that their diets are adequate to maximize growth. The superior performance of birds fed the higher E: P ratios (i.e. 65 MJ ME/kg protein) in this experiment than that of Faulkner, (1993) and NRC (1994) suggests that, when the dietary protein content is increased beyond that required to meet the AA requirement of the broiler, there should be adequate dietary energy intake to ensure that the inputs of dietary protein can result in lean tissue gain and be utilised efficiently.

The current study has shown that when protein sources of low biological value (i.e. UPS) are used in practical feeding and there is no access to synthetic AA's, the dietary protein content would have to be increased for the requirements of the essential AA's to be met. This will require an increase in the dietary ME levels for the protein to be efficiently utilised. There are however indications that this results in suboptimal performance (Wethli *et al.*, 1975; Moundras *et al.*, 1993; Boorman and Ellis, 1996). Wethli *et al.* (1975) indicated that when the dietary protein content is in excess of the bird's requirements, high protein *per se* couldn't be regarded as the cause of any impairment of maximum response. It has been shown in this study that it is possible to obtain better performance by feeding extra energy.

Some metabolic explanations for the effect of surplus AA's were provided by Moundras *et al.* (1993) and Morris *et al.* (1999). Moundras *et al.* (1993) suggested that the undersupply of AA's such as threonine to body tissues may be responsible for the lowered growth rates of rats fed diets loaded with excess protein beyond the needs of the rat, whilst Morris *et al.* (1999) postulated that since the liver needs to get rid of surpluses of AA's the activities of numerous catabolic enzymes (i.e. threonine-serine dehydratase) are increased resulting in the loss of some AA's and growth depression. The decreased availability of some critical AA's

physiologically necessary in the control of protein intake (Moundras *et al.*, 1993) adversely influences the efficiency of protein utilisation when diets loaded with excess AA's are fed.

Furthermore, it is worth considering that excess protein ingestion induces a marked increase in the broiler chicken's dietary requirement for choline (Ketola and Nesheim, 1974; Molitoris and Baker, 1976). Consequently, there is an increased requirement for methyl groups that are needed to support uric acid synthesis under conditions that require large amounts of N to be excreted (Baker, 1991). Choline, which is bio-synthesized in the presence of S-adenosylmethionine, functions in phospholipid synthesis, acetyl choline formation, and, as betaine, in the remethylation of homocysteine to methionine (Baker, 1991). Therefore, until the need for choline for these functions is met, it is not possible to elicit maximum response by feeding protein in excess of the bird's requirements for AA's as evidenced in these enquiries.

CONCLUSIONS

The principle that diets supplying an array of nutrients that closely parallel the bird's nutrient needs give rise to an improved biological performance and efficiency of protein utilisation was confirmed in broiler chickens aged 10 to 24 days of age. The poorer performance observed for birds fed the BPS in the lower E: P ratios suggests that when the dietary protein content is increased beyond that required to meet the amino acid requirements of a broiler, the energy content should also be increased to ensure that sufficient energy is available for the efficient utilisation of dietary protein. There were no significant differences between the slopes of e_p vs. E: P ratio for birds fed the BPS and the UPS. The equation describing the overall relationship between E: P and e_p was $0.012009 (\pm 0.00029)E: P$. The linear increase in the slope of the ascending part of the curve relating E: P and e_p , suggests that each unit increase in energy relative to protein results in a proportional unit increase in the amount of protein deposited. It also evident that alterations of the biological performance (weight gain and FCE), carcass composition and quality, and the efficiency of protein utilisation in broiler chickens is achievable through a number of dietary routes. This includes changes to dietary protein quality (and hence AA's), daily food allocation and dietary energy to protein ratios.

CHAPTER 3:

**THE INFLUENCE OF PROTEIN QUALITY (PQ), ENERGY TO
PROTEIN (E: P) RATIO AND DAILY FOOD ALLOCATION (DFA) IN
THE PERIOD 23 TO 37 DAYS.**

ABSTRACT

The effect of excess dietary crude protein on the efficiency of utilization of protein (e_p) by broiler chickens as influenced by protein quality (PQ), energy to protein (E: P) ratio and daily food allocation (DFA) in the period 23 to 37 days was measured.

Two series of feeds differing in PQ, i.e. balanced (BPS) and unbalanced protein mixture (UPS), and covering a range of six E: P ratios (viz.: 32.5 to 65 MJ ME/kg) were offered at three feed allocations (*ad lib.* 0.8 and 0.6 of *ad lib.* intake) to chickens. The lysine concentrations of the BPS and the UPS ranged from 12.5 to 24.9 and from 6.0 to 12.1 g/kg respectively. Two hundred and forty chicks were randomly assigned either to an initial slaughter group (eight birds/experiment) or to one of the thirty-six dietary treatments with six replications.

For birds fed diets in the BPS, increasing the dietary E: P ratios (reducing protein contents) in isoenergetic diets increased carcass protein and e_p . In spite of the similar protein intakes of birds fed diets in the UPS and BPS (31.4 versus 30.8 g/d), birds fed diets in the UPS at *ad lib.* intake recorded significantly ($p < 0.01$) lower protein gains (9.4 versus 12.3 g/d) than those fed the BPS.

The slopes obtained from fitting a linear regression model to the e_p 's for the BPS and the UPS at the three levels of daily food allocation were 0.0114 (± 0.0002), 0.0116 (± 0.0002), 0.0134 (± 0.0003) and 0.0104 (± 0.0003), 0.0103 (± 0.0002), 0.0112 (± 0.0003) respectively. The combined slope for birds fed the BPS and the UPS were 0.0121 ± 0.001101 and 0.0106 ± 0.00049 respectively. With the exception of birds fed BPS 0.6 of *ad lib.* intake, there were no significant differences between the slopes of e_p vs. E: P ratio for birds fed either the BPS or UPS at other feed allocations.

Keywords : excess protein, daily food allocation, efficiency, and broiler.

INTRODUCTION

It was highlighted in Chapter 2 that diets supplying an array of nutrients that closely parallel the bird's nutrients needs give rise to a better biological performance. Furthermore, the poorer performance observed for birds fed balanced protein mixtures (BPS) at lower energy to protein (E: P) ratios suggests that when the dietary protein content is increased beyond that required to meet the amino acid (AA) requirements of a broiler, the energy content should also be increased to ensure that sufficient energy is available for the efficient utilisation of dietary protein. The problems associated with the feeding of excess protein (and hence AA's) in chick diets are the depression in growth rates and the impaired utilisation of dietary protein. Excesses of protein beyond the needs for protein synthesis are constantly degraded; this degradation increases as dietary protein level increases (Scott *et al.*, 1976). Thus the utilisation of dietary protein is less than 100% efficient and decreases with increasing excesses of dietary protein.

Our previous studies provide evidence confirming the model of Kyriazakis and Emmans (1992a, b) in broiler chickens in the starter phase of growth. This model saw the efficiency of protein utilisation, e_p , as a function of the metabolizable energy to digestible crude protein ratio (ME:DCP) in the feed on offer. It is uncertain whether the same concepts would derive the same responses for birds offered similar dietary treatments in the age group 23 to 37 d. These older birds may be less sensitive to effects of excesses of protein because older birds may have relatively greater protein reserves with which to buffer the effects of poor dietary AA patterns (Boorman, 1980).

The effect of excess dietary crude protein on the efficiency of utilization of protein (e_p) by broiler chickens as influenced by protein quality (PQ), E: P ratio and daily food allocation (DFA) in the period 23 to 37 days was examined in this study. This study will also determine if the results observed for younger birds is applicable to older birds.

MATERIALS AND METHODS

This experiment was a repeat of the study reported in Chapter 2 with a few modifications. Older birds (23 to 37 days of age) were also used in this experiment in comparison to the previous experiment reported in Chapter 2. Two series of feeds (Table 2.1) differing in protein quality (PQ) and covering a range of six E: P ratios were offered at three daily food

allocations (*ad lib.* 0.8 and 0.6 of *ad lib.*) to male broiler chickens 23 to 37 days of age. In order to obtain six replications of each of the 36 treatments, a facility with 216 cages would have been required. Eighty single cages were available for the study, so the 36 treatments could be replicated only twice at a time. Consequently the 36x2 treatments were replicated three times (treated as blocks) at intervals of three weeks to obtain six replications of each treatment. The same batch of ingredients was used to mix diets for the three periods. The measures of response were feed intake (g/d), bodyweight gain (g/d), feed conversion efficiency (FCE) (g gain/kg food intake), rates of lipid (LR) and protein retention (PR) (g/d), and the net efficiency of protein utilisation (e_p).

Birds and housing

Two hundred and forty male Ross broiler chickens of uniform size were used in the experiment. During the pre-test period (0-21 days post hatching) the chicks received a standard commercial starter feed (240 g crude protein/kg) *ad lib.* At 21 days of age, 90 chicks were placed in pairs in 3-tier battery cages in the experimental unit, to accustom them to the facilities, after being weighed to the nearest gram. At 23 days of age, 80 birds were assigned either to an initial slaughter group or to one of the 36 treatments, such that the average starting weight and weight range was similar for each treatment in each experiment. The selected birds were randomly allocated to individual cages supplied with continuous artificial lighting. The house temperature was monitored daily throughout the duration of the trial.

Experimental feeds and design

The same diets as described in Chapter 2 were used in the study, one being based on a balanced, and the other on an unbalanced (UPS) AA mixture (Table 2.1). These were each fed at six E: P ratios and at three daily food allocations.

The two basal diets were formulated to contain 13 MJ AME/kg and 400 g CP/kg. The AA specifications applied were those suggested by the EFG (1998) broiler growth model for broilers aged 23 to 37d. The mean lysine requirement over this period is 10.6 g lysine/kg. The basis of these requirements is that a broiler will be growing body and feather protein, of known AA composition, at a rate determined by its potential growth rate. The crude protein concentrations within each series were 400, 360, 320, 280, 240 and 200 g CP/kg respectively, all with the same energy content of 13 MJ AME/kg resulting in six E: P ratios (viz.: 32.5, 36.1, 40.6, 46.4, 54.2 and 65.0 MJ ME/ kg protein).

Dietary treatments

(i) *Ad libitum* treatments

Birds allocated to these treatments were given free and continuous access to one of the 12 dietary treatments. Feed consumption was measured daily by weighing the food and trough at the start and end of each 24-hour period.

(ii) Restricted treatments

Birds designated to these treatments were restricted to 0.8 or 0.6 of the average consumption of the respective *ad lib.* treatments determined from the previous day. The allocated feed was divided into two portions, with the first being given in the morning at 7 a.m. and the other in the afternoon at 2 p.m. The same levels of restriction were applied for each of the two protein series.

Management and slaughter procedure

At 23 d of age, eight birds of the same average weight (0.895 ± 0.06 kg) as recorded for the chicks assigned to the experimental diets were selected at random and euthanatized by carbon dioxide asphyxiation. The carcasses were placed in gas-impermeable plastic bags and frozen at -4°C pending processing at a later stage. These birds were used to determine the initial composition data at 23d posthatching. All surviving experimental birds were fed twice daily and weighed on a full-fed basis once weekly. At the conclusion of the experiment, all birds were weighed and killed by asphyxiation with carbon dioxide to prevent loss of blood. The carcasses were frozen in freezer bags at -4°C for later analysis. All frozen carcasses were allowed to thaw in sequence, chopped into smaller pieces and were ground individually in a mincer. Each ground sample was then thoroughly mixed and a sub-sample of approximately 500g was taken from each carcass for moisture determination and subsequent chemical analyses.

Lipid retention, energy retention, heat production and the net efficiency of protein utilisation (e_p)

Lipid, energy retention, heat production and the net efficiency of protein utilisation (e_p) above maintenance were calculated as in the previous study. Protein digestibility values for the various raw materials used were obtained from the European Table of Energy Values for

Poultry Feedstuffs (1986). The d_{cp} for the BPS and UPS series were 0.8330 (Feeds BPS1...BPS6) and 0.8857 (Feeds UPS1...UPS6). The proportion of total digested protein which can be incorporated into body protein, 'v', for the BPS and UPS were 1.00 (Feeds BPS1...BPS5), 0.89 (Feed BPS6) and 1.00 (Feeds UPS1 and UPS2), 0.91, 0.80, 0.68, and 0.57 (UPS3...UPS6). The ideal AA balance for a bird of between 23 and 37 days of age was obtained from the EFG broiler simulation model (1998), which makes use of a factorial approach to calculate the AA's required for the growth of body and feather protein at the rates appropriate for a given genotype at a given degree of maturity.

Statistical design and analysis

The aim of this study was to test the hypothesis that the efficiency with which dietary crude protein is used for growth in broiler chickens is affected by protein quality (PQ), energy to protein (E: P) ratio and daily food allocation (DFA). The statistical design of the investigation was a 2x6x3 factorial design with three factors: protein series (two levels), E: P ratio (at six levels) and three DFA's (*ad lib*, 0.8 and 0.6 of *ad lib*. intake). Since these factors represent quantitative variables, the most informative method of analysis of this experiment is to consider the response y as a function of the E: P ratio at each DFA and for each protein series separately. The response y is expected to improve with increasing E: P ratios in a linear manner and could become curvilinear as the maximum is approached. A plateau is ultimately reached, where the input being investigated does not limit performance and there is no response to further increases of the input (Morris, 1998).

Regression analyses were used to define the relationship between the variables and the various factors. Linear regression equations were also determined for each protein series at each DFA for e_p vs. E: P ratio. At zero dietary energy to protein content, e_p must be assumed to be zero, thus simple linear equations derived from the experiments were forced through the origin as giving the best estimates of the relationship between e_p vs. E: P ratio. Each individually penned bird was considered as an experimental unit.

RESULTS

All the results presented and regression analyses conducted are from pooled data over six replications. Table 3.1 summarises values for the mean responses in food intake (FI), average daily live-weight gain (ADG), daily live-weight gain per kg food (FCE), protein retention (PR) and e_p .

Table 3.1. *The effect of dietary protein quality, E: P ratios and daily food allocation on the biological performance and the efficiency of protein utilisation (e_p), of male broiler chickens from 23 to 37 days of age.*

¹ Dietary treatment	E: P ratio	² Daily food allocation	Feed intake (g/d)	Weight gain (g/d)	FCE (g gain/kg food intake)	Protein intake (g/d)	PR (g/d)	e _p	
BPS ³	1	32.5	1.0	84.3	41.2	489	35.0	10.0	0.38
	2	36.1	1.0	106.5	53.9	506	36.7	12.9	0.42
	3	40.6	1.0	100.1	49.5	495	33.3	12.7	0.50
	4	46.3	1.0	109.0	54.5	500	30.0	12.5	0.53
	5	54.2	1.0	107.5	51.8	482	28.3	12.7	0.64
	6	65.0	1.0	115.0	57.0	496	21.7	13.0	0.69
			Mean	103.7	51.3	495	30.8	12.3	0.53
	7	32.5	0.8	65.1	29.1	447	26.7	8.5	0.42
	8	36.1	0.8	81.5	30.9	380	26.7	9.2	0.40
	9	40.6	0.8	80.6	33.6	417	23.3	9.0	0.45
	10	46.3	0.8	86.1	36.7	427	23.3	10.2	0.54
	11	54.2	0.8	85.8	42.6	496	20.0	10.8	0.69
	12	65.0	0.8	86.4	40.5	468	18.3	10.0	0.71
			Mean	80.9	35.6	439	23.1	9.6	0.53
	13	32.5	0.6	47.6	18.1	381	20.0	7.5	0.50
	14	36.1	0.6	59.8	22.5	377	20.0	7.3	0.43
	15	40.6	0.6	57.9	28.4	490	16.7	8.8	0.64
	16	46.3	0.6	65.4	27.8	425	17.0	8.0	0.59
	17	54.2	0.6	63.3	25.9	424	16.7	8.0	0.71
	18	65.0	0.6	63.3	25.4	402	10.0	7.8	0.85
			Mean	59.6	24.9	417	16.7	7.9	0.62
UPS ⁴	19	32.5	1.0	100.9	51.5	511	40.0	11.2	0.32
	20	36.1	1.0	100.5	50.1	499	38.3	10.0	0.32
	21	40.6	1.0	117.0	52.3	447	36.7	11.7	0.41
	22	46.3	1.0	109.0	46.1	423	26.7	8.2	0.48
	23	54.2	1.0	104.9	44.5	424	26.7	9.2	0.67
	24	65.0	1.0	105.4	34.4	327	20.0	6.2	0.64
			Mean	106.3	46.5	438	31.4	9.4	0.47
	25	32.5	0.8	79.1	34.1	431	30.0	8.5	0.32
	26	36.1	0.8	81.1	34.8	430	28.3	8.5	0.36
	27	40.6	0.8	93.2	33.8	363	26.7	7.5	0.33
	28	46.3	0.8	85.5	27.8	325	26.7	6.7	0.43
	29	54.2	0.8	80.8	30.8	381	16.7	7.5	0.71
	30	65.0	0.8	83.2	20.7	249	16.7	4.7	0.66
			Mean	83.8	30.4	363	24.2	7.2	0.47
	31	32.5	0.6	58.1	23.3	402	23.3	6.8	0.35
	32	36.1	0.6	59.6	25.0	420	20.0	6.0	0.33
	33	40.6	0.6	68.6	25.3	368	20.0	6.5	0.40
	34	46.3	0.6	62.9	20.1	320	16.7	5.8	0.52
	35	54.2	0.6	59.3	20.0	338	10.0	3.8	0.79
	36	65.0	0.6	61.1	13.9	228	17.8	5.6	0.50
			Mean	61.6	21.3	346	17.8	5.6	0.50

(Table 3.1 continued on next page)

Source of variation	SEM	1.5	0.94	7.48	0.54	0.19	0.01
PQ	NS	NS	***	*	NS	**	NS
DFA	NS	NS	NS	NS	*	NS	NS
E:P	**	**	***	**	NS	***	***
DFA*PQ	NS	NS	*	NS	NS	NS	NS
EP*PQ	***	***	***	***	NS	***	NS
EP*DFA	NS	NS	NS	NS	NS	NS	NS

The **mean** of each response due to daily food allocation are highlighted in bold. ¹Each treatment was replicated six times in three experiments that followed the same format and used the same compounded feeds. The protein contents within each feeding level were 400, 360, 320, 280, 240 and 200 g CP/kg. ² Proportion of *ad lib.* . ³Balanced Protein Series; ⁴ Unbalanced Protein Series, *, P<0.05, **, P<0.01, ***, P<0.001

Food intake, daily live weight gain and efficiency of food utilization

The birds used for this experiment were older and heavier than those used in our previous investigation but the pattern of responses was similar to that obtained with corresponding diets. E: P ratios significantly influenced the FI (P<0.001), ADG (P<0.001) and FCE (P<0.01) but this was dependent on protein quality. Protein quality significantly influenced weight gain (P<0.001) and FCE (P<0.05).

The food intake of birds fed diets in the BPS increased initially with a decrease in dietary protein content or an increase in E: P ratio, this then dropped at an E: P ratio of 54.2 to the level of birds fed the 36.1 MJ ME/kg protein. The lowest food intake amongst the *ad lib.* fed birds occurred on the 400 g CP/kg feed with the highest lysine concentration of 24.9g/kg. The food intake of birds offered unrestricted access to the UPS increased up to a maximum at an E: P ratio of 40.6 MJ ME/kg protein and then dropped to a level higher than that for birds fed the highest protein concentration or lowest E: P ratio of 32.5 MJ ME/kg protein. Food intakes, and therefore protein intakes were greater for birds fed the UPS than those fed the BPS. But this was not statistically significant. As the dietary lysine concentration increased, so did the ADG increase linearly (P<0.0001) until a maximum ADG was attained. Thereafter it declined or showed no significant change. The BPS series significantly (P<0.0001) enhanced the birds' growth rate in comparison to the UPS series at all levels of DFA. For birds fed BPS diets, ADG at *ad lib.* 0.8 and 0.6 of *ad lib.* intake improved by 4.8, 5.2 and 3.6 g/d respectively compared with birds fed the UPS. There was a significant (P<0.01) reduction in body weight gain between birds fed *ad lib.* and those allocated food at 0.6 of the previous *ad lib.* intake.

Food conversion efficiency to 37 d of age for birds fed the BPS responded initially to increasing E: P ratio to about 36.1 MJ ME/kg protein, thereafter it showed no consistent trend. As E: P ratio increased from 32.5 to 65 MJ ME/kg protein; FCE declined progressively from 511 to 327 for birds fed the UPS. FCE at the three levels of food allocation (i.e. *ad lib.* 0.8 and 0.6 of *ad lib.* intake) for the BPS was improved by 11.5 17.3 and 17.0 % respectively compared with birds fed the UPS. Feed restriction reduced FCE, resulting in a significant ($p<0.01$) difference in conversion between the birds that were fed *ad lib.* and those fed 0.6 of the *ad lib.* intake.

Protein, lipid and energy retention

The mean composition of the initial slaughter group (23 days old) at 0.895 ± 0.06 kg liveweight was 707 ± 8.55 g moisture /kg, 150.4 ± 5.85 g protein /kg, 24.33 ± 0.74 MJ ME/kg GE. The following equation was used to calculate the energy retained only as protein and lipid: $ER = h_p \cdot PR + h_l \cdot LR$, where h_p and h_l are the heats of combustion of protein and lipid. The relationship between dietary PQ, E: P ratio and daily food allocation (DFA) on LR and H are shown in Table 3.2. Figure 3.1 and 3.2 summarises the principal responses of excess dietary protein on e_p as influenced by dietary treatments. There were slight differences in the responses of some birds offered the same dietary treatments. This probably arose because individual birds had different starting weights. It was thus appropriate to use the initial body weight of each bird as a covariate. The rate of change in PR at the three daily food allocations (i.e. 1, 0.8 and 0.6 of *ad lib.*) for the BPS was improved by 2.9, 2.4 and 2.3 g/d respectively in comparison to birds fed the UPS. Conversely, the main effect of feed allocations for the UPS resulted in the LR being 6.1, 3.3 and 2.7 g/d higher than those of the birds offered diets in the BPS. This was dependent on the interaction with PQ. Birds fed diets in the UPS retained comparatively more energy at each level of DFA (i.e. 238.8, 126.8 and 105.5 kJ/d) than those offered the BPS. Energy to protein ratio significantly influenced PR ($P<0.001$), but this was dependent on PQ. There was also a significant ($P<0.05$) interaction between E:P and PQ for ER.

Birds fed diets in the BPS and UPS had similar protein intakes of 30.8 and 31.4 g/d but significantly different ($P<0.0001$) protein gains of 12.3 and 9.4 g/d respectively. The daily rates of PR were highest for birds offered unrestricted access to each protein series, but declined with a decrease in daily food allocation.

Table 3.3. *The effect of dietary protein quality, E: P ratios and daily food allocation on the lipid gain, energy retention and heat production of male broiler chickens from 23 to 37 days of age.*

Dietary treatment	E: P ratio	² Daily Food Allocation	Lipid gain (g/d)	Energy retained (kJ/d)	Heat production (kJ/d)
Balanced protein series					
1	32.5	1.0	4.7	201.7	896.7
2	36.1	1.0	7.4	311.7	1075.0
3	40.6	1.0	5.7	243.3	1060.0
4	46.3	1.0	9.6	396.7	1021.7
5	54.2	1.0	8.7	361.7	1036.7
6	65.0	1.0	15.6	636.7	860.0
		Mean	8.63	358.6	991.6
7	32.5	0.8	2.8	128.3	716.7
8	36.1	0.8	4.3	183.3	876.7
9	40.6	0.8	3.9	168.3	880.0
10	46.3	0.8	5.0	213.3	910.0
11	54.2	0.8	7.6	316.7	798.3
12	65.0	0.8	9.5	386.7	735.0
		Mean	5.53	232.7	819.5
13	32.5	0.6	1.8	78.3	540.0
14	36.1	0.6	2.6	111.7	663.3
15	40.6	0.6	2.0	100.0	661.7
16	46.3	0.6	2.8	115.6	611.7
17	54.2	0.6	2.6	111.3	708.3
18	65.0	0.6	4.7	195.0	625.0
Unbalanced protein series		Mean	2.74	Mean	635.0
19	32.5	1.0	11.9	485.0	825.0
20	36.1	1.0	10.4	425.0	881.7
21	40.6	1.0	17.8	720.0	798.3
22	46.3	1.0	15.0	604.3	1091.7
23	54.2	1.0	16.7	673.3	690.0
24	65.0	1.0	16.1	676.7	721.7
		Mean	14.7	597.4	771.0
25	32.5	0.8	7.2	295.0	730.0
26	36.1	0.8	8.5	350.0	708.3
27	40.6	0.8	9.0	366.7	845.0
28	46.3	0.8	7.8	316.7	793.3
29	54.2	0.8	9.4	383.3	666.7
30	65.0	0.8	11.0	445.0	638.3
		Mean	8.83	359.5	730.3
31	32.5	0.6	2.8	120.0	636.7
32	36.1	0.6	5.5	233.3	550.0
33	40.6	0.6	5.9	241.7	648.3
34	46.3	0.6	5.9	241.7	578.3
35	54.2	0.6	6.7	268.3	500.0
36	65.0	0.6	6.0	240.0	553.3
		Mean	5.45	224.2	577.8
		SEM	0.351	14.0	14.0

(Table 3.3 continued on next page)

Source of variation			
PQ	NS	NS	NS
DFA	NS	NS	*
E:P	NS	NS	***
DFA*PQ	NS	NS	NS
EP*PQ	NS	*	NS
EP*DFA	NS	*	NS

The **mean** of each response due to daily food allocation are highlighted in bold. ¹Each treatment was replicated six times in three experiments that followed the same format and used the same compounded feeds. The protein contents within each feeding level were 400, 360, 320, 280, 240 and 200 g CP/kg. ² Proportion of *ad lib.* *, P<0.05, ***, P<0.001

Birds fed diets in the BPS continued to achieve higher protein gains than those offered the UPS as dietary E: P ratio increased or with a reduction in protein concentration. The lowest protein gain of 5.6 g/d was recorded at the highest E: P ratio of 65 MJ ME/kg protein for birds fed diets in the UPS.

Heat production

Energy to protein ratio and DFA had a significant (P<0.001) effect on H. As the dietary E: P ratios increased from 36.1 to 65, 32.5 to 46.5 and 32.5 to 54.2 MJ ME/kg protein for birds fed diets in the BPS, the H within each level of daily food allocation decreased from 1075 to 860, increased from 716 to 910 and 540 to 708 kJ/d respectively for birds fed diets at *ad lib.* 0.8 and 0.6 of *ad lib.* intake. For birds fed diets in the UPS, increases in dietary E: P ratios from 32.5 to 54.2, 40.6 to 65 and 40.6 to 54.2 MJ ME/kg protein, resulted in an inconsistent decrease in H (kJ/d) for birds fed *ad lib.*, a reduction from 845 to 638 and 648 to 500 for birds fed 0.8 or 0.6 of the *ad lib.* intake respectively. Birds fed diets in the BPS had lower ER (kJ/d) but higher H (kJ/d) of 220.6, 86.74 and 57.12 at the three levels of DFA than those fed diets in the UPS (Table 3.2).

Birds fed diets in the BPS retained less energy (kJ/d) as lipid (viz: 341.22, 218.46 and 108.90) in comparison to those fed the UPS (viz: 580.14, 349.14 216.48). Conversely, birds fed diets in the UPS retained less energy (kJ/d) as protein (viz: 173.27, 133.09, 105.80) in comparison to those fed the BPS (viz: 226.32, 176.95, 145.36) at the three levels of feed allocation. There was a decrease in heat production, due to the lower energy intake, as the feeding level decreased.

The net efficiency of protein utilisation above maintenance, e_p

The e_p values were calculated from the equation of Kyriazakis and Emmans (1991b), which is similar to that proposed by ARC (1981). However, in this study maintenance protein (MP) was calculated on an ideal protein basis as suggested by Emmans and Fisher (1986) rather than on a crude protein basis. The e_p values calculated for each level of feed allocation within each protein series are shown in Table 3.3. A regression was fitted to these data and the full response surface for e_p was: $0.229 - 0.278 \text{ PQ} - 0.303 \text{ DFA} + 0.0242 \text{ E: P} + \text{Wt0} + 0.169 \text{ DFA} \times \text{PQ} + 0.003 \text{ E: P} \times \text{PQ} - 0.005 \text{ E: P} \times \text{DFA}$ ($R^2 = 0.77 \pm 0.08$),

where Wt0 is the initial weight of the birds.

Table 3.3. *The slope and maximum efficiency of protein utilisation (e_p) of male broiler chickens aged 23 to 37 days obtained by fitting a linear regression model to the response data.*

¹ DFA	Slope (kg/MJ)	s.e. of slope coefficient	Adj. R^2	P- value	Predicted inflection at E:P ratio (MJ ME/kg protein)	Maximum e_p observed	Maximum e_p predicted
Balanced Protein Series (BPS)							
1.0	0.0114	0.0003	0.66	0.000	70.29	0.69	0.73
0.8	0.0116	0.0002	0.73	0.000	68.86	0.71	0.75
0.6	0.0134	0.0004	0.58	0.000	59.51	0.85	0.87
Mean	0.0114	0.0003	0.66	0.000			
Unbalanced Protein Series (UPS)							
1.0	0.0104	0.0003	0.65	0.000	76.88	0.67	0.68
0.8	0.0104	0.0003	0.68	0.000	77.28	0.71	0.67
0.6	0.0112	0.0003	0.75	0.000	71.33	0.79	0.72
Mean	0.0107	0.0002	0.71	0.000			

¹Regressions were from treatments that had been replicated six times; s.e. Standard error.

The linear effects were highly significant ($P < 0.001$, $R^2 = 0.74$). A test of significance was performed on the data to establish the relationship between the response variable e_p and the E: P ratio. A plot of the data also indicated that the straight line fit was satisfactory for the relationship between e_p and the E: P ratio. The response of e_p to dietary E: P ratios are shown in Figs.3.1 and 3.2. With the intercept set to zero for each level of feed allocation, linear regression equations were fitted to the data. The efficiency of protein utilisation was significantly ($p < 0.001$) affected by protein series and E: P ratio. Birds fed the BPS had significantly ($P < 0.05$) higher e_p values in comparison to those offered the UPS. The highest e_p for each protein series occurred at a feed allocation of 0.6 of the previous *ad lib* intake and

an E: P ratio of 65 MJ ME/kg protein for birds fed the BPS but not for those fed the UPS where e_p was highest at 54.2 MJ ME/kg protein. Conversely the lowest e_p occurred in birds fed *ad lib* at 32.5 MJ ME/kg protein. Within each level of feed allocation and at each E: P ratio, e_p values were higher for birds a restricted diet.

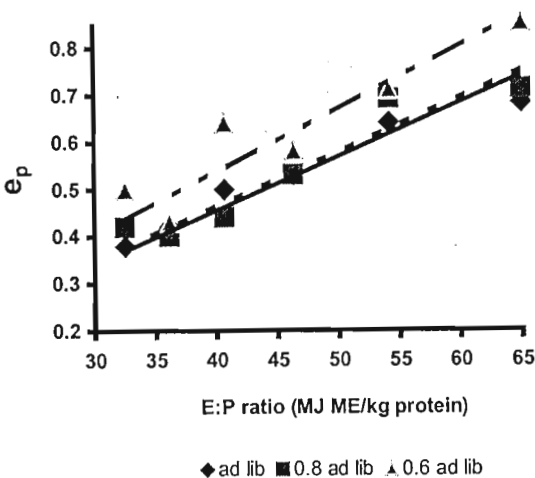


Figure.3.1. The effect of protein quality (PQ), E: P ratio and daily food allocation (DFA) on e_p of birds fed the BPS.

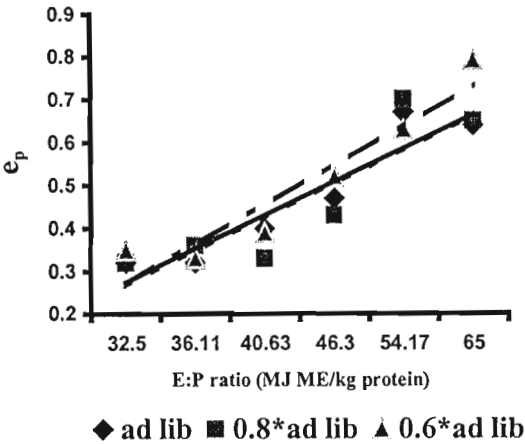


Figure. 3.2. The effect of PQ, E: P ratio and DFA on e_p of birds fed diets in the UPS.

A heteroscedastic t-test using Minitab (1998) was performed on the data to establish whether the slopes differed significantly from each other. The slopes (Table 3.4) were significantly greater ($P<0.05$) for birds fed diets in the BPS at 0.6 of the proportion of *ad lib.* intake than

for birds on the other food allocations. The linear regression coefficients between birds fed diets in the BPS and UPS at either 0.8 or 0.6 of the *ad lib.* intake respectively did not differ significantly from each other ($P=0.12$, $P=0.06$). The slopes for these birds were 0.0114 vs. 0.0104 and 0.0116 vs. 0.0104 respectively. The inflection points for the BPS and UPS at each level of feed allocation were estimated as in Chapter 2 and are shown in Table 3.3. This is not shown on the Figure 3.1 and 3.2 as the points occur outside the range of experimental data.

A stepwise multiple regression technique was performed on the e_p data for the two growth periods (10 to 24 and 23 to 37d) to establish whether the slopes of the ascending part of the regression of e_p on E:P differed significantly from each other. The dummy variable capturing the effect of age group, $adum$ was assigned a value of 0 for the period 10 to 24 and a value of 1 for the period 23 to 37d respectively. The variable capturing the effect of protein series, $pdum$ was assigned a value of 0 or 1 for the BPS and UPS respectively. The created variable measuring whether the different age groups or protein series have different slopes is 'aslopes' and 'pslopes', respectively. Besides E:P there are product terms and two dummy variables enabling the running of a multiple regression analysis.

The complete Model fitted for e_p is given by

E:P $adum$ $pdum$ $aslopes$ $pslopes$ $apint$

Where $aslopes = adum \times E:P$ (testing whether slopes differ between age groups);

$pslopes = pdum \times E:P$ (testing whether slopes differ between protein series);

$apint = adum \times pdum$ (testing the interaction of age and protein series on the intercept)

$apslopes = apint \times E:P$ (testing the interaction of age and protein series on the slope).

The criterion for a variable being entered into the model was set at $P=0.10$. The results of the stepwise regressions are given in Table 3.4

Table 3.4. *Estimated regression coefficients obtained by fitting the responses with a regression model of the form : EP adum aslopes*

Variable	Parameter estimate	Standard error	t-value	p-value
Intercept	-0.33624	0.04877	-6.90	0.0001
e _p	0.02031	0.00102	19.95	0.0001
adum	0.36633	0.06094	6.01	0.0001
pdum	-0.05536	0.01404	-3.94	0.0001
aslopes	-0.00946	0.00129	-7.34	0.0001
SEM	0.074			
Adj R ²	0.85			

Slopes did not differ for broilers fed the BPS and UPS between 10 to 24 d and 23 to 37 d. However age of the bird (aslopes) significantly ($P < 0.001$) affected the slope of e_p vs. E:P ratio

DISCUSSION

The principal nutritional factors that may influence body and protein growth are the AA composition of the protein, the dietary protein content, the associated E: P ratio of the ration, and the daily nutrient allocation. Few quantitative results have been published on the efficiency of protein utilisation (e_p) when feeding diets with concentrations of proteins beyond those required to meet the AA requirement of the broiler. Having shown in Chapter 2 that growth and e_p are impaired in birds 10 to 24 d of age fed high-protein diets (i.e., protein concentrations >350g CP/kg), this study was set up to investigate if the results observed for younger birds would be observed in older birds 23 to 37 d of age.

Amino acid balance: effects on the biological performance

Excess protein was shown to impair the performance of chicks 23 to 37 d of age. The sub optimal performance measured was in agreement with reports in Chapter 2. Maximum weight gain was less for birds fed the UPS than for those fed with the balanced mixture of proteins regardless of the age of the bird, even if excess dietary protein were fed in order to meet the requirements for essential amino acids. Observations in this study indicate that the

deleterious effects of excess dietary protein (i.e. regardless of protein balance) are not buffered by the presence of excess protein reserves in the mature bird.

Diets used in these series of experiments contained excess protein (and hence AA's), therefore by increasing their food intake, birds fed the UPS would have consumed extra energy and protein beyond their requirement for growth. Ferguson *et al.* (2000b) underlined that, it is not always possible for animals fed diets with very low lysine: protein ratios of less than 0.03 g/kg to increase their food intakes. This may be due to the high heat increment (HI) associated with the digestion of low amino acid: high-energy diets or because the capacity of the gut constrains maximum food intake. This was evidenced in this experiment by the lack of increase in food intake by birds fed the UPS at dietary E: P ratios between 54.2 and 65 MJ ME/kg protein. H is associated with an increase in PR (Mac Leod, 1997), thus birds depositing protein at a higher rate will be expected to have a problem with heat disposal. However, heat disposal was not the main problem limiting growth rate on diets loaded with excesses of poorly balanced proteins. This is because heat production was greater for birds fed the BPS (991.6 kJ/d) than for the UPS (771.0 kJ/d), but these birds had a better average daily weight gain (51.3 vs. 46.5 g/d respectively).

The relationship between dietary protein and lysine concentration for broiler growth has not been satisfactorily quantified for protein concentrations in excess of 300 g/kg. The range of lysine to CP ratios in the BPS and UPS were 0.061 to 0.062 and 0.015 to 0.031 respectively. Thus the ratios in the UPS were lower than the minimum lysine: CP ratio of 0.0587 recommended by Morris *et al.* (1999) for optimum biological performance (FI, ADG and FCE) of broiler chickens. This may account for the depression in food intake and body growth of birds fed the UPS. High protein diets with an imbalanced AA profile are extensively catabolised in order to prevent their accumulation in the blood. This results in the loss of some critical glucogenic AA's (i.e. threonine, serine, glycine and glutamine) required by the peripheral tissues (Moundras *et al.*, 1993). The extensive catabolism of diets in the UPS could have produced a large direct signal to the brain for appetite depression. The subsequent reduction in food intake enables the bird to (i) prevent the accumulation of some AA's (i.e. Aromatic AA's) at physiologically intolerant levels (ii) control protein intake at a level that assists in maintaining physiologically tolerant concentrations of indispensable AA's in the brain (Moundras *et al.*, 1999). It is possible that birds fed the BPS had a lower plasma AA concentration allowing them to ingest a slightly greater amount of food than those on the UPS. Excess protein was therefore catabolised to a greater extent for birds fed the UPS than

the BPS instead of being used for body growth in these birds. According to Moundras *et al.* (1993), the high rate of catabolism is an adaptive response, which could be of physiological relevance in the control of food intake.

Investigations of the effect of feeding high levels of low-quality proteins in growing chickens were carried out by Wethli *et al.* (1975). They reported that diets containing 420 g CP/kg of either herring meal or groundnut meal supplemented with lysine and methionine, gave good growth rates and FCE's. The fact that birds fed diets containing excess protein but supplemented with lysine and methionine increased their growth rate (Wethli *et al.*, 1975), implies that such diets were not unpalatable or toxic. Thus in the current study, the reduced performance of birds fed high concentrations of unsupplemented dietary protein cannot be attributable to an insufficient intake of essential amino acids (EAA's), since feed consumption was already substantial to allow for the ingestion of adequate amounts of EAA's (i.e. lysine). The impairment of maximum response of birds fed diets in the UPS may be due to an unfavourable balance of ingested protein mixtures (AA's). Moundras *et al.* (1993) indicated that there is a fall in the concentrations of glucogenic AA's and an increased availability of branched chain AA's which causes an imbalanced AA supply on feeding high protein diets. The addition of lysine and methionine to groundnut meals (Wethli *et al.*, 1975) or the supplementation of high protein diets with essential amino acids (Morris *et al.*, 1999) resulted in the restoration of performance to almost maximum level. In practice it may be possible to feed high concentrations of low quality proteins but growth will be suboptimal unless diets are supplemented with EAA's. It has been shown that excess protein can impair the performance of birds 10 to 24 or 23 to 37 d of age fed the BPS in the lower E: P ratios. Based on these observations, it is suggested that, when broiler chickens are offered diets containing typical ingredients in which the dietary protein content is increased beyond that required to meet the AA requirements of a broiler, the energy content should also be increased. This is necessary in order to make sufficient energy available for the efficient utilisation of dietary protein.

Amino acid balance: effects on protein and lipid retention and heat production.

Measurements of PR of birds fed higher dietary protein in the BPS and UPS showed a decrease in the magnitude of responses. This is because these diets provide large amounts of un-utilizable protein (AA's). For birds offered the BPS, below an E: P ratio of approximately 54.17 MJ ME/kg protein, there was insufficient energy available to deposit maximum protein

resulting in lower protein deposition as E: P ratios were decreased further to 32.5 MJ ME/kg protein. The protein intake of birds offered unrestricted access to diets in the BPS at E: P ratios between 32.5 and 65 MJ ME/kg protein decreased from 35 to 21.7 g/d whilst protein retention increased from 10 to 13 g/d. The fact that protein deposition is highest at the lowest protein concentration (200 g CP/kg, 12.5g/kg lysine) could imply that sufficient energy was available at this E: P ratio of 65 MJ ME/kg protein for PR. Energy is required for protein turnover and retention (Boorman, 1980). Thus at the highest dietary CP content (400g CP/kg, 32.5 MJ ME/kg protein, 24.9 g/kg lysine) insufficient dietary energy appeared to have an adverse effect on the full utilization of ingested protein for PR. There are several reports indicating that requirements for AA's are positively correlated with the dietary protein level (Morris *et al.*, 1987,1992). With an increasing dietary excess of dietary CP supply (i.e. 400 g CP/kg) there is a reduction in the utilisation of the first limiting AA. This accounts for the reduced performance at the very high dietary CP levels in the BPS.

When broilers are offered *ad lib.* access to UPS diets and at DFA of 0.8 of *ad lib.* the protein intakes at E: P ratios between 32.5 and 46.3 MJ ME/kg protein decreased from 40.0 to 26.7 and 30.0 to 26.7g/d respectively, whilst PR decreased from 11.2 to 8.2 and 8.5 to 6.7g/d respectively. PR was highest for broilers that were fed diets containing dietary lysine at 12.1g/kg, which is higher than the requirements as suggested, by the EFG growth model (1998) and NRC (1994). The content of 6.0 g lys/kg diet at the lowest dietary protein concentration of 200 g CP/kg is far below the requirement for maximum protein gain. The reduction in PR of birds fed diets in the UPS with E: P ratios increasing from 32.5 to 65 MJ ME/kg protein is due to a decrease in the dietary lysine content in comparison with birds fed similar E: P ratios in the BPS.

Energy retained as protein for birds provided with *ad lib.* access to diets in the BPS and the UPS were 226.32 and 173.27 kJ/d respectively, conversely less energy was retained as lipid for birds fed BPS diets than in those on imbalanced diets (341.22 vs. 580.14 kJ/d). Depositing excess fat was a consequence of trying to maximise protein gain. There was no need for birds fed balanced diets to deposit excess lipid and therefore they did not need to consume excess energy. Birds on the UPS on the highest protein diets did not need to over consume energy in order to consume sufficient amounts of the limiting amino acids. The lysine: CP ratios were very low and within the range 0.015 to 0.03. The simplest explanation is that a greater amount of heat is produced per MJ of energy stored as protein than for each MJ stored as lipid. This is partly due to the fact that PR (g/d) in the whole body of the broiler

chicken is significantly more complex in functional terms than LR in the adipose tissue. Where the dietary AA profiles on offer are present in amounts more than sufficient to support maximum protein growth, which the birds are capable of, surplus AA's are deaminated. This is a relatively inefficient process in comparison to the supply of energy from a carbohydrate or fat source. Consequently, the energy requirement increases for all ATP-consuming metabolic processes.

It was expected that birds fed imbalanced diets would lose the maximum amount of heat possible because of the imbalanced nature of the feeds, but they lost less heat than those offered the BPS. Feed intake of birds fed the UPS was not significantly different from those fed the BPS. Apart from there being an increase in heat production due to the inefficiency of reactions by which absorbed nutrients are metabolised, the linking of one amino acid to another requires the expenditure of four pyrophosphate "high energy" bonds (McDonald *et al.*, 1991). The latter authors also underlined the fact that if ATP provides these "high energy" bonds through glucose oxidation, about 2.5 MJ of energy would be lost as heat for each kg of protein formed. Since birds fed diets in the UPS deposited smaller amounts of protein, not as much heat was produced as a result of the digestive and synthetic processes in comparison to those fed the BPS.

Daily food allocation: effect on biological performance and carcass compositional changes.

The negative effects of restricted feed intake on body weight gain or growth has been extensively reported in Chapter 2. The impairment of growth rate due to feed restriction has been attributed to reductions in thyroxine, insulin-like growth factors (IGF) and growth hormone (Gonzales *et al.*, 1998). Birds fed the UPS *ad lib.* retained a similar amount of protein as those fed the BPS at an intake of 0.8 of *ad lib.* (viz: 9.4 vs. 9.6 g/d) but retained more lipid than birds allowed unrestricted access to the BPS (viz: 14.7 vs. 8.63 g/d).

Protein retention for birds fed diets in the UPS declined with increasing E: P ratio while energy retention increased (Table 3.2). Conversely PR for birds fed diets in the BPS increased with increasing E: P ratio and feeding level. Diets in the UPS contained a decreasing amount of lysine as E:P ratios increased from 32.5 to 65 MJ ME/kg protein. The lysine: CP ratios decreased from 0.03 to 0.015, which was considerably lower than the recommended ratios of Morris *et al.* (1987) of 0.053 and Morris *et al.* (1999) of 0.058 for maximum performance (FCE and growth). These authors suggested that specifying the lysine

requirements for chicks, as a proportion of the dietary protein will ensure that when diets containing higher than normal protein mixture concentrations are fed, an upward adjustment is made to the minimum lysine concentration of the diet for maximum broiler performance to be achieved.

The net efficiency of protein utilisation above maintenance (e_p).

The efficiency of protein utilisation was better for birds restrictively fed at 0.6 of *ad lib* than for those fed *ad lib* or 0.8 of *ad lib*. Restricted birds utilised a greater proportion of the energy available for protein synthesis, while very little was retained as fat. In comparison to birds fed BPS diets, those fed the UPS were less efficient due to some dietary protein being absorbed in a form unavailable for protein synthesis or to physiological losses (Simon, 1989). Ledin (1983) reported a lower rate of passage of feed on restricted feeding in rabbits resulting in a better digestibility for energy whilst digestibility depression occurred with increasing intake. Additionally, when the diet remains in the gastrointestinal tract for a longer period of time, this increases the chances for the digestive enzymes to act. The better protein efficiency for restricted birds observed in the current study may be due in part to the increased digestibility of dietary protein and /or to a reduction in endogenous losses.

The efficiency of protein utilisation was considerably lower for birds (i) fed UPS diets than those on BPS and (ii) and for birds fed diets containing the highest-protein concentrations (400 g CP/kg). This is in agreement with reports by Moundras *et al.* (1993) who examined the metabolic effects of consuming excessive amounts of high-protein diets in rats. They indicated that an increase in dietary protein concentration up to 600 g CP/kg, leads to a reduced availability of some glucogenic AA's to the peripheral tissues and a depressed concentration of these AA's in the liver tissues. This was accompanied by a reduction in both muscle and liver glycogen concentrations. These effects were attributed to a 45-fold increase of liver threonine-serine dehydratase activity. It is possible that a similar mechanism exists in broiler chickens fed high-protein diets and is responsible for the poor performance of birds fed protein mixtures at 400 g CP/kg. This is evidenced by reports by Scott *et al.* (1976) that the efficiency of protein utilisation is not 100% efficient because of the occurrence of a constant AA degradation that increases with an increase in dietary protein levels beyond the requirement of the broiler chicken. Small excesses of lysine, for instance have been reported to induce marked increases in kidney arginase activity and increased arginine degradation. A consequence of this is the lysine-arginine antagonism in which growth is impaired.

The induction of AA catabolism, following the intakes of excess protein is an adaptive response that may be of physiological relevance in the control of dietary protein intake but may negatively influence whole-body protein metabolism in the chicken. There is evidence from the studies of Kadowaki *et al.* (1989) in rats that dietary CP intakes at levels greater than required for maximum growth are not associated with an increase in protein synthesis. Thus the reduction in growth rate and efficiency of protein utilisation in birds fed diets containing excess CP may be related to some energy wastage, that is associated with the catabolism of excess AA's through glyconeogenesis. The drop in the concentrations of AA's such as threonine and other glucogenic AA's (Moundras *et al.*, 1993), may explain the depressed growth and efficiency of protein utilisation observed for birds fed high-concentrations of excess dietary CP. A consequence of the fall in concentrations of some AA's is the imbalanced supply of AA's that cannot be used for protein synthesis resulting in a reduction in protein utilisation.

Regardless of growth period, the e_p 's of birds fed diets in the BPS were superior to those of birds fed UPS diets. A decrease in daily food allocation to 0.6 of *ad lib*, improved e_p for birds fed the BPS (0.62) and the UPS (0.50) at 23 to 37 d of age. The measurements of e_p showed differences between the different age groups. These effects could be due to differences in the requirements for protein (and hence AA's) for muscle or feather growth. For instance, in the young chick the sulphur AA's make up a greater proportion of the feather protein than in the muscle. The relative rates of soft tissue and feather growth have been reported to differ for different growth periods (Boorman, 1980), thus the proportion of AA's required for growth will change with age. This will account for some variation in e_p between these two age groups.

The predicted inflection points shown in Table 3.4. occur at higher E: P ratios than those determined by Faulkner (1993) and Kyriazakis and Emmans (1992a, b). However, the ascending slopes for birds fed diets in the BPS at 0.8 and 0.6 of the previous *ad lib*. intakes of 0.012 and 0.013 were not significantly different from those determined by Kyriazakis and Emmans (1992a, b) or Faulkner (1993), of 0.0112 and 0.0128 respectively. The slopes for the birds offered the UPS were lower than those determined for birds offered the BPS. Birds restricted had a limited energy intake and efficiently utilised any protein ingested resulting in a larger value for the slope. All the predicted intercept values estimated for birds fed the UPS (viz: 76.8, 77.3, 71.0) and that of the *ad lib*. fed birds (viz: 70.3) were similar to that reported for pigs (viz: 73 MJ ME/kg protein at an e_p of 0.814) fed diets varying in protein and energy

contents (Kyriazakis and Emmans, 1992b). The mean slopes determined in this experiment for birds fed balanced and unbalanced diets of 0.0114 ± 0.00026 and 0.0107 ± 0.00018 respectively did not differ significantly from the overall mean slope of 0.01201 ± 0.00029 for birds aged 10 to 24 d of age in the previous study.

Assuming that, in commercial practice, a 200-g/kg protein and a 13.4 MJ ME/kg diet is fed *ad lib.* to broilers in the finisher period (NRC, 1994), this yields a ratio of 66.9 MJ ME/kg protein. The inflection points determined for birds fed diets in the BPS at *ad lib.* and an intake of 0.8 of previous *ad lib.* intake of 70.26 and 68.59 MJ ME/kg protein are similar to that used in commercial practice. This suggests that broilers in the grower period are being fed close to their optimum for efficient use of dietary protein. It is recommended that when formulating feeds for broilers, the E: P ratio should always be kept above a minimum level of 66.9 MJ ME/kg protein.

The poorer performance observed for birds fed the BPS in the lower E: P ratios in the current study and our previous study (Chapter 2), suggests that when excess dietary protein is fed, the energy content should also be increased to ensure that sufficient energy is available for the efficient utilisation of dietary protein. In addition, these results have important implications in areas where there is a scarcity of high quality protein sources and there is limited access to synthetic AA's.

CONCLUSIONS

It can be concluded that the dietary protein quality, daily food allocation and E: P ratio influence e_p . The minimum lysine:CP ratio recommended for maximum body growth is 0.0587. The range of lysine to CP ratios in the BPS and UPS were 0.061 to 0.062 and 0.015 to 0.031, respectively. Thus the ratios in the UPS were lower than recommended and accounted for the poor biological performance of birds fed these diets. When broilers are fed diets varying in protein quality, protein accretion would be at a maximum if additional energy was supplied to ensure that there is sufficient energy available for the efficient utilisation of dietary protein, and hence AA's. If protein retention is at a maximum, the additional dietary energy supplied will result in an increased lipid deposition. The intake of dietary energy below the bird's requirement results in lipid catabolism and a decreased body lipid content. There is also a minimum range of E: P ratios (i.e. 68.59 to 70.26 MJ ME/kg diet for balanced protein mixtures) that should be maintained if e_p is to be maximised.

The observed nutritional interactions (i.e. PQ x EP, EP x DFA) are very important in poultry nutrition. For instance the use of balanced protein mixtures at high E:P ratios was found to improve both the biological performance of birds and the efficiency of protein utilisation. An understanding of the basis of these interactions offers a chance of developing practical approaches to avoiding the interactions if they are deleterious or exploiting them if they are beneficial. Understanding the biochemical and physiological basis of nutrient partitioning could result in new nutritional approaches in controlling the quality of lean tissue growth and the overall e_p with which dietary ME is utilised for increased protein tissue deposition.

CHAPTER 4:

THE INFLUENCE OF DIFFERENT METABOLIZABLE ENERGY (ME) CONTENTS AND DIETARY ENERGY TO PROTEIN RATIOS IN BROILERS BETWEEN 10 AND 24 DAYS.

ABSTRACT

A study was conducted to evaluate the effects of feeding high levels of dietary protein (250 to 500 g/kg diet) at varying metabolizable energy (ME) contents (11 to 14 MJ ME/kg) on the biological performance and the net efficiency of protein utilisation (e_p) of chick's aged 10 to 24d. Lysine concentrations of 9.7 to 27 g/kg were utilised.

The growth rate (weight gain and FCE) exhibited a significant ($P<0.05$) improvement to increases in dietary ME. There were no significant interactions between dietary crude protein concentration (CP) and ME on the responses of birds to all parameters measured. The efficiency of protein utilisation e_p was significantly affected by CP ($p<0.05$) and E:P ratio ($P<0.001$).

The ascending slopes for the relationship between e_p and E:P ratio at 95% confidence interval for broiler chickens in the starter phase of growth (10 to 24 d) is best described by a linear model of the form $e_p = 0.01309 \pm 0.00015 \text{ E:P}$ (Adjusted $R^2 = 0.749 \pm 0.0604$). There were no significant differences ($P<0.05$) between slopes for birds fed the 12, 13 and 14 ME series. The slope for birds fed the 11 MJ ME was not significantly different ($P=0.19$) from that of birds fed the 12 MJ ME diets but was significantly different from the slopes for birds fed the 13 ($P<0.01$) and 14 ($P<0.05$) ME MJ dietary regimes.

The superior performance of birds offered diets in the higher ME series and high E: P ratios suggests that when dietary protein content is in excess of requirement, the dietary energy content should be increased to provide sufficient energy for the ingested protein to be efficiently utilized.

Keywords: excess protein, energy to protein ratio, efficiency, and chick growth.

INTRODUCTION

It was highlighted in Chapters 2 and 3 that diets in which protein is in excess, result in protein not being efficiently utilised. This is in agreement with reports for chicks (Harper *et al.*, 1970; Wethli *et al.*, 1975; Morris *et al.*, 1987; Abebe and Morris, 1990) and rats (Harper *et al.*, 1970) that an excess of AA's depresses growth rate and protein utilisation. Abebe and Morris (1990) fed chicks with protein concentrations ranging from 140 to 280-g/kg diets, with the lysine content varying at each protein concentration. These authors concluded that excess protein needs to be balanced by increasing the specification for critical AA's to maximize growth. The addition of extra lysine to maintain a dietary ratio of 50 g lysine/kg CP allowed the chicks to grow well on diets containing up to 280 g CP/kg diet. The implication of this observation is that, when birds are fed excess protein, the excess load of absorbed AA's exerts an imbalancing effect (Morris *et al.*, 1987). This requires an increased supply of the limiting AA's if maximum growth is to be achieved.

In Chapters 2 and 3 considerable attention has been given to the influence of dietary protein quality, daily food allocation and E: P ratio on e_p in broiler chickens. These experiments were based on dietary regimes (e.g. BPS and UPS) in which energy (E) was kept constant and protein (P) varied, thus altering the E:P ratios. The data indicated that performance and e_p of broilers is influenced considerably by variations in energy and nutrient (e.g. protein) inputs during growth. The reduction in e_p with decreasing E: P ratio observed in the previous studies is of great significance in nutrition. This is because at very low E: P ratios or high dietary CP concentrations, broilers may not be able to consume enough dietary energy for conducting processes related to protein digestion and metabolism. Normal protein growth and e_p will therefore be reduced.

Broiler chickens in our previous experiments were fed protein mixtures either balanced (BPS) or unbalanced (UPS) at 13 MJ ME/kg. It is uncertain whether feeding the BPS at varying ME contents (i.e. 11, 12, 13 and 14 MJ ME/kg) will derive similar responses for broilers 10 to 24 d of age. Lean tissue accretion is an energy demanding process, this implies that intakes of protein mixtures can only result in lean tissue gain or protein retention if there is sufficient dietary energy for the ingested protein to be utilised. According to Wiseman (2001), the effects of ME cannot be regarded in isolation from E:P. Emphasis in this chapter will be

placed on the relationship between E:P ratio and e_p , and not the effect of varying ME *per se* in dietary regimes fed to broiler chickens.

The objective of the study reported here was to investigate the effects of excess dietary protein on the net efficiency of utilisation of protein by broiler chickens as influenced by dietary regimes in which E:P ratio is altered by varying energy and protein simultaneously. This relationship would provide additional information on the slope of the ascending curve and permit the derivation of a model relating e_p to the dietary E: P ratio.

MATERIALS AND METHODS

This experiment was a repeat of the study reported in Chapter 2 with a few modifications. A total number of six replications per feeding treatment were achieved by splitting the experiment into two periods, with a three-week interval between periods. Similar procedures were followed in both periods. The study was designed to measure the response of broiler chickens (10 to 24 d of age) offered *ad lib.* access to high protein diets containing 11 to 14 MJ ME/kg. Variables measured included average daily body weight gain (ADG), food intake (FI), food conversion efficiency (FCE), heat production (H), rates of lipid (LR) and protein retention (PR) and the net efficiency of protein utilisation above maintenance (e_p).

Birds and housing

One thousand four hundred and fifty broiler chickens of uniform size were used in the experiment, but this was divided into two periods. During the pre-test period (0-9 days post hatching) the chicks received a standard commercial starter feed (220 g CP/kg, 12 g lysine/kg, 120 g/kg moisture, 25 g/kg fat, 8 and 7 g/kg Ca and P respectively) *ad libitum*. At nine days of age, ten chicks were placed in the one-tier battery cage in the experimental unit, to accustom them to the facilities, after being weighed to the nearest gram. At the commencement of the experiment (ten days of age), ten birds were assigned to an initial slaughter group (to allow calculation of nutrient retention). In addition ten birds per pen were randomly assigned to one of the 24 treatments (viz: four ME series, six CP levels per ME series), such that the average starting weight and weight range was similar for each treatment. As a consequence, six replicates of ten chicks per each of the 24 treatments were obtained. Birds were given free access to feed and water. Group body weight and feed consumption were determined weekly. Small amounts of feed were allocated twice daily to the birds to

prevent feed wastage. The house temperature was monitored and recorded daily throughout the fourteen-day trial. Temperatures were kept within the range 18 to 30°C. Artificial lighting was used to provide illumination for 24h/d. The design was a conventional 4x6 factorial with three replicates in each period of the trial.

Experimental feeds and design

Prior to mixing, diets were formulated to contain the desired composition using the Winfeed feed formulation programme. The four basic diets employed are shown in Table 4.1 and the analysed amino acid contents in Table 4.2.

Table 4.1. *The raw ingredient composition and nutrient contents of basal diets used in the experiment.*

Raw ingredient (g/kg)	Basal diet			
	1	2	3	4
Maize	27.00	585.60	100.00	580.63
Sugar	45.00		-	
Starch	45.00			
Maize gluten	120.00	80.00	100.00	55.96
Soybean meal 50	98.79	26.99	118.19	75.51
SPI 66 ¹	454.30	67.43	352.34	0.00
Fishmeal 65	109.97	106.09	108.67	104.59
² SF O/C 37	1.51	48.66	158.46	138.96
Sunflower oil	81.37	62.92	45.29	0.00
Limestone	7.70	10.07	6.83	9.80
³ NaHCO ₃	0.56	1.07	0.74	1.23
⁴ Vit+Min	2.50	2.50	2.50	2.50
⁵ MCP	6.30	8.67	6.98	9.18
Filler				21.64
Calculated Nutrients (g/kg):				
Dry matter	917.67	895.72	919.67	879.30
Crude protein	500.00	250.00	500.00	250.00
AMEn (MJ/kg)	14.00	14.00	11.00	11.00
Fat	100.00	100.00	67.96	38.02
Crude fibre	24.17	23.07	42.96	33.72
Ash	55.31	32.83	50.34	30.57
Calcium	10.00	10.00	10.00	10.00
Phosphorus (avail.)	5.00	5.00	5.00	5.00
Sodium	1.50	1.50	1.50	1.50
Chloride	1.80	1.80	1.80	1.80

¹SPI 66, Soya protein isolate; ²SF O/C 37, Sunflower Oil Cake; ³NaHCO₃, sodium bicarbonate

⁴Vit+Min, Vitamin + mineral premix; ⁵MCP Monocalcium phosphate

To reduce faecal organic matter (FOM) and thus heat stress, diets were made using highly digestible feed ingredients. Furthermore to minimise losses of nitrogen in the excreta, total essential AA's (TEAA's) were minimised, thereby reducing as far as possible with the ingredients available, the excessive amounts of AA's in the feed. Apart from reducing excreta N, this would also have reduced the amount of heat required for the excretory process.

Table.4.2. *The analysed amino acid composition (g/kg) of the basal feeds used in the experiment.*

	Basal feeds			
	1	2	3	4
Amino acid				
Lysine	27.0	11.1	23.7	9.7
Methionine	8.4	5.0	7.4	4.4
Threonine	17.6	8.5	15.5	7.4
Tryptophan	5.6	2.1	4.9	1.8
Arginine	30.8	12.1	26.8	10.3
Histidine	12.1	5.8	10.6	5.0
Isoleucine	22.7	10.1	19.8	8.5
Leucine	45.8	24.6	39.8	20.3
Phenylalanine	26.3	11.7	22.7	9.5
Phenylalanine + tyrosine	18.5	16.8	18.4	17.0
Valine	23.9	11.4	20.9	9.8

Diets were formulated to contain an excess of well-balanced protein mixtures of approximately 250 and 500 g CP/kg at 11 and 14 MJ AME_n/kg respectively. Dietary treatments encompassing four energy levels (viz: 11, 12, 13 and 14 MJ AME_n/kg) and six dietary crude protein levels (viz: 250, 300, 350, 400, 450 and 500 g CP/kg) making up a total of 24 E: P ratios were obtained according to the dilution principle described by Gous and Morris (1985). This required the successive dilution with a low-protein "dilution" mixture of a concentrated protein mixture ("summit") to produce a series of diets of decreasing protein contents within each energy series. In order to obtain intermediate diets having crude protein contents of 450, 400, 350, 300, the 500 and 250 g CP/kg diets were blended in the following ratios: 0.8:0.2, 0.6:0.4, 0.4:0.6, and 0.2:0.8 respectively. Dietary crude protein was provided in excess of the requirements for broiler chickens in this phase of growth (EFG, 1998). The same batch of ingredients was used to mix diets for the two halves of the experiment.

Management and slaughter procedure

Similar procedures were followed as in our previous study in Chapter 2. Twenty birds (ten of each sex) with weights close to the group mean were selected at random at the start of the experiment and used to determine the initial body composition at ten days post hatching. These birds were killed by asphyxiation with carbon dioxide and kept frozen at -4°C. At the trials conclusion, 5 broilers per replicate were killed by asphyxiation with carbon dioxide and also kept frozen in plastic bags. The entire carcass (including feathers) was later cut into smaller pieces, homogenised and kept until further processing for proximate analysis. In order to obtain a homogenous paste, samples were passed through a butcher's mincer three times. All ground samples were then thoroughly mixed and sub-sampled for chemical analyses. Representative samples of 200 to 300g were freeze dried to determine carcass moisture. Freeze dried samples were then reground in a blender before protein analysis were run. The procedure (AOAC, 1990) was applied to duplicate carcass samples to obtain the crude protein and gross energy (GE) contents. Similar procedures were adopted for older birds.

Retention of lipid (LR), energy (ER) and heat production (H) and the efficiency of protein utilisation (e_p).

A more detailed description of the procedures and calculations of lipid retention (LR), energy (ER), heat production (H) and the net efficiency of protein utilisation (e_p) above maintenance have been given elsewhere (Chapter 2). The calculated e_p was expressed as a function of the E: P ratio in the feed (MJ ME/kg digestible CP).

Protein digestibility values for the various raw materials used were obtained from the European Table of Energy Values for Poultry Feedstuffs (1986). The d_{cp} for the four ME series 14, 13, 12 and 11 ranged from 71.9 to 78.6, 77.9 to 79.8, 78.8 to 83.0 and 81.4 to 83.0. The proportion of total protein digested which is able to be incorporated into body protein ' v ' were 0.82 for the 13, 12 and 11 ME series and 0.93 for the 14 ME series. The ideal AA balance for a bird of between 10 and 24 days of age was obtained from the EFG broiler simulation model (1998). The inflection points for each ME series were estimated on the assumption that the maximum achievable e_p was 0.8 (Morris *et al.* 1987; Kyriazakis and Emmans, 1992a).

Statistical design and analysis

The objective of this study -was to measure the responses to different E: P ratios. A response model in which E:P ratio can be used to predict the responses was determined using SAS (1990). Multiple regression analysis was conducted using individual data points to determine the relationship between both E: P ratio and the measured responses. Linear and quadratic terms were tested for significant differences. Linear regressions of e_p on E: P ratios were also determined for each ME series. Similar statistical procedures as reported in Chapter 2 were utilised in analysing data.

RESULTS

The mean composition of the initial slaughter group at 10 days of age at an average weight of 220 g was 722.9-g moisture /kg, 156.9-g protein /kg and 22.38 MJ ME/kg GE. The weight of each bird at the start of the investigation was used to adjust all variable means and acts as a crucial blocking factor. Data for each group of birds (e.g. 10 birds/group) per replicate were used for statistical analysis. All regression analyses conducted, and the results presented, are from pooled data of six replicates of ten chicks per each of the 24 treatments.

Food intake (FI), average daily live-weight gain (ADG) and efficiency of food utilization (FCE).

The relationship between E: P ratios on the biological response variables are shown in Table 4.3. The growth rate (Weight gain and FCE) exhibited a significant ($P<0.05$) improvement to increases in dietary ME. There were no interactions between CP and ME on the responses of birds to all parameters measured. Food intakes increased linearly ($P<0.05$) as E: P ratio was increased. FI was highest for birds fed diets in the lowest ME series (54.9 g/d) and showed a decreasing trend as dietary ME or E: P ratio decreased. E:P ratio significantly ($P<0.001$) influenced ADG. ADG decreased after an E: P ratio closer to 30 to 35 MJ ME/kg protein for birds fed diets in the 11 and 12 MJ ME series. FCE increased to a maximum at E: P ratios of 35, 40, 45 and 50 MJ ME/kg protein for birds fed diets in the 11, 12, 13 and 14 MJ ME series and thereafter declined.

Table 4.3. *The effect of dietary ME series and E:P ratios on the biological performance and the efficiency of protein utilisation (e_p), of male broiler chickens from 10 to 24 days of age.*

¹ Dietary treatment	E: P ratio (MJ ME/kg protein)	Dietary crude protein (g/kg)	Feed intake (g/d)	Weight gain (g/d)	FCE (g gain/kg food intake)	Protein intake (g/d)	PR (g/d)	e_p
11 MJ ME/kg								
1	22	500	44.28	23.71	538.28	22.14	5.13	0.29
2	24	450	51.27	27.73	549.13	23.07	5.86	0.32
3	28	400	51.09	29.68	583.41	19.45	6.28	0.41
4	31	350	60.05	34.83	582.79	21.02	7.20	0.44
5	37	300	60.85	33.37	549.34	18.25	6.95	0.49
6	44	250	61.87	33.75	544.03	15.47	7.04	0.72
		Mean	54.90	30.51	557.83	19.90	6.41	0.44
12 MJ ME/kg								
7	24	500	42.92	22.61	526.79	21.46	4.95	0.29
8	26	450	49.54	26.93	537.77	22.29	5.72	0.32
9	30	400	50.51	30.48	608.17	19.23	6.41	0.42
10	34	350	56.85	33.23	586.92	19.90	6.88	0.45
11	40	300	58.29	34.87	602.23	17.49	7.03	0.53
12	48	250	68.74	37.08	539.21	17.19	7.21	0.71
		Mean	54.48	30.87	566.85	19.59	6.37	0.45
13 MJ ME/kg								
13	26	500	45.22	24.96	555.93	22.61	5.55	0.32
14	29	450	47.80	28.96	613.67	21.51	6.21	0.38
15	32	400	50.07	32.54	653.29	19.04	6.50	0.45
16	37	350	56.68	35.34	625.64	19.84	6.99	0.47
17	43	300	60.42	39.17	647.95	18.13	7.32	0.54
18	51	250	65.70	40.98	628.83	16.94	7.80	0.76
		Mean	54.31	33.66	620.89	19.68	6.73	0.49
14 MJ ME/kg								
19	28	500	43.48	26.29	611.22	21.74	5.46	0.37
20	31	450	46.28	30.52	660.37	20.83	6.19	0.42
21	35	400	54.51	34.47	641.87	20.64	6.91	0.47
22	40	350	53.24	35.77	674.82	18.64	6.97	0.51
23	47	300	57.61	39.23	680.29	17.82	8.22	0.64
24	56	250	62.92	41.03	653.57	15.73	7.94	0.73
		Mean	53.01	34.55	653.69	19.23	6.94	0.52
		SEM	0.89	0.59	6.96	0.0003	0.09	0.012
Source of variation								
	E:P		*	***	*	NS	***	***
	E:P ²		NS	*	NS	NS	*	NS

The mean of each response due to ME series are highlighted in bold. ¹Each treatment was replicated eight times.

²E:P; Energy to protein ratio. * P<0.05, *** P<0.001.

In spite of the fact that food intakes, and therefore protein intakes were greater for birds fed the lower energy series, FCE tended to be better as the dietary energy level increased.

ADG of birds fed diets in the 14, 13, 12 and 11 MJ ME series were 34.6, 33.7, 30.8 and 30.5 g/d respectively. Birds fed diets in the 14, 13 and 12 MJ ME series gained 11.69, 9.35 and 1.15% more weight than those fed diets in the 11 MJ ME series, but ate 3.45, 1.07 and 0.78% less food. Subsequently, birds on the 14, 13 and 12 MJ ME series had 14.7, 10.2 and 1.59% better FCE than those fed diets in the 11 MJ ME series. FCE was highest for birds fed diets in the 14 MJ ME series (653.7 g gain/kg food intake) and lowest for the 11 MJ ME series (557.8 g gain/kg food intake).

Protein (PR), lipid (LR), energy (ER) retention and heat production (H).

The daily rate of change in PR, LR, ER and heat production (H) in response to dietary treatments in which excess protein are present in amounts not needed to maximize biological performance are shown in Table 4.3 and 4.4. There were no significant effects of either ME or CP or the interaction between CP and ME or ME and E:P on these variables. Birds fed diets in the 11 MJ ME series had average tissue moisture content of 0.97, 1.60 and 1.93% greater than birds fed diets in the 12, 13 and 14 MJ ME series. Whilst percentage carcass protein and protein intake for birds fed the 11 MJ ME series were 1.55, 1.12, 3.81 and 2.95, 4.46, 5.89% greater than that for birds fed diets in the 12, 13 and 14 MJ ME series respectively.

Protein retention was significantly affected by E:P ($P<0.001$) and $E:P^2$ ($P<0.01$). Protein retention was greater for birds fed diets in the 14 MJ ME series (i.e. 6.95 g/d) in comparison to 6.37 and 6.41g/d observed in the 13, 12 and 11 MJ ME series respectively. Birds fed diets in the 14 and 13 MJ ME series retained 7.76 and 4.72% more protein in comparison to those fed the 11 MJ ME series. PR increased to a maximum of 7.20 g/bird d and 8.22 g/bird d at E:P ratios of 31 and 47 MJ ME/kg protein for birds fed the 11 and 14 MJ ME series respectively. For birds fed diets in the 11 and 12 MJ ME series, PR increased linearly to 7.21 and 7.80 g/bird d at E:P ratios of 48 and 51 MJ ME/kg protein respectively.

The main effect of LR and ER of birds fed diets in the 14, 13 and 12 MJ ME series showed an improvement of 15.23, 16.60, 18.58% and 14.48, 15.55, 16.75% respectively over birds fed diets in the 11 ME series. The ME intake of birds fed diets in the 11 MJ ME series was 0.78,

1.07 and 3.45% greater than for birds fed diets in the 12, 13 and 14 MJ ME series respectively.

Table 4.4. *The effect of dietary ME series and E:P ratios on lipid retention, energy retention and heat production of male broiler chickens from 10 to 24 days of age.*

¹ Dietary treatment	E: P ratio (MJ ME/kg protein)	Dietary crude protein (g/kg)	Lipid retention (g/d)	Energy retention (kJ/d)	Heat production (kJ/d)
11 MJ ME/kg					
1	22	500	1.29	58.28	517.36
2	24	450	1.79	79.33	587.16
3	28	400	1.82	81.12	583.10
4	31	350	2.49	108.59	672.08
5	37	300	2.56	111.22	679.88
6	44	250	2.43	106.09	698.16
		Mean	2.06	90.77	622.96
12 MJ ME/kg					
7	24	500	1.45	64.39	493.56
8	26	450	1.86	81.81	562.23
9	30	400	2.06	90.57	566.08
10	34	350	2.68	115.95	623.12
11	40	300	3.13	133.82	623.96
12	48	250	3.98	167.66	725.98
		Mean	2.53	109.03	599.16
13 MJ ME/kg					
13	26	500	1.53	68.22	519.58
14	29	450	1.59	71.66	549.79
15	32	400	1.81	80.88	570.08
16	37	350	2.75	118.89	617.92
17	43	300	2.87	123.84	661.92
18	51	250	4.29	181.39	672.70
		Mean	2.47	107.48	598.67
14 MJ ME/kg					
19	28	500	1.64	72.51	492.76
20	31	450	1.72	77.23	524.41
21	35	400	2.43	106.01	602.64
22	40	350	2.20	97.36	594.79
23	47	300	2.80	122.69	626.18
24	56	250	3.78	161.04	656.91
		Mean	2.43	106.14	582.95
		SEM	0.105	4.23	8.80
Regression					
² E:P			NS	NS	NS
E:P ²			NS	NS	NS

The **mean** of each response due to ME series over the range of E:P ratios are highlighted in bold.

¹Each treatment was replicated eight times, ²E:P; Energy to protein ratio, NS not significant

Birds fed diets in the 14, 13, 12 and 11 MJ ME series had an average H of 582.9, 598.6, 599.2 and 622.9 kJ/d respectively. Birds fed diets in the 11 MJ ME series produced 3.82, 3.91 and 6.42% more heat than birds fed diets in the 12, 13 and 14 MJ ME series respectively.

The net efficiency of protein utilisation, e_p

Birds fed diets in the 14 and 13 MJ ME series were 13.46 and 8.16% more efficient in utilising dietary protein above maintenance in comparison to those fed diets in the 12 MJ ME series. While those fed diets in the 14 MJ ME series were 5.77% more efficient than those fed the 13 MJ ME series. Birds fed diets in the 14, 13 and 12 MJ ME series were 15.38, 10.20 and 2.23% more efficient in utilising dietary protein above maintenance in comparison to those fed diets in the 11 MJ ME series. Regression equations with predictors ME, CP and the interaction between them were fitted to the data for the marginal efficiency and net efficiency but showed no significant effects. From a statistical point of view it was found that the data from six replications of each treatment under each ME series, could be adequately described by fitting linear regression functions using E: P ratio as a predictor. The linear regression equations derived were forced through the origin as giving best estimates of the slopes. This is because at zero energy to protein contents e_p is assumed to be zero. The efficiency of protein utilisation was plotted as a function of the E: P ratio for each ME series (Figure 4.1).

The slopes of the linear regression equations fitted to the data are presented in Table 4.5. The E: P ratio significantly ($P < 0.0001$) influenced e_p within each ME series. The slopes for birds fed diets in the 11, 12, 13 and 14 MJ ME series were 0.0135 ± 0.00035 , 0.0129 ± 0.00037 , 0.0127 ± 0.00031 and 0.0132 ± 0.00025 respectively. The combined slope for all dietary treatments was 0.01309 ± 0.00015 (Adjusted $R^2 = 0.749 \pm 0.0604$). There were no significant differences in the slopes between ME series and the mean slope for all dietary treatments. The highest e_p for each ME series occurred at the highest E: P ratio within each ME series. These were 0.723 ± 0.083 , 0.714 ± 0.062 , 0.768 ± 0.075 and 0.728 ± 0.076 for the 11, 12, 13 and 14 MJ ME series at E: P ratios of 44, 48, 52 and 56 MJ ME/kg protein respectively.

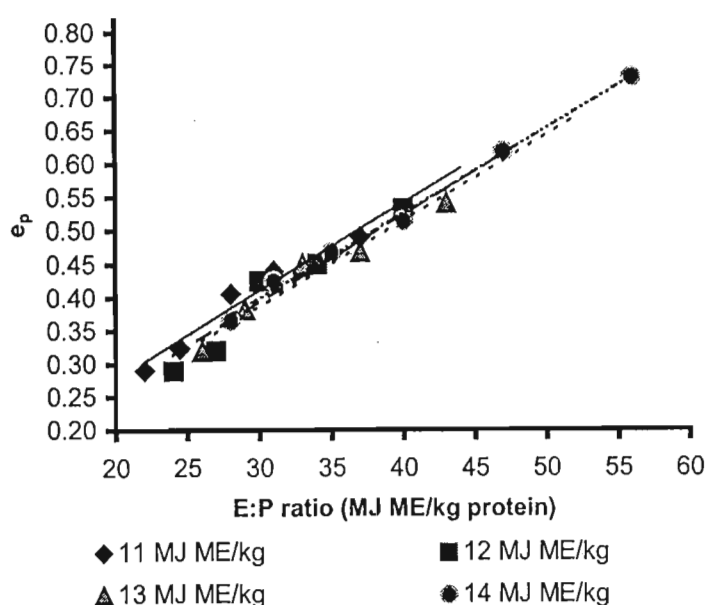


Figure.4.1 Effect of E:P ratio on the net efficiency of protein utilisation of broiler chicks 10 to 24 d of age

The lowest e_p occurred in birds fed diets at the lowest dietary E: P ratios within each ME series. These were 0.292 ± 0.024 , 0.289 ± 0.043 , 0.321 ± 0.038 and 0.364 ± 0.045 for the 11, 12, 13 and 14 MJ ME series at E: P ratios of 22, 24, 26 and 28 MJ ME/kg protein respectively.

Table 4.5. The slope and net efficiency of protein utilisation (e_p) of male broiler chickens aged 10 to 24 days obtained by fitting a linear regression model to the response data.

Dietary treatment ¹	Slope (kg/MJ)	s.e.	Adj. R ²	P-value	Predicted inflection at E:P ratio (MJ ME/kg protein)	Maximum e_p observed
ME series						
11	0.0135	0.00035	0.59	<0.001	58.88	0.72
12	0.0129	0.00038	0.60	<0.001	61.83	0.71
13	0.0127	0.00031	0.57	<0.001	62.93	0.76
14	0.0132	0.00025	0.79	<0.001	60.69	0.73
Mean	0.0131	0.00016	0.75	<0.001	61.12	

¹Regressions from each treatment, which was replicated eight times with, intercepts set to zero;

s.e. standard error.

DISCUSSION

Whilst the effects of varying energy to protein (E: P) ratios on pig performance are well documented (Kyriazakis and Emmans, 1991a, b), in a general sense the description of these effects in the broiler chicken remains unresolved. In the investigations reported in Chapters 2 and 3, birds were fed similar E: P ratios at a dietary ME of 13 MJ ME/kg. The data showed that biological performance and e_p was influenced by the associated energy to protein ratio of the feed. Similar results reported in the present study are applicable only to the range of experimental feeds employed (i.e. 250 to 500 g CP/kg at varying ME contents) and may give rise to errors if extrapolated for other dietary treatments outside this range and of a markedly different AA composition.

Effects on the biological performance

The results point to the fact that essential nutrients serving as substrate (i.e. dietary protein or AA's) are required to be present in the diet in a satisfactory ratio to energy in order for birds fed such diets to be able to satisfy their nutrient requirements. The findings of the current investigation confirm the principle that deviations from the critical balance between energy and protein (i.e. very low E: P ratios) result in undernutrition and poor biological performance of birds fed such diets.

The biological performance of birds fed diets in the high E: P ratio was superior to that of birds fed lower E: P ratios in agreement with data reported in Chapters 2 and 3. This is in spite of the differences in absolute values between the present study and previous studies. There was an increase in feed intake as E: P ratio increased (Table 4.2). Consequently, there was an increase in ME intake resulting in an increased fat deposition at higher E: P ratios. At low levels of protein concentration (250 g CP/kg), the extra energy allowance (i.e. increasing the dietary energy content) had a negligible effect on biological performance. At moderately high CP concentrations (generally in the range 250 to 350 g CP/kg) weight gain and FCE improved with increasing ME series in comparison to dietary CP levels of 500 g CP/kg. These considerations imply that at excess protein intake, ME availability could possibly limit biological performance. Growth rate was low for birds fed the 11 MJ ME series because there was not enough energy to enable the ingested protein to be deposited. The excess protein ingested is presumably deaminated and the nitrogen excreted resulting in a comparatively

lower biological performance and higher heat production of birds fed diets in the 11 MJ ME series.

The lowered average daily food intake and ADG at very low E: P ratios of 22, 24, 26 and 28 MJ ME/kg protein at 500 g CP/kg is related to the comparatively low protein retention (5.13, 4.94, 5.55 and 5.46 g/bird d) and the inability of birds fed these diets to lose sufficient heat to the environment. As dietary E: P ratios increase from 22 to 56 MJ ME/kg protein so did the biological performance. This is as a consequence of more energy being made available to deposit the ingested protein. At low E: P ratio i.e. when excess protein is fed, the excretion of excess nitrogen increases since the demands for tissue replacement are met. Any excess glucogenic AA's are thus subjected to oxidation in the tricarboxylic acid cycle after the removal of nitrogen (McDonald *et al.*, 1991). This is a wasteful process resulting in a curtailment of maximum response of birds fed diets containing excess protein, the effect being much more severe at high protein concentrations.

Protein (PR) and lipid (LR) retention and Heat production (H).

Increasing dietary protein (i.e. reducing the dietary E: P ratio), regardless of ME series, resulted in a reduction in PR, LR and H. This is in agreement with results reported in Chapters 2 and 3. Protein retention is known to be dependent on the amount of energy supplied up to some limit (Edwards and Campbell, 1991). The present study confirmed the curtailment of maximum response in PR of birds offered diets containing large excesses of protein and when diets of the lower ME series are fed. Morris *et al.* (1999) reported that birds offered *ad lib* access to diets containing excess protein between 12 and 13 MJ ME/kg, could not take in enough energy to support a higher protein deposition. The present study showed increases in PR for birds fed diets in the 13 and 14 MJ ME series over those fed the 11 MJ ME series. However, the daily intake of energy for birds fed the 11 MJ ME series was inadequate for carrying out the processes necessary for protein metabolism and deposition.

Not unexpectedly the rate of LR increased as ME and E: P ratio increased. It has been demonstrated that if the supply of protein exceeds the broilers need, but energy intake is constrained, a broiler can grow protein at a maximum but its rate of LR is lower. Thus within the 13 to 14 MJ ME series, PR increased as E: P ratio increased as long as the intrinsic capacity for protein deposition was not fully utilised. In line with observations by Kyriazakis and Emmans (1992a) the rate of PR was not constant at constant CP intake.

There was an increase in ER at high dietary E: P ratios because of the increase in LR. Heat production was associated with the deposition of protein and or fat. Heat production of the birds fed the 12, 13 and 14 MJ ME series were 3.8, 3.9 and 6.4% less than that of birds fed the 11 MJ ME series. This is in contrast to LR, which increased by 18.6, 16.8 and 15.2% respectively and ER, which increased by 16.8, 15.6, and 14.5%, respectively, over that of birds fed the 11 ME series. The question of whether heat dissipation limits growth of birds fed diets containing unsupplemented excess protein was examined by Morris *et al.* (1999). In the current study food intakes, growth rates and HPR on the highest protein diets (i.e. 500 g CP/kg) were lower than on other dietary treatments. However, Morris *et al.* (1999) considered diets containing lower levels of surplus protein (220 to 300g CP/kg) and indicated that growth rate was depressed without a reduction in food intake when birds were fed diets containing excess protein beyond the AA requirements of the bird. The supplementation of the high protein diets with the first limiting AA's increased the growth rate and possibly the rate of protein deposition. Consequently these authors concluded that heat disposal was not responsible for the curtailment of growth on diets containing surplus (AA's) but rather resulted in the loss of the limiting AA's.

The heat output of birds fed the low ME series was increased because the birds had to metabolise and excrete large amounts of excess unutilised dietary AA's. A considerable amount of energy (i.e. 622.95 kJ/d) was lost in the form of heat during these metabolic processes. The strategy of the nutritionist under warm tropical conditions of heat stress would be to balance the dietary energy to protein contents to reduce heat load. It is also essential to minimize the TEAA's of the diets, in order to prevent an impairment of broiler performance under moderate increases in environmental temperatures.

The net efficiency of protein utilisation above maintenance (e_p).

The net efficiency of protein utilisation above maintenance (e_p) was shown to be a function of the E:P ratio of the feed in agreement with our previous investigations in Chapter 2 and 3. The data also suggest a curtailment of maximum response in e_p at high dietary concentrations of up to 500 g CP/kg. Wethli *et al.* (1975) indicated that when dietary protein content is increased beyond that required to meet the AA requirement of a broiler, the impairment of response was not due to high dietary protein per se. For instance, they observed that growth could not be maximised when soybean meal and groundnut meal are utilised as a supplement to cereal based diets even though very high dietary CP concentrations were fed to birds. They

indicated that AA's in poorer quality oil seed cakes occur in disproportion to the requirements of the chick such that the utilisation of the limiting AA is reduced. The e_p values calculated are similar to those shown by Kyriazakis and Emmans (1992a, b) but opposite to the views of Stranks *et al.* (1988) who indicated that e_p is constant and independent of energy supply. The calculated e_p increased with increasing E: P ratio. The results confirm the previous reports in Chapter 2 and 3 and that of Morris *et al.* (1999) that protein utilisation would be lowered if the E: P ratio was reduced below a critical value.

The predicted inflection points were shown to occur at E: P ratios of 58.88, 61.83, 62.93, and 60.69 MJ ME for the 11, 12, 13, and 14 ME series respectively. These inflection points are not significantly different from that determined for birds of a similar age offered unrestricted access to diets in the balanced protein series (i.e. 59.2 MJ ME/kg protein) in Chapter 2. The inflection point was lowest for birds fed diets in the 11 ME series and highest for the 14 ME series. The combined inflection point was 61.12 MJ ME/kg protein.

The value for the overall slope obtained by fitting a linear regression model for the relationship between e_p and E: P ratio is 0.0131 kg/MJ. This estimate for the slope is similar to that determined by Faulkner (1993) of 0.0141 kg/MJ for the pooled estimate of both male and female broiler chickens 7 to 21d of age. The ascending slopes for each ME series are not significantly different from those observed in Chapter 2. It can be deduced from the values of the inflection points for birds in the starter phase of growth in this experiment (61.12 MJ ME/kg protein) and those offered unrestricted access to the BPS (59.20 MJ ME/kg protein) that below an E: P ratio of approximately 60.16 ± 1.36 MJ ME/kg protein, there is not sufficient energy available to deposit the dietary protein. It is expected that below this value, there is the catabolism of excess protein to restore an energy: protein value (or release energy) for the remaining protein to be used for protein retention. The linear increase in e_p with an increase in E: P ratio suggests that each unit increase in ME intake relative to dietary protein gives rise to a proportional increase in the amount of protein deposited. The relationship between e_p and E: P ratio for broiler chickens in the starter phase of growth (10 to 24 d) is best described by a linear model of the form $e_p = 0.0114 \text{ E: P}$.

CONCLUSION

The superior growth performance and the net efficiency of protein utilisation (e_p) of birds fed the higher ME series and high E: P ratios indicate that, high e_p 's are attainable only when energy resources needed to support the use of proteins for protein synthesis are adequate.

CHAPTER 5:

THE INFLUENCE OF DIFFERENT METABOLIZABLE ENERGY (ME) CONTENTS AND ENERGY TO PROTEIN RATIOS IN BROILERS BETWEEN THE AGES OF 23 TO 37 DAYS

ABSTRACT

An investigation was carried out with diets, in which the protein (and hence amino acid's, AA's) was not limiting but in excess (250 to 500 g CP/kg diet) was fed to broiler chickens from 23-37d old. The lysine concentrations of the diets ranged from 0.038-0.054 g lysine/kg CP. Two hundred and twenty broiler chickens were randomly assigned either to an initial slaughter group (12 birds) or to one of the twenty-four dietary treatments with eight replications.

Broiler chickens are not tolerant of very high dietary crude protein contents, unless dietary energy content increases to ensure that sufficient energy is available for the efficient utilisation of excess dietary protein.

A linear regression model was fitted to the data of efficiency of protein utilisation (e_p) as a function of the E: P ratio. When the data were combined, the slope of the ascending part of the curve was 0.0149 ± 0.0002 .

It is concluded that e_p is not influenced only by the dietary crude protein, indicating that it is the ratio of energy to protein in the diet that is important and not the actual amounts that are consumed each day.

Keywords : energy: protein ratio, efficiency, and broiler

INTRODUCTION

In Chapter 2 to 4 the effects of balanced vs. unbalanced diets, *ad lib.* vs. restricted feeding, high ME vs. low ME diets on biological performance and the efficiency of protein utilisation were examined in broiler chickens 10 to 24 d and 23 to 37d of age. Diets used in these investigations contained excess protein being designed to decrease the E:P ratio below a critical value such that the e_p was compromised. The model of Kyriazakis and Emmans (1992a, b) relating e_p to E: P ratio of the feed was confirmed in growing broiler chickens. The special importance of dietary E: P ratios, not at high E: P ratios but at low E: P ratios (i.e. when excess protein is fed) were highlighted in these studies. A consequence of feeding unbalanced protein mixtures is the suboptimal performance of birds. Why then would anyone want to feed birds diets of high protein contents? It is only when synthetic AA's are unavailable or the cost is prohibitive that large amounts of these poor quality proteins are fed. This is done in order to provide minimum levels of essential AA's in the diet. The data from these series of studies provide some answers to the question why at high protein contents the e_p appears to drop off? It is suggested that it is necessary to increase the dietary energy content to ensure that sufficient energy is available for the efficient utilisation of excess dietary protein.

The objective is to measure the responses of broiler chickens 23 to 37d of age to the different E:P ratios and ME levels. This study will provide more information on the slope of e_p vs. E: P ratio (the ascending curve). In addition the effect of dietary ME levels were examined to observe whether the results observed in previous studies could be corroborated.

MATERIALS AND METHODS

This study was a repeat of one of the study reported in Chapter 4. However, the growth period covered in the present enquiry was from the 23 to 37 d of age. The techniques used were similar to those reported in the Materials and Methods in the previous chapter

General procedures

Housing, handling, feeding and slaughter procedures were in accord with the policies of the University of Natal Committee Code Of Ethics on Laboratory Animal Care. A total number of eight replications per feeding treatment were achieved by splitting the experiment into two

periods, with a three-week interval between periods. Similar procedures were followed in both periods. The design was a conventional 4x6 factorial with four replicates in each period of the trial.

Birds and housing

Two hundred and twenty commercial male broiler chickens of uniform size were used in the experiment. During the pre-test period (0-21 days post hatching) the chicks received a standard commercial starter feed (240 g CP/kg) *ad lib*. At day 23 posthatching, 104 birds were individually weighed. Four individually penned chicks were then randomly assigned to one of the 24 experimental diets (four birds/treatment), such that the average starting weight and weight range was similar for each treatment. The initial slaughter group consisted of eight birds to allow calculation of nutrient retention. Food and water was available *ad lib*. for the fourteen-day feeding period. The house temperature was monitored and recorded daily throughout the trial. Temperatures were kept within the range 20 to 30°C. Artificial lighting was used to provide illumination for 24h/d.

Experimental feeds and design

The experimental feeds and design were similar to those reported in Chapter 4. The four basic diets employed are shown in Table 4.1 and the amino acid contents in Table 4.2. These diets were formulated to contain an excess of well balanced protein mixtures of approximately 500 and 250 g CP/kg at 14 and 11 MJ AME_n/kg respectively. The four energy levels (viz: 11, 12, 13 and 14 MJ AME_n/kg) and six dietary crude protein levels (viz: 250, 300, 350, 400, 450 and 500 g CP/kg) making up a total of 24 E:P ratios were obtained according to the dilution principle described by Gous and Morris (1985). Excesses of amino acids were kept to a minimum by minimising the total essential amino acid (TEAA) content of the basal diets. The same batch of ingredients was used to mix diets for the two halves of the experiment.

Management and slaughter procedure

Management, slaughter and chemical analysis procedures were the same as those employed in Chapter 2, except that birds were individually penned in the current enquiry.

Retention of lipid, energy, heat production and the efficiency of protein utilisation (e_p).

A more detailed description of the procedures and calculations of LR, ER, H and e_p have been given elsewhere (Chapter 2). Protein digestibility values for the various raw materials used were obtained from the European Table of Energy Values for Poultry Feedstuffs (1986). The d_{cp} for the four ME series 14, 13, 12 and 11 ranged from 71.9 to 78.6, 77.9 to 79.8, 78.8 to 83.0 and 81.4 to 83.0. The proportion of total protein digested which is able to be incorporated into body protein ' v ' were 0.9 for the 13, 12 and 11 ME series and 1.0 for the 14 ME series. The ideal AA balance for a bird of between 23 and 37 days of age was obtained from the EFG broiler simulation model (1998).

Statistical design and analysis

The statistical procedures were the same as those employed in Chapter 2. A response model in which the predictor E: P ratio was used to predict the variate was used. This was achieved by making use of multiple regression analysis in which E: P was used to predict growth. Linear regression equations for e_p were also determined for each ME series using SAS (1990). The weight of each bird at the start of the investigation was used to adjust all variable means and acts as a crucial blocking factor.

RESULTS

The results presented are for birds fed diets in which the protein is not limiting, but in excess. The mean composition of the initial slaughter group at 23 days of age and 0.786 ± 0.043 kg live weight was 679.3 ± 13.32 g moisture /kg, 171.3 ± 9.5 g protein /kg, 24.33 ± 0.55 MJ ME/kg GE. The relationships between E:P ratios at various dietary energy levels on the biological performance and protein utilisation are shown in Table 5.1.

Table 5.1. *The effect of dietary ME and E:P ratios on the biological performance and the efficiency of protein utilisation (e_p), of male broiler chickens from 23 to 37 days of age.*

¹ Dietary treatment	² E:P ratio (MJ ME/kg protein)	Dietary crude protein (g/kg)	Feed intake (g/d)	Weight gain (g/d)	FCE (g gain/kg food intake)	Protein intake (g/d)	PR (g/d)	e_p
11 MJ ME/kg								
1	22	500	79.6	43.1	540.9	41	10.7	0.34
2	24	450	92.5	53.3	576.6	42	12.4	0.38
3	28	400	102.0	61.1	599.1	39	13.9	0.44
4	31	350	105.5	64.9	615.0	37	13.9	0.49
5	37	300	106.5	61.0	573.0	32	12.9	0.53
6	44	250	116.4	61.9	532.0	29	13.6	0.68
		Mean	100.4	57.6	572.8	36.7	12.9	0.48
12 MJ ME/kg								
7	24	500	80.4	46.4	576.7	34	10.2	0.35
8	26	450	90.0	52.5	582.6	36	10.6	0.39
9	30	400	89.8	55.8	621.6	35	12.7	0.46
10	34	350	91.0	65.2	686.1	32	13.1	0.55
11	40	300	98.0	62.3	635.9	29	14.0	0.64
12	48	250	115.0	67.5	587.2	28	13.5	0.67
		Mean	94.0	58.3	615.0	32.3	12.4	0.51
13 MJ ME/kg								
13	26	500	79.3	51.0	642.7	40	11.9	0.40
14	29	450	85.0	53.9	634.2	39	12.4	0.42
15	32	400	85.3	57.7	676.6	34	13.0	0.52
16	37	350	94.7	66.4	701.5	33	14.4	0.60
17	43	300	108.3	72.5	669.1	32	15.1	0.64
18	51	250	113.1	66.9	591.4	28	12.3	0.65
		Mean	94.3	61.4	652.6	34.3	13.2	0.54
14 MJ ME/kg								
19	28	500	78.0	46.3	593.8	39	11.1	0.42
20	31	450	86.7	53.7	619.3	39	12.6	0.47
21	35	400	90.1	63.4	704.1	36	13.4	0.53
22	40	350	92.7	68.7	741.5	33	14.6	0.64
23	47	300	102.6	69.4	676.3	30	14.1	0.65
24	56	250	101.6	65.8	647.4	26	13.0	0.70
		Mean	92.0	61.2	663.7	33.8	13.1	0.56
		SEM	1.31	1.09	6.95	0.10	0.01	0.01
Source of variation								
² E:P			**	***	***	NS	***	***
E:P ²			NS	***	***	NS	***	***

The **mean** of each response due to ME series are highlighted in bold. ¹Each treatment was replicated eight times. ²E:P; Energy to protein ratio. ** P<0.01, *** P<0.001.

Food intake (FI), live-weight gain (ADG) and efficiency of food utilization (FCE).

Using an anova to separate the treatment comparison into main effects of E:P, ME and CP and the interactions was not appropriate to examine the response data. Regressions using E:P ratio as a predictor showed that it significantly improved the fit in average daily live-weight gain (ADG)($P<0.001$) and daily live-weight gain per kg food intake (FCE)($P<0.001$).

The predictor $E:P^2$ also significantly influenced the fit in ADG ($P<0.001$) and FCE ($P<0.001$) but not FI ($P=0.07$). This curvilinear response (Fig 5.1 and 5.2) implies that ADG and FCE increases with increasing E: P ratio until the required E:P ratio was reached. A further increase in E: P ratio above this value has no beneficial effect on the response.

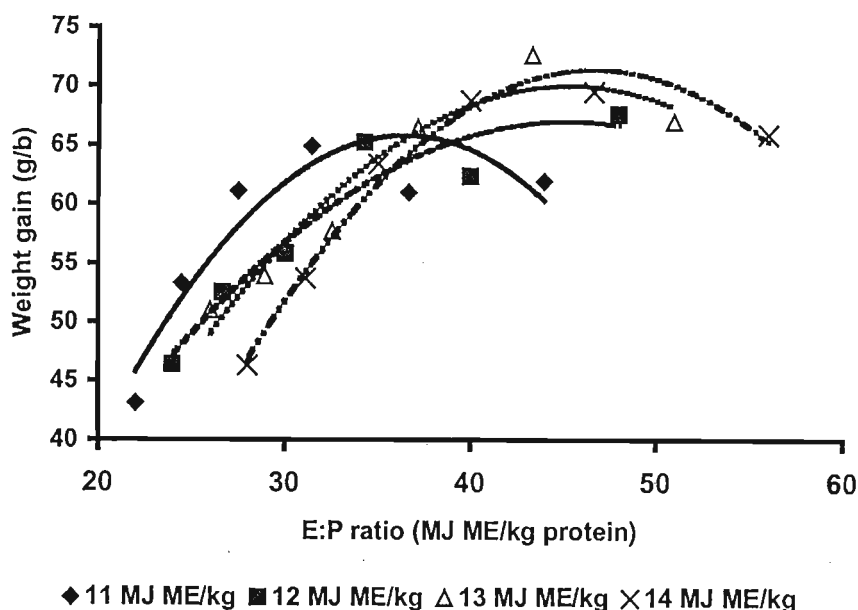


Figure 5.1. The effect of ME and E: P ratios and on weight gain of chicks from 23 to 37days of age.

Food intake tended to be lower for birds fed diets in the higher ME series. Between 22 to 38 MJ ME/kg protein, weight gain was greater for birds fed the 11 ME series but at E: P ratios exceeding 38 MJ ME/kg protein birds fed the higher ME series had better growth rates.

There was also an improvement in FCE (Figure 5.2) as dietary ME was increased. Food conversion efficiency (FCE) increased to a maximum at E: P ratios of 31, 34, 37 and 40 MJ ME/kg protein for birds fed diets in the 11, 12, 13 and 14 MJ ME series and thereafter

declined. In spite of the fact that food intakes, and therefore protein intakes were greater for birds fed the lower energy series, FCE tended to be better as the dietary energy level increased. Weight gain improved by 1.22% for birds fed the 12 MJ ME series over those fed diets containing 11 MJ ME and 5.31% for birds fed the 13 MJ ME diets over birds fed the 12 MJ ME diets. However an increase in dietary ME from 13 to 14 MJ ME/kg resulted in a decrease (0.36%) in weight gain

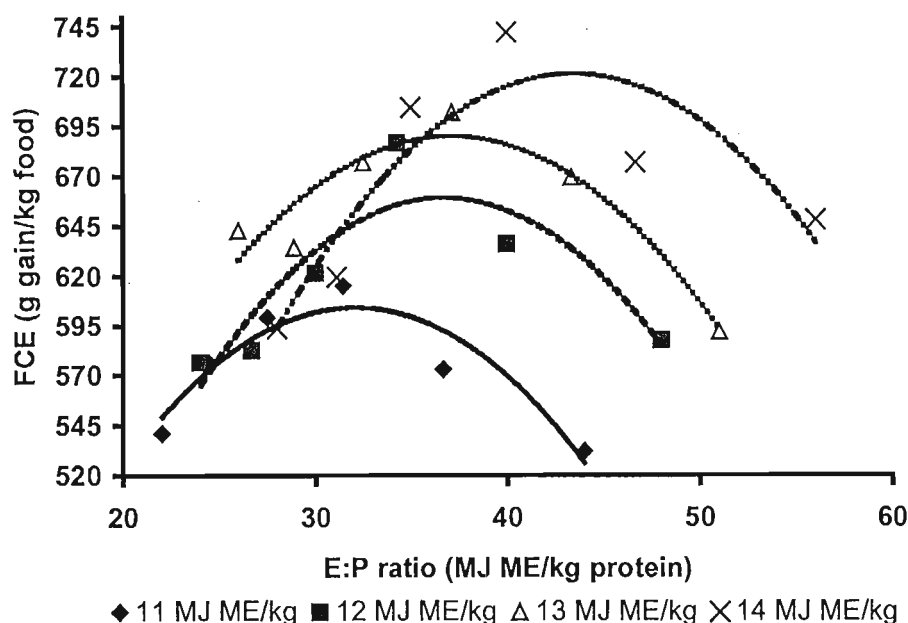


Figure 5.2. The effect of ME and E: P ratios and on FCE of chicks from 23 to 37days of age.

Protein, lipid, and energy retention and heat production

The daily rate of change in PR, LR, ER and heat production (H) are shown in Table 5.1 and 5.2. Protein retention for birds fed the 12 MJ ME series showed an improvement of 4.03% over that of birds fed the 11 MJ ME series and 6.45% for birds fed the 13 MJ ME series over the 12 MJ ME series. However, an increase in dietary ME from 13 to 14 MJ ME/kg resulted in a slight and insignificant decrease (0.76%) in PR. Protein retention increased to a maximum at E: P ratios of 28, 40, 37 and 40 MJ ME/kg protein for birds fed diets with 11, 12, 13 and 14 MJ ME/kg respectively and thereafter declined. There were highly significant effects of E: P and $E:P^2$ on PR ($P<0.001$) but not on LR.

Table 5.2. *The effect of dietary ME and E:P ratios on lipid retention, energy retention and heat production of male broiler chickens from 23 to 37 days of age.*

¹ Dietary treatment	E:P ratio (MJ ME/kg protein)	Dietary crude protein (g/kg)	Lipid retention (g/d)	Energy retention (kJ/d)	Heat production (kJ/d)
11 MJ ME/kg					
1	22	500	5.22	223.75	835.00
2	24	450	5.90	252.50	961.00
3	28	400	6.51	275.00	996.00
4	31	350	8.45	355.00	1022.00
5	37	300	7.28	306.25	1065.00
6	44	250	8.43	352.50	1168.00
		Mean	6.97	294.17	1007.83
12 MJ ME/kg					
7	24	500	4.03	171.25	703.00
8	26	450	6.01	252.50	800.00
9	30	400	6.44	272.50	878.00
10	34	350	7.89	331.25	853.00
11	40	300	6.98	292.50	971.00
12	48	250	13.19	541.25	937.00
		Mean	7.42	310.21	857.00
13 MJ ME/kg					
13	26	500	5.85	248.75	782.00
14	29	450	6.67	280.00	852.00
15	32	400	8.79	366.25	753.00
16	37	350	9.52	396.25	828.00
17	43	300	11.40	473.75	926.00
18	51	250	17.41	707.50	773.00
		Mean	9.94	412.08	819.00
14 MJ ME/kg					
19	28	500	5.55	233.75	781.00
20	31	450	7.08	297.50	831.00
21	35	400	8.95	373.75	796.00
22	40	350	9.50	398.75	822.00
23	47	300	11.40	471.25	847.00
24	56	250	14.80	603.75	738.00
		Mean	9.55	396.46	802.50
		SEM	0.395	0.02	0.02
Source of variation					
² E:P			NS	NS	**
E:P ²			NS	NS	**

The mean of each response due to E:P ratio are highlighted in bold. ¹Each treatment was replicated eight times, ²E:P; Energy to protein ratio. ** P<0.01.

Energy retention was significantly affected by E: P ($P<0.05$) and $E:P^2$ ($P<0.0001$). The main effect of LR and ER showed an increase of 7.38, 6.11, 1.71% and 6.46, 31.81, 3.92% respectively as dietary ME increased from 11 to 14 MJ ME/kg. However, H decreased by 14.98, 4.43 and 2.01% as dietary ME increased from 11 to 12, 12 to 13 and 13 to 14 MJ ME/kg respectively. Energy to protein ratio had a significant ($P<0.01$) effect on H. An increase in the dietary E: P ratios from 22 to 44, 24 to 40, 26 to 43 and 28 to 47 MJ ME/kg protein for birds fed diets with 11, 12, 13 and 14 MJ ME respectively increased heat production. The H of birds fed diets in each of these energy levels increased from 835 to 1168, 703 to 971, 782 to 926 and 781 to 847 respectively.

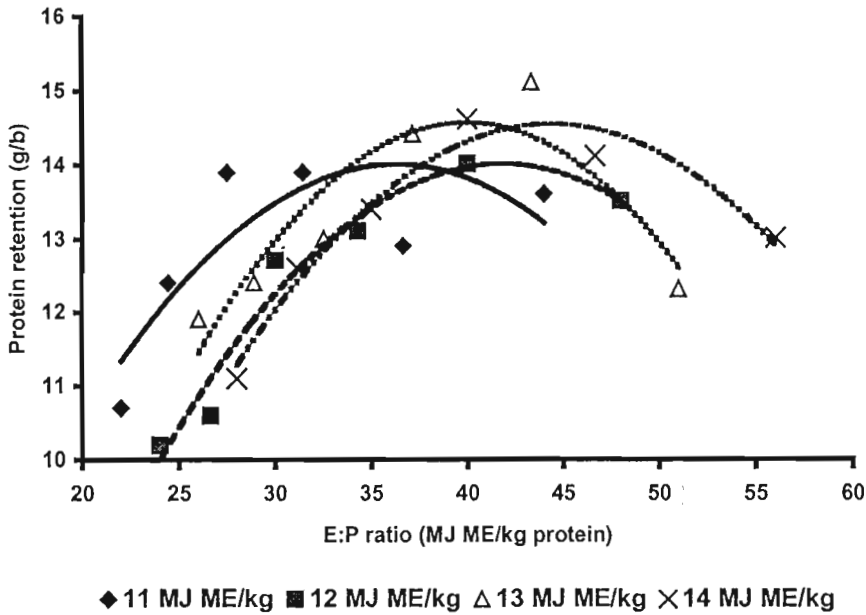


Figure 5.3. The effect of ME and E: P ratios on protein retention of chicks between 23 to 37days of age.

The net efficiency of protein utilisation, e_p

Linear regression equations for each ME series were fitted to the data (Table 5.3). The e_p as a function of the E: P ratio was described well by a straight line for each MJ ME series. The slopes of each best-fitted line are shown in Figure 5.4. The first five diets of the 13 and 14 MJ ME diets were used to fit a regression line for these ME series, since the linear slope only occurred over this range. All slopes were significantly different from zero. The mean slope range at 95% confidence interval for the model without the intercept was 0.0136 to 0.0156

with an overall mean of 0.0149 ± 0.00020 . There were no significant differences between slopes for birds fed diets in the 11, 12 and 13 MJ ME series. There were significant differences ($P < 0.05$) between slopes for birds fed diets in the 11 and 14, 12 and 14 MJ ME series. The highest e_p for each ME series occurred at the highest E: P ratio. Conversely the lowest e_p occurred in birds fed diets at the lowest dietary E: P ratios within each ME series. The lowest e_p was recorded with birds fed diets in the 11 MJ ME/kg series

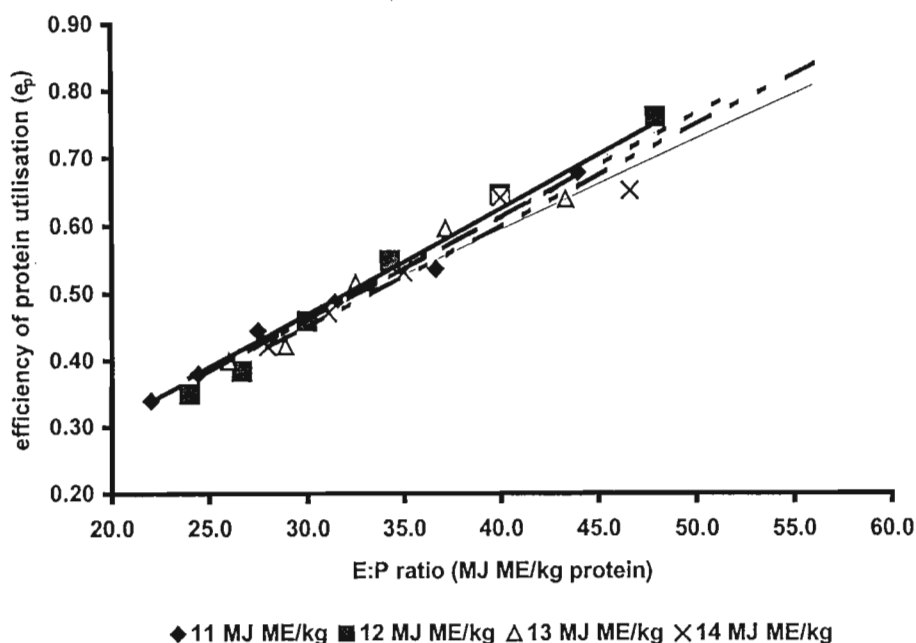


Figure 5.4. The effect of ME and E:P ratios on the net efficiency of protein utilisation of male chicks between 23 to 37 days of age.

Table 5.3. The slope and net efficiency of protein utilisation (e_p) of male broiler chickens aged 23 to 37 days obtained by fitting a linear regression model to the response data.

Dietary treatment ¹	Slope (kg/MJ)	s.e. of slope coefficient	Adj. R ²	P-value	Predicted inflection at E:P ratio (MJ/kg)	Maximum e_p observed
ME series						
11	0.0153	0.00020	0.98	<0.001	52.29	0.6764
12	0.0156	0.00025	0.98	<0.001	51.28	0.7573
13	0.0153	0.00029	0.95	<0.001	52.29	0.6515
14	0.0136	0.00019	0.98	<0.001	58.82	0.7027
Mean	0.0149	0.00020	0.97	<0.001	53.67	0.6969

¹Regressions from each treatment, which was replicated eight times with, intercepts set to zero; s.e. Standard error.

A comparison between the slopes for the e_p responses of birds for both growth periods was obtained by fitting the responses with a stepwise multiple regression model following similar procedures described in Chapter 3. A summary of the results of the regressions is given in Table 5.4.

Table 5.4 *Parameter estimates obtained by fitting the e_p responses of birds for both growth periods (10 to 24 and 23 to 37d) with a stepwise multiple regression model of the form: EP_{adum} aslopes*

Variable	Parameter estimate	Standard error	t-value	p-value
ME 11				
Intercept	-0.1290	0.04127	-3.12	0.0025
E:P	0.0181	0.00146	12.38	0.0001
adum	0.1478	0.06184	2.39	0.0192
aslopes	-0.0036	0.00193	-1.87	0.0651
SEM	0.0655			
Adj R^2	0.78			
ME 12				
Intercept	-0.1290	0.0413	-3.12	0.0025
E:P	0.0171	0.0012	14.54	0.0001
SEM	0.0575			
Adj R^2	0.87			
ME13				
Intercept	-0.0913	0.0450	-1.90	0.0606
E:P	0.0157	0.0013	12.42	0.0001
adum	0.2480	0.0631	3.93	0.0002
aslopes	-0.0053	0.0017	-3.19	0.0020
SEM	0.0667			
Adj R^2	0.75			
ME 14				
Intercept	0.0030	0.0432	0.07	0.9458
E:P	0.0131	0.0011	12.39	0.0001
adum	0.1737	0.0570	3.05	0.0001
aslopes	-0.0032	0.0014	-2.27	0.0262
SEM	0.0605			
Adj R^2	0.77			
Overall comparison of slopes for the two growth periods (10 to 24 d and 23 to 37d)				
Intercept	-0.0528	0.0226	-2.33	0.0204
E:P	0.0150	0.0006	24.19	0.0001
adum	0.1424	0.0298	-3.14	0.0018
aslopes	-0.0026	0.0008	-3.14	0.0018
SEM	0.0673			
Adj R^2	0.78			

The slopes did not differ between age groups (10 to 24 d and 23 to 37 d) when broilers were fed the 11 and 12 MJ ME diets. For the 13 and 14 MJ ME diets, the slopes were depressed by

advancing maturity. The results presented in Table 5.4 show that, in the case of the overall comparison between the slopes for both growth periods, the intercept is negative and the age of the bird (as slopes) significantly affected the slopes. The overall mean slope of 0.015 was greater than 0.013 reported for birds 10 to 24 days of age fed excesses of dietary protein at varying ME contents.

DISCUSSION

In Chapters 2 to 4 a problem arose when chicks were offered high levels of protein 350 to 500 g CP/kg without changing the ME content of the diet. It may be possible to solve this problem, by providing sufficient energy in the feed to process this excess protein. A method of determining this would be to offer birds a range of feeds varying in E:P ratio all in the high range of DCP (as there is little point in conducting these measurements in the area where protein is limiting), and progressively increase ME content from 11 to 14 MJ ME/kg.

The uniqueness of the current investigation is the comparatively larger number of dietary treatments (i.e. 24) replicated eight times. In the current study the dietary energy and protein contents were higher than recommended for maximum biological performance. The reason was to obtain a wide range of dietary E:P ratios that can still give rise to maximal performance and e_p . Excesses of amino acids were kept to a minimum by minimising the total essential amino acid (TEAA) content of the basal diets. Diets of the lower E:P ratios also contained higher levels of lysine than recommended.

Effects on the biological performance

This experiment has shown that the biological performance of broiler chickens is influenced by variations in E:P ratios during growth. It has also been demonstrated that the birds responses to dietary ME concentration cannot be regarded in isolation from E:P ratio. The data indicate that growth is an energy demanding process. The implication is that, when birds are fed high protein contents, the dietary energy content should be increased to ensure that sufficient energy is available for the efficient utilisation of excess dietary protein. It was of interest to investigate the effects of altering both dietary energy (E) and protein (P) simultaneously on broiler performance. Reducing dietary E at constant P contents narrowed energy to protein ratios. There are severe consequences of feeding diets of very low E:P ratios for biological performance. The data indicate that a low-energy diet (11 MJ ME) or low E:P ratio is associated with a poorer daily weight gain and FCE. It is clear from Table 5.1, that at a dietary protein concentration of 250g CP/kg, the extra energy allowance (i.e.

increasing the dietary energy concentration) had a negligible effect on biological performance. At a dietary protein concentration of 300g CP/kg, weight gain and FCE improved with an increase in dietary energy concentration. These findings are in agreement with results in Chapter 4, in which growth was improved in response to higher dietary E: P ratios or energy concentration in younger birds.

Care has to be taken when assessing the response of the model outside the data range. In spite of this, the model provides a biologically acceptable response over the large range of E: P ratios studied. The lowered food intake and ADG at very low E: P ratios between 22 to 24 MJ ME/kg protein could be related to the comparatively low protein retention (10.2 to 10.7 g/d) and the inability of birds to lose sufficient heat to the environment as a result of the energy to protein imbalance. The same is true of E: P ratios above 51 MJ ME/kg protein where ADG decreased.

Lipid and protein retention and heat production.

Broiler chickens fed diets in the higher ME series and high E: P ratios showed a higher LR. The data for PR was best described by E:P ($P<0.001$) and $E:P^2$ ($P<0.001$). ME significantly ($P<0.05$) influenced PR but the interactions between ME and E:P were of no significance ($P=0.554$). At low dietary ME contents broiler chickens were unable to consume sufficient energy to achieve normal growth, and thus were unable to deposit excess lipid in their body tissues. For birds fed low ME concentrations, a large proportion of the energy consumed was used for maintenance (basal metabolism and normal activity) with less available for growth. Not unexpectedly the rate of LR increased as ME and E: P ratio increased. The data demonstrate that excess protein, or a relatively low dietary E: P ratio reduces LR (Table 5.2) in agreement with reports by Bartov and Plavnik (1998). However the data do not support the observations of Bartov and Plavnik (1998) that diets varying in their energy and CP levels, but having the same E: P ratio, produce a similar LR. For instance, LR at an E: P ratio of 28 MJ ME/kg protein for birds on 11 MJ ME/kg (450 g CP/kg) and 12 MJ ME/kg (500 g CP/kg) were 5.90 and 4.03 g/bird, respectively.

The feeding of increasing amounts of excess protein, at a given ME, led to an increase in PR until a maximum value was reached. Further increments in protein concentration did not result in an increase in PR. Increases in PR only occurred when more dietary energy was supplied. Subsequently there was a further increase in PR to a maximum at a higher crude protein concentration. Protein retention was therefore driven by ME intake when the supply

of protein was above the needs of the broiler for maximum PR. This is in line with previous observations that under conditions where dietary protein is in excess of requirement, PR is a function of ME intake. The rate of PR and LR was higher for birds fed diets in the 13 and 14 MJ ME series over the 11 and 12 MJ ME series. These findings are in agreement with the results of Harper (1983) and data reported in Chapter 4. The rate of PR was not constant at constant CP intake. In the formulation of practical diets for poultry, it is thus necessary to ensure that there is sufficient energy to support the metabolic reactions involved in growth, maintenance of normal physical activity and body temperature (Scott *et al.*, 1976).

There was an increase in ER at the high E: P ratios within each ME series as a consequence of the increase in LR. Above a certain critical E: P ratio the bird continues to partition more of the available energy towards lipid deposition than towards protein deposition because PR is at a maximum. Heat production of the birds showed a downward trend as dietary ME series increased from 11 to 14 MJ ME/kg, in contrast to ER and LR, which increased. It can be deduced from the results that levels of dietary protein in excess of its AA requirement depress growth performance and reduces LR in the low MJ ME series. This effect, however, is not limited to birds in the starter phase but also those in the grower/finisher stage of growth. Wiseman (2001) suggested that when birds are fed excesses of dietary protein at low dietary ME concentrations (i.e. 10.78 MJ ME/kg, 46.9 MJ ME/kg protein), dietary energy is used in the catabolism of excess protein through gluconeogenesis rather than being used mainly in protein synthesis. The associated reduction in carcass fat content offers a possible means of altering carcass quality by feeding high protein diets, but this has deleterious consequences such as an increased N excretion (Wiseman, 2001) and a profound depletion of some glucogenic AA's (i.e. threonine, serine and glycine) required for protein synthesis (Moundras *et al.*, 1993).

The net efficiency of protein utilisation above maintenance (e_p).

In calculating e_p the digestible protein intake and protein retention were considered. The net efficiency with which dietary protein is used above maintenance in this study was found to be a function of the availability of dietary energy at constant protein intake. The calculated e_p increased with increasing E: P ratio. These results are in agreement with studies by Kadowaki *et al.* (1989) that showed that intakes of dietary protein in excess of that required for maximal growth are not associated with any significant increase in the efficiency of protein synthesis.

The predicted inflection points occurred at a lower E: P ratio for the 11, 12 and 13 MJ ME series (51.28, 52.29, 52.29 MJ ME/kg protein) but at a higher E: P ratio for the 14 MJ ME series (58.82 MJ ME/kg protein) than the value of 54.14 MJ ME/kg protein reported by Faulkner (1993). The values for the ascending slopes for the 11, 12 and 13 MJ ME series of 0.0153, 0.0156 and 0.0153 respectively were higher than that determined by Faulkner (1993) of 0.0141 kg/MJ, whilst the values for the 14 MJ ME series of 0.0136 was similar to that determined by Faulkner (1993). These values are higher than the value of 0.0112 kg/MJ reported by Kyriazakis and Emmans (1992a), for pigs. There were no significant differences between slopes for birds fed diets in the 11, 12 and 13 MJ ME series. However, significant differences between slopes were observed for birds fed diets in the 11 and 14, 12 and 14 MJ ME series. The overall mean slope of 0.015 was greater than 0.013 reported for birds 10 to 24 days of age fed excesses of dietary protein at varying ME contents. Differences between these values may be due to age differences, species differences and possibly the variability in the AA analysis and feed characteristics used in the different studies.

CONCLUSIONS

High protein diets, even when balanced in all essential AA's, affected biological performance of the broiler chickens in terms of average daily live weight gain and food conversion efficiency. Excess protein is catabolised and therefore used less efficiently for lean-tissue deposition. The growth depression caused by excesses of dietary protein, and hence amino acids are also due to the lack of sufficient energy for the efficient utilisation of excess dietary protein. Broiler chickens fed on diets containing a lower concentration of energy (i.e. 11 or 12 MJ ME series) did not increase their food intake sufficiently, and therefore the energy contribution for increased protein retention was inferior to that in chicks fed higher energy diets. This accounts for the poorer growth rates of birds fed diets containing a lower energy concentration (i.e. 11 MJ ME series). The efficiency of protein utilisation (e_p), is not influenced only by the dietary crude protein content, indicating that it is the ratio of energy to protein in the diets that is important and not the actual amounts of each consumed each day.

CHAPTER 6:

THE EFFECTS OF EXCESS DIETARY PROTEIN, PROTEIN QUALITY AND DAILY FOOD ALLOCATION LEVEL ON THE GROWTH, MUCOSAL STRUCTURE AND DIGESTIVE FUNCTION IN BROILER CHICKENS 10 TO 24 DAYS OF AGE.

ABSTRACT

Two series of feeds differing in protein quality (PQ), i.e. balanced (BPS) and unbalanced (UPS) protein series, and covering a range of protein contents (400, 300 and 200 g CP/kg) at 13 MJ ME/kg were offered at two daily food allocation (*ad lib.* or 0.75 of *ad lib.* intake) to assess the effects on the growth, visceral organ development, mucosal structure and digestive function in broiler chickens

The crypt depth of chicks on *ad lib.* feeding regime was higher on the BPS than on the UPS. Feed restriction negatively affected the development of the crypt in the jejunum. The protein content of the jejunal mucosa was higher ($P<0.001$) for birds fed *ad lib.* on UPS than on the BPS.

Maltase ($P<0.001$), sucrase ($P<0.01$) and Alkaline phosphatase activities were significantly lower in chicks offered *ad lib.* access to UPS.

The lack of energy for conducting the various functions related to protein metabolism and deposition may be partly responsible for the poor performance of birds

Keywords: broiler, protein quality, daily food allocation, intestinal development

INTRODUCTION

Protein deposition in broiler chickens is a process that requires a relatively large amount of energy and is, to some extent, dependent on bird-related factors such as the development of the gastrointestinal tract (GIT). Apart from the commonly assessed effects of energy to protein ratio (E:P ratio) on the biological performance of broiler chickens, the study of causal connections at the GIT/organ level has been largely ignored or underestimated. Nutrient processing by the GIT determines the amount of nutrient that is available to the internal tissues for metabolism. The GIT utilises some of the nutrients ingested for self-renewal (Webster, 1980; Reynolds *et al.*, 1991) and the efficiency of nutrient supply to the internal tissues would be dependent on dietary factors, including E: P ratios. Preliminary work on the effect of varying E:P ratios on the biological performance and gastrointestinal development of broiler chickens have been reported by Swatson *et al.* (2000). It was evident that changes in GIT development and biological performance do occur due to changes to dietary energy to protein ratios. Some of the effects were partly explained by changes in the pattern and rate of development of the GIT, as has been recently reported (Iji, *et al.*, 2001a, b).

There is little information regarding the effects of dietary nutrients, especially energy and protein quality/ daily food allocation (DFA) level, on the development of the GIT and overall growth of poultry. Dietary protein has been associated with the regulation of insulin-like growth factors (IGF) and somatotropin and thus, body growth and fat deposition in broiler chickens (Caperna *et al.*, 1999; Kita and Okumura, 1999). Kita *et al.* (1996) also found that feed restriction for 4-7 days reduced plasma IGF, as did the consumption of low-protein diets.

The aim of the present study was to test the hypothesis that body growth and GIT function are dependent on dietary energy and protein content, protein quality and the daily food allocation. The effects of these factors on the development of visceral organs associated with digestion and nutrient absorption were also studied.

MATERIALS AND METHODS

The materials and methods used in this experiment were similar to that reported in Chapter 2. Two series of feeds differing in protein quality (PQ) and covering a range of three crude protein levels (i.e. 200, 300 and 400) at a constant energy content of 13 MJ ME/kg, were offered at two levels of daily food allocation (DFA: *ad lib.* or 0.75 of *ad lib.*) to Ross male

broiler chickens, from 10 to 24 days of age (Table 2.1). The calculated amino acid composition (g/kg diet) are shown in chapter 2 (Table 2.2). The dietary treatments were replicated six times.

Birds and housing

Seven hundred and twenty Ross broiler chickens of uniform size were used for the study. During the pretest period (0-9 days posthatching) the chicks received a standard commercial starter feed (240 g crude protein/kg) *ad lib*. Day-old chicks were placed in groups of ten in single-tier battery cages in the experimental unit, to accustom them to the facilities after being weighed to the nearest gram. At 10 days of age, birds were randomly assigned either to one of the 12 feeding treatments, such that the average starting weight and weight range (0.180 ± 0.017 g) were similar for each treatment. The experimental birds were given *ad lib*. access to water and continuous artificial lighting. The house temperature was monitored and recorded daily throughout the duration of the trial.

Experimental diets and design

Two dietary protein series were used in this experiment, one being based on a balanced (BPS), and the other being based on an unbalanced (UPS) amino acid mixture as in Chapter 2 (Table 2.1). These were each fed at three protein levels and at two daily food allocations (DFA). The crude protein contents within each series were 400, 300 and 200 g CP/kg, respectively; the 300 and 200 g CP/kg diets were made by appropriately blending the summit (400 g CP/kg diet) and dilution (protein free) feeds. The blending ratio between the summit and dilution diets for compounding the 300 and 200 g CP /kg diets were 0.75:0.25 and 0.50:0.50 respectively. Feeds had the same energy content of 13 MJ AME/kg, resulting in three dietary E:P ratios, viz.: 32.5, 43.3 and 65.0 MJ ME/kg protein.

Dietary treatments:

(i) Ad libitum treatments

Birds assigned to these treatments were given free and continuous access to one of the 12 dietary treatments. Feed consumption was measured daily by weighing the food at the start and end of each 24-hour period.

(ii) Restricted treatments

Birds designated to these treatments were restricted to 0.75 of the average consumption of the respective *ad lib.* treatments. The allocated feed was divided into two portions, with the first being given in the morning at 7 a.m. and the other in the afternoon at 2 p.m. The same levels of restriction were applied for birds on each of the two protein series.

Bird management procedure

Birds were fed twice daily and weighed once weekly. In the case of the *ad lib.* fed treatments, feed remaining in the trough at the end of 24 hours was collected and weighed daily, the average of these intakes over equivalent treatments being used to calculate the amounts to be offered to the birds on the restricted treatments. At the conclusion of the experiment, all birds and feed remaining were weighed

Sample collection

At the end of the feeding period, one bird per cage, selected at random was slaughtered through asphyxiation with CO₂ and dissected. The joint weights of the proventriculus and gizzard as well as the weight of the small intestine were recorded, with the contents. The pancreas, liver and spleen were also weighed. Tissue samples (5 cm long) were taken from the proximal region of the jejunum and flushed with ice-cold saline. Duplicate samples representing each dietary treatment were then snap-frozen in liquid nitrogen and used for digestive enzyme assay. A subsample (1 cm long) was fixed in neutral buffered formalin and used to assess the morphometry of the intestinal mucosa.

Histology

Tissue slices for histological examination were processed by serial dehydration with ethanol, clearing with histolene and embedded in paraffin wax. Sections were cut from the waxed tissue on a Leitz 1512 microtome (Ernst Leitz Westlar GmbH, Austria), cleared of wrinkles by floating on warm water (45-50°C) prior to mounting on 10 % poly-L-lysine coated slides. The slides were stained by Lilee Meyer's haematoxylin, counter-stained with eosin yellow and mounted in DePeX medium.

Slides were viewed on an Olympus BH-2 microscope and digitised using video image software, Video Pro (Leading Edge, Bedford Park, South Australia). Images were viewed

(optical lens No. 4) to measure the crypt depth, villus width at the crypt-villus junction, villus height and villus apical width. Apparent villus surface area was estimated through trigonometry (Iji, *et al.*, 2001a). Fifteen villi were assessed per sample.

Measurement of digestive enzymes

The intestinal tissue homogenate was prepared as described by Shirazi-Beechey *et al.* (1991). The tissue was cut into an ice-cold buffer (100 mM mannitol, 2 mM Tris/HEPES, pH 7.1) and the mucosa was then stripped into the buffer using a swirl mixer at high speed for one minute. The mixture was homogenised at medium speed for thirty seconds. Sub-samples of the homogenate were taken into Eppendorf tubes, frozen in liquid nitrogen and stored in a deep freezer (-20°C) for enzyme analysis.

Enzyme assays were conducted on fixed substrate concentrations established in studies on other species and previously standardised with poultry (Iji, *et al.*, 2001b). Biochemical assays were conducted for maltase (EC. 3.2.1.20), sucrase (EC. 3.2.1.26) and alkaline phosphatase (AP, EC. 3.1.3.1).

The specific activities of enzymes were measured according to methods previously described for other species (Miller *et al.*, 1960; Dahlqvist, 1964; Holdsworth, 1970). Assays were, however, conducted at a temperature of 39°C. The protein content of the jejunal mucosa was measured according to the method described by Bradford (1976).

Statistical design and analysis

Data were analysed by both the general linear model (GLM) and regression of Minitab (1998). The data were regressed, using varying levels of protein quality (PQ) and daily food allocation (DFA), and dietary protein concentration as independent factors. Differences between mean values were determined by the use of least significant difference.

RESULTS

Feed intake and utilization

Feed intake was significantly influenced ($P < 0.001$) by protein balance, dietary crude protein and DFA, as well as the interaction between protein balance and excess dietary protein (Table 6.1). At a dietary protein content of 200 g/kg, the birds consumed less feed on the UPS diets

than on the BPS diets. This effect was only observed with birds on diets containing excess dietary protein (300 and 400 g CP/kg) and on restricted feed intake. Birds on the balanced diets and fed *ad lib.* consumed less feed ($P<0.001$) as dietary protein content increased. There was no effect of varying crude protein content on feed intake on the UPS diets when fed *ad lib.*

Table 6.1 Feed intake, weight gain and feed conversion efficiency (FCE) of broiler chickens reared on diets varying in protein content (CP) and protein quality (PQ) between 10 to 24 days of age.

Protein Quality	CP (g/kg)	DFA	Feed intake (g/d)	Weight gain (g/d)	FCE (g weight gain/ kg feed)
BPS	200	1.0	64.9 ^a	29.8 ^{ab}	459.0 ^{bc}
		0.75	48.7 ^{cd}	19.1 ^{de}	391.9 ^{de}
UPS	200	1.0	48.6 ^{cd}	17.0 ^{ef}	350.9 ^e
		0.75	36.4 ^{fg}	11.2 ^g	306.7 ^g
BPS	300	1.0	58.1 ^b	30.9 ^a	531.2 ^a
		0.75	43.6 ^e	18.7 ^{de}	428.7 ^{cd}
UPS	300	1.0	53.4 ^{bcd}	22.7 ^c	424.9 ^{cd}
		0.75	40.1 ^g	13.9 ^f	347.4 ^f
BPS	400	1.0	50.9 ^{cd}	26.9 ^b	528.7 ^a
		0.75	38.2 ^{fg}	14.6 ^f	381.2 ^e
UPS	400	1.0	47.4 ^{de}	22.7 ^c	466.0 ^b
		0.75	35.6 ^g	13.8 ^f	390.8 ^{de}
		SEM	1.73	1.02	15.05
<i>Source of variation</i>					
PQ			***	***	***
CP			***	***	***
DFA			***	***	***
PQ x CP			***	***	***
CP x DFA			NS	NS	**
PQ x DFA			NS	***	**
PQ x CP x DFA			NS	NS	NS

Mean values on the same column with different superscripts are significantly different (** $P<0.01$; *** $P<0.001$).
NS - not significant.

DFA, daily food allocation (proportion of *ad lib.*)

BPS, balanced protein series

UPS, unbalanced protein series

The interactions between dietary PQ and crude protein, and between PQ and DFA, on body weight gain were significant ($P<0.001$). Therefore, at both DFA's, there was a reduction ($P<0.001$) in the body weight gain of birds on the unbalanced diets, at protein contents of 200 and 300 g CP/kg diet. At the 400 g CP level, this effect was observed only on the balanced

diets. Body weight gain declined ($P<0.001$) with an increase in dietary crude protein on the balanced diets; the reverse was the case on the unbalanced diets.

For birds reared on the 200 and 300 g CP/kg diets, FCE was poorer ($P<0.001$) on the unbalanced protein diets than on the balanced diets. On the 400 g CP/kg diets, this effect was noticeable only in chicks that were fed *ad lib*. FCE also increased ($P<0.001$) with increasing dietary CP content although the trend for the chicks on the balanced protein diet, on a restricted regime was not consistent. FCE was also influenced ($P<0.01$) by the interactions between crude protein and DFA, PQ and DFA as well as between PQ and crude protein content ($P<0.001$). As expected, birds on restricted feeding gained significantly less weight ($P<0.001$) and had a poorer FCE than those on the *ad lib*. feeding regime.

Visceral organ weight

The weights of the visceral organs from birds on the various diets are shown in Table 6.2.

Table 6.2: The effect of protein quality (PQ) dietary protein content (CP) and daily food allocation (DFA) on weight (g/100 g body weight) of visceral organs.

Protein quality	CP (g/kg)	DFA	Gizzard	Small intestine	Pancreas	Spleen	Liver
BPS	200	1.0	4.2 ^b	4.5 ^{bc}	0.30 ^{bc}	0.14 ^{ab}	3.2 ^b
		0.75	4.2 ^b	5.3 ^{ab}	0.30 ^{bc}	0.21 ^a	3.9 ^{ab}
UPS	200	1.0	5.2 ^b	4.3 ^c	0.30 ^{bc}	0.09 ^b	3.3 ^b
		0.75	5.4 ^b	5.3 ^{ab}	0.40 ^{bc}	0.17 ^a	4.2 ^{ab}
BPS	300	1.0	4.1 ^b	4.5 ^{bc}	0.40 ^{bc}	0.12 ^{ab}	3.4 ^b
		0.75	4.9 ^b	5.2 ^{ab}	0.50 ^{ab}	0.13 ^{ab}	3.3 ^{ab}
UPS	300	1.0	5.3 ^b	4.7 ^{bc}	0.40 ^{bc}	0.11 ^b	3.6 ^{ab}
		0.75	5.9 ^a	5.2 ^{ab}	0.40 ^{bc}	0.17 ^a	4.0 ^{ab}
BPS	400	1.0	4.1 ^b	5.6 ^a	0.50 ^{ab}	0.14 ^{ab}	3.7 ^{ab}
		0.75	4.5 ^b	5.1 ^{ab}	0.60 ^a	0.22 ^a	4.3 ^a
UPS	400	1.0	4.3 ^b	4.8 ^{ab}	0.30 ^c	0.13 ^{ab}	3.9 ^{ab}
		0.75	4.3 ^b	4.9 ^{ab}	0.40 ^{bc}	0.16 ^{ab}	3.3 ^b
		SEM	0.48	0.34	0.046	0.033	0.35
Source of variation							
PQ			***	NS	***	NS	NS
CP			***	NS	***	NS	NS
DFA			NS	***	***	***	*
PQ x CP			*	NS	***	NS	NS
CP x DFA			NS	*	NS	NS	NS
PQ x DFA			NS	NS	NS	NS	NS
PQ x CP x DFA			NS	NS	NS	NS	NS

Mean values on the same column with different superscripts are significantly different (* $P<0.05$); *** $P<0.001$).

NS - not significant.

DFA, daily food allocation (proportion of *ad lib*)

For chicks on diets containing 300 g CP/kg, the combined weight of the proventriculus and gizzard on the BPS was significantly lower ($P<0.01$) than that of chicks on the UPS. There was also a significant ($P<0.001$) effect of excess dietary protein on the proventriculus and gizzard, but only for birds fed the restricted diets. The weight of the small intestine was influenced ($P<0.001$) by variation in DFA ($P<0.001$) and interactions between crude protein and DFA ($P<0.05$) in chicks. The weight of the pancreas was lowest ($P<0.001$) in chicks on the unbalanced diets, especially when dietary protein was 400 g CP/kg. In chicks fed restricted amounts of the balanced protein diets, pancreatic weight increased ($P<0.001$) with an increase in dietary protein level. The weight of the spleen was influenced ($P<0.001$) by DFA. There was also a significant ($P<0.05$) effect of DFA on the weight of the liver but this was not consistent.

Intestinal mucosal morphometry

The crypt depth of chicks on *ad lib.* feeding regime was higher ($P<0.01$) on the balanced diets than on the unbalanced diets (Table 6.3).

Table 6.3: *The effect of protein quality (PQ), crude protein (CP) and daily food allocation (DFA) on the morphometry of jejunal mucosa.*

Protein Quality	CP (g/kg)	DFA	Crypt depth (μm)	Villus height (μm)	Villus surface area (mm^2)
BPS	200	1.0	558.9 ^{ab}	1251.6 ^a	0.28 ^a
		0.75	537.6 ^{ab}	1014.3 ^{ab}	0.25 ^{ab}
UPS	200	1.0	402.5 ^c	875.1 ^b	0.20 ^{ab}
		0.75	454.2 ^b	847.2 ^b	0.18 ^b
BPS	300	1.0	548.4 ^{ab}	1196.1 ^{ab}	0.27 ^a
		0.75	563.2 ^{ab}	1021.9 ^{ab}	0.26 ^{ab}
UPS	300	1.0	522.5 ^{abc}	1010.6 ^{ab}	0.21 ^{ab}
		0.75	464.4 ^{bc}	939.9 ^{ab}	0.19 ^{ab}
BPS	400	1.0	629.8 ^a	1144.5 ^{ab}	0.27 ^a
		0.75	523.3 ^{abc}	1047.6 ^{ab}	0.25 ^{ab}
UPS	400	1.0	580.1 ^{ab}	907.7 ^{ab}	0.24 ^{ab}
		0.75	534.1 ^{ab}	918.0 ^{ab}	0.22 ^{ab}
		SEM	121.47	298.00	0.078
<i>Source of variation</i>					
PQ			**	***	***
CP			*	NS	NS
DFA			NS	NS	NS
PQ x CP			NS	NS	NS
CP x DFA			NS	NS	NS
PQ x DFA			NS	NS	NS
PQ x CP x DFA			NS	NS	NS

Mean values on the same column with different superscripts are significantly different (* $P<0.05$); ** $P<0.01$; *** $P<0.001$). NS - not significant. DFA, daily food allocation (proportion of *ad lib.*)

Crypt depth was also influenced ($P<0.05$) by variation in crude protein content independent of PQ. Villus height was reduced ($P<0.001$) on the unbalanced diets. Although apparent villus surface area was influenced ($P<0.001$) by protein balance, there was no trend.

Activities of digestive enzymes in the jejunum

There were significant interactions between PQ, CP level, and DFA on AP ($P<0.05$). As CP increased from 200 to 400 g CP/kg, mucosal protein content (mg/g tissue) decreased from 126.2 to 63.1 and 92.4 to 63.1 for birds fed the BPS at *ad lib* and 0.75 of *ad lib* intake. In contrast, as CP increased from 200 to 400 g CP/kg, mucosal protein content (mg/g tissue) for birds allowed *ad lib* access to the UPS diets increased from 115.7 to 165.2. When the diet contains excess protein (i.e. at protein contents of 300 and 400 g CP/g diet) and at unrestricted feeding, the protein content of the jejunal mucosa was higher ($P<0.001$) on the unbalanced than on the balanced diets (Table 6.4).

Table 6.4 *The effect of protein quality (PQ), crude protein (CP) and daily food allocation (DFA) on mucosal protein content and activities of digestive enzymes in the jejunum.*

Protein Quality	CP (g/kg)	DFA	Protein ¹	Maltase ²	Sucrase ²	AP ³
BPS	200	1	126.2 ^a	2.2 ^{ab}	0.07 ^{ab}	1.2 ^{ab}
		0.75	92.4 ^{bc}	2.0 ^{ab}	0.06 ^{abc}	1.2 ^{ab}
UPS	200	1	115.7 ^a	1.9 ^{ab}	0.07 ^a	0.8 ^b
		0.75	98.9 ^{abc}	2.1 ^{ab}	0.05 ^{abc}	0.9 ^b
BPS	300	1	72.9 ^{bc}	2.3 ^{ab}	0.07 ^a	1.5 ^a
		0.75	95.4 ^{bc}	1.6 ^{ab}	0.04 ^{bc}	1.0 ^{ab}
UPS	300	1	164.9 ^a	1.1 ^b	0.04 ^{bc}	0.6 ^b
		0.75	98.8 ^{abc}	1.8 ^{ab}	0.04 ^{bc}	1.1 ^{ab}
BPS	400	1	64.9 ^c	2.5 ^a	0.06 ^{ab}	1.8 ^a
		0.75	63.1 ^a	2.7 ^a	0.06 ^{ab}	1.3 ^{ab}
UPS	400	1	165.2 ^{bc}	1.1 ^b	0.03 ^c	0.6 ^b
		0.75	96.6 ^{bc}	1.7 ^{ab}	0.04 ^{bc}	1.1 ^{ab}
		SEM	19.81	1.3	0.011	0.21
Source of variation						
PQ			***	***	**	***
CP			NS	NS	*	NS
DFA			**	NS	NS	NS
PQ x CP			**	*	NS	NS
CP x DFA			NS	NS	NS	NS
PQ x DFA			**	*	NS	***
PQ x CP x DFA			*	NS	NS	*

¹ mg/g tissue; ² μmole glucose/mg protein/minute; ³ μmole nitrophenol/mg protein/minute.

Mean values on the same column with different superscripts are significantly different (* $P<0.05$; ** $P<0.01$; *** $P<0.001$). NS - not significant.

DFA, daily feed allocation (proportion of *ad lib*)

DFA also had a significant ($P<0.01$) effect on jejunal protein content in birds that received balanced diets containing 200 or 400 g CP/kg, mucosal protein being reduced in birds on restricted feeding. The specific activity of maltase was lower ($P<0.001$) in chicks on the unbalanced diets but this was observed only on diets containing 400 g CP/kg and on *ad lib.* feeding regime. There was also a reduction ($P<0.05$) in sucrase activity with increasing dietary CP content, in birds fed *ad lib.* on the unbalanced diets. There were also significant interactions between PQ and DFA ($P<0.001$) and between the three factors ($P<0.05$). At excess nutrient (protein) intake, PQ affected ($P<0.001$) the specific activity of AP, this tending to be lower in chickens on the unbalanced diets than in those fed the balanced diets.

DISCUSSION

The general response of poultry to feed supply and quality is well documented (Zaghari *et al.* 1996; Carew *et al.* 1998; Tan *et al.* 1999) but the mechanisms underlying these effects have not been adequately studied. An understanding of the effects of these factors is made more difficult by the fact that they operate in synergy with each other rather than as independent factors. The results of this study reveal many interactions between some of the dietary factors. For example, feed intake responded differently on account of protein quality (PQ) as well as dietary protein content. Feed intake was affected by PQ but the response depended on the level of dietary protein. Similarly, the response in growth to dietary protein content was only observed on the balanced diets fed *ad lib.* These responses are partly due to the fact that the effects of imbalances are felt only at low protein contents, which is why balance experiments (NPU, PER, etc) are conducted at contents of about 80 to 100 g protein/kg.

Weight gain showed an increase of 1.1 g/bird d when dietary protein contents increased from 200 g to 300 CP/kg and for birds fed balanced diets, when protein content was increased further, from 300 to 400 g CP/kg, weight gain decreased by 4 g/bird/d. However, birds fed UPS diets, showed an increase in weight gain of 5.7 g/bird d when dietary protein contents increased from 200 g CP/kg to 300 or to 400 g CP/kg. The depression in growth of birds fed the BPS when the dietary protein content increased beyond that required to meet the amino acid requirement of the broiler (i.e. 400 g CP/kg) has also been reported by Morris *et al.* (1999). It is suggested that the dietary energy content of 13 MJ ME/kg could be increased further to ensure that sufficient energy is available for the excess dietary protein to be utilised efficiently for growth. For birds fed the UPS the proportion of amino acids in the total protein

digested that can be incorporated into body protein (v) for the 400 to 200 g CP/kg ranged from 0.96 to 0.48. Thus only 48% of the ingested protein is utilised for growth on the low protein diet (i.e. 200 g CP/kg in the UPS) partly accounting for the lowered growth of birds fed these diets. The negative effects of restricted feed intake on body weight gain or growth has been extensively reported by previous researchers (Khantaprab *et al.*, 1997; Gonzales *et al.*, 1998; Tan *et al.*, 1999). Gonzales *et al.* (1998) partly attributed this effect to reductions in thyroxin, IGF and growth hormone. The effects of low protein supplies have been ameliorated by supplementation with methionine and cysteine, as long as other indispensable amino acids are sufficient (Bunchasak *et al.*, 1997). In the present study, FCE increased with an increase in dietary protein content. This confirms the reports by Zaghari *et al.* (1996) who observed a positive effect of increasing dietary protein content on FCE and body weight. In a comparative study, Ramlah *et al.* (1996) also reported positive effects of mild feed restriction on FCE.

Differences in the weight of visceral organs may partly explain some of the underlying mechanisms in the overall response to dietary factors, such as those observed in the present study. In the current study, AAB had some effect on the weight of the proventriculus and gizzard while dietary protein content had a positive effect on the weight of the pancreas under a restricted feeding regime. The increase in the weight of the pancreas in chicks that were reared on diets containing excess protein may be due to the need for increased secretion of pancreatic proteases that target protein. In a previous study, Leeson and Zubair (1997) observed an increase in the weight of the liver in chickens that were fed a high protein diet. There are controversies with regard to the effects of feed restrictions on the development of visceral organs (Picard *et al.*, 1999; Ramlah *et al.*, 1996). Generally, organs associated with nutrient derivation may not respond to poor nutrition due to the fact that such organs are preferentially developed in early life (Iji *et al.*, 2001a). In some instances, feed restriction has been observed to lead to an increase in the weights of visceral organs (Zubair and Leeson, 1994), probably as a result of birds trying to increase their potential for digestion and absorption.

There is a dearth of data on the effects of dietary factors, especially those evaluated in the current study, on intestinal morphology and digestive function. The results indicate some negative effects of feed restriction on the development of the crypt in the jejunum. The crypt is the region associated with renewal of the mucosa, both structurally and functionally (Syme and Smith, 1982; Holt *et al.*, 1985; Smith *et al.*, 1990). However, the effect of feed restriction

on the crypt did not, result in shorter villi, except at the lowest level of dietary protein 200 g CP/kg. The absence of any negative effects on the villus may be due to the fact that villus growth is regulated by both cell formation in the crypt and rate of migration and extrusion. It is not known how DFA or protein balance affects the later processes (migration and extrusion). The absence of negative effects of the UPS on mucosal protein content suggests that tissue protein synthesis or loss was not affected by the amino acid ratios. However, this result is, contradicted by the negative effect of feed restriction on mucosal protein content in the jejunum.

The effects of the dietary treatments on digestive enzyme activities appear to be dependent on the nature of the enzyme. The key enzymes assessed in the present study, maltase and sucrase, are carbohydrases and were chosen because of the relatively high levels of carbohydrates in poultry diets. The activities of sucrase and AP were negatively affected by dietary protein content. There was no significant interaction between CP and PQ for either sucrase or AP. Thus their effects were independent of each other. This finding is in contrast to research reports on the rat (Sonoyama *et al.*, 1994) where the activity of an aminopeptidase, a protease was low on low protein diets while there were no effects of protein level on the activities of sucrase and AP.

CONCLUSIONS

The overall superior growth performance of birds fed the BPS confirmed the principle that diets that supply an array of nutrients that closely parallel the broiler's nutrient requirements give rise to best performance. Feed restriction negatively influenced the development of the jejunal crypt, a region that regulates the renewal of the mucosa. The changes in the activities of some of the digestive enzymes may also explain the effects of protein quality and feed supply on body growth. The link between intestinal structure and function and biological performance is an area that warrants further investigations.

CHAPTER 7:

THE BIOLOGICAL PERFORMANCE, STRUCTURAL AND FUNCTIONAL DEVELOPMENT OF THE GASTROINTESTINAL TRACT (GIT) OF BROILER CHICKENS (10 TO 24 d) IN RESPONSE TO DIETS VARYING IN METABOLIZABLE ENERGY CONTENT.AND ENERGY TO PROTEIN RATIOS

ABSTRACT

The biological performance, structural and functional development of the gastrointestinal tract of broiler chickens (10 to 24 d) in response to diets varying in energy to protein contents (E:P ratios) was undertaken. The E: P ratios and lysine concentrations of the diets ranged from 22 to 56 MJ ME/kg protein and 9.7 to 27 g/kg respectively.

There was an indication of reduced feed intake, but improved biological performance in response to higher dietary energy concentration partly due to the availability of adequate amounts of energy for conducting the various functions related to protein deposition and metabolism.

Mucosal protein, the specific activity of maltase, sucrase and alkaline phosphatase declined with an increase in dietary metabolizable energy content from 12 to 14 MJ ME/kg. However the activity of sucrase, maltase and AP increased with increasing E: P ratios within each ME series.

The superior performance of birds fed the higher ME series and high E: P ratios suggests that when the dietary protein content is increased beyond that required to meet the amino acid requirements of a broiler, the energy content should be increased to ensure that sufficient energy is available for the efficient utilisation of dietary protein.

Keywords: energy to protein ratio, intestinal growth, efficiency, broiler.

INTRODUCTION

It is generally assumed that changes in productivity observed in broilers are moderated by nutrient metabolism at the level of the internal body tissues and by alteration to the processing of energy and nutrients, including protein. Nutrient processing by the gastrointestinal tract (GIT) determines the amount of nutrient that is available to the internal tissues for metabolism. The GIT also utilizes part of the ingested nutrients for self-renewal (Webster, 1980; Reynolds *et al.*, 1991). The efficiency of nutrient supply to internal tissues would be dependent on dietary factors, including dietary energy and protein contents.

The GIT of the chicken is anatomically complete in the embryonic phase (Moran, 1985). However it develops functionally to a peak within a few days after hatching (Nitsan *et al.*, 1991; Iji *et al.*, 2001a,b). Sulistiyanto *et al.* (1999) indicated that the intestinal villi, which are known to play a crucial role in digestion and absorption, are underdeveloped at hatching. The growth of villi is genetically dependent, and is stimulated by the presence of external factors, including dietary nutrients, to attain maximum capacity at about 10 d after hatching (Moran, 1985; Noy and Sklan, 1995). Noy and Sklan (1995) suggested that nutrient availability in chicks developed concomitantly with the growth of digestive organs and the increase in enzyme activities. The implication is that to ensure maximum growth, the utilization of balanced amounts of nutrient: energy ratios are a prerequisite to allow the GIT to reach optimum capacity during the early growth period (viz: 1-21 d) in the chick.

This investigation is part of a series of studies designed to examine the effects on biological performance, functional and structural development of the broiler chicken when fed diets with excess protein. Morris *et al.* (1999) indicated that excess protein, could impair the utilization of the first limiting amino acid and depress growth. These authors postulated that, when dietary protein content is increased to about 300 g CP/kg, which is beyond that required to meet the amino acid (i.e. AA) requirement of the broiler, the liver needs to get rid of excess incoming amino acids by subjecting them to oxidation in the tricarboxylic acid cycle after the removal of nitrogen. Consequently the activity of numerous catabolic enzymes is increased resulting in some of the limiting amino acids being lost. The objective of this study was to examine the capacity of the broiler chick to utilize diets containing excess protein but varying in ME content and to determine the response of the GIT to these dietary treatments.

MATERIALS AND METHODS

Birds and Design

Four hundred and eighty Ross commercial male broiler chicks were housed, 10 birds to a cage, in single-tiered, wire-floored brooder cages. Each cage was equipped with its own feeder and two nipple drinkers. Food and water were provided *ad lib*. Four basal diets (Table 4.1) were formulated to contain either 250 or 500 g crude protein/kg and 11.0 or 14.0 MJ AME/kg. Appropriate blending produced 12 dietary mixtures varying in E: P ratio. Four energy levels (11, 12, 13 and 14 MJ AME/kg) and three protein levels (250, 400, 500 g/kg) were used in the experiment. The experimental design was therefore a 4 x 3 factorial design, yielding 12 E:P ratios varying from 22 to 56 MJ AME/kg protein. Birds (initial body weight 208 ± 14.4 g) were randomly allocated to the multi-bird cages.

There were four replicates per treatment. The chicks were fed one of the 12 diets between 10 and 24 days of age. The food allocated to each pen of birds at the start of each week and that remaining at the end of the week was recorded, as were the weights of the chicks. At the end of the 14-d experimental period, three birds per cage were slaughtered through asphyxiation with carbon dioxide. The birds were dissected and the GIT removed. The empty weights of the proventriculus plus gizzard, duodenum, jejunum, ileum and caeca were measured, to obtain the tissue weight. These sections of the GIT were cut open then flushed with distilled water to get rid of food particles or digesta and the empty weight measured. The pancreas and liver were also weighed.

Analysis of digestive enzyme activities

Brush-border membrane vesicles (BBMV) were prepared in line with the method described by Shirazi-Beechey *et al.* (1991). The preparation of BBMV entails hypotonic shock and rupture of the cell, followed by serial centrifugation and elimination of unwanted cell fractions. The end product is devoid of contamination from the pancreas or digesta. The specific activities of brush-border membrane-bound carbohydrases, maltase (EC. 3.2.1.20) and sucrase (EC. 3.2.1.26) were measured, using a modification of the method described by Dahlqvist (1964). The incubation mixture was freshly prepared 100 mM maltose or sucrose, respectively in succinate buffer (4 mM sodium succinate, 90 mM sodium chloride, pH 6.0). Homogenates or vesicles, 25 μ l were incubated in 475 μ l of substrate-buffer for 30 minutes at

39°C. Incubation was terminated by pipetting in 5 folds of 0.2 % Triton X-100 (w/v) in 0.5 M Tris buffer, pH 7.02 at 39°C. Incubation released glucose, which was then estimated by the GOD-Perid test kit (glucose oxidase, EC. 1.1.3.4) (Roche Diagnostics, Indianapolis, USA). The amount of glucose released was determined colorimetrically at 610 nm after 30 minutes of colour development at room temperature. The activities of total protease and alkaline phosphatase (AP, EC. 3.1.3.1) were also measured according to techniques used by Holdsworth (1970) and Kopecny and Wallace (1982).

Data analysis

Data were analysed using both the general linear model (GLM) and regression analysis of Minitab (1998). The data were regressed, using varying level of ME and CP. Differences between mean values were determined by the use of least significant difference.

RESULTS

The response of the birds fed protein in excess of their requirements, in terms of feed consumption and utilization is shown in Table 7.1.

Table 7.1 *Effects of energy and excess dietary protein at varying dietary energy to protein (E:P) ratios on the biological performance of broiler chicks 10 to 24 d of age.*

Energy (MJ ME/kg)	Dietary Protein (g/kg)	E:P ratio (MJ ME/ kg protein)	Weight gain (g/day)	Feed intake (g/day)	FCE (g wt gain/kg feed)
11	500	22.0	20.7 ^d	45.4 ^c	460 ^c
	400	27.5	26.7 ^{cd}	49.9 ^{bc}	530 ^{bc}
	250	44.0	36.1a ^{bc}	61.8 ^{ab}	580 ^{ab}
		Mean	27.8	52.4	523.3
12	500	24.0	23.0 ^d	46.2 ^c	500 ^c
	400	30.0	28.6 ^{cd}	53.0 ^{bc}	540 ^{bc}
	250	48.0	38.0 ^{abc}	64.7 ^{ab}	590 ^{ab}
		Mean	29.9	54.6	543.3
13	500	26.0	26.0 ^d	48.3 ^c	540 ^{bc}
	400	32.5	32.1 ^{cd}	50.0 ^c	640 ^a
	250	52.0	43.8 ^a	65.3 ^{ab}	670 ^a
		Mean	34.0	54.5	616.7
14	500	28.0	26.3 ^d	43.2 ^c	610 ^{ab}
	400	35.0	36.3 ^{bc}	57.8 ^{bc}	630 ^{ab}
	250	56.0	37.3 ^{bc}	54.5 ^{bc}	680 ^a
		Mean	33.3	51.8	640.0
		SEM	3.70	4.44	34.0
Energy			*	NS	***
Protein			***	***	***
Energy x protein			NS	NS	NS

a, b, c - Mean values in the same column not sharing a superscript are significantly different (*P<0.05; ***P<0.001). SEM - standard error of difference between mean values. Mean values of main effects are highlighted in **bold**

The interactions between the factors for all growth responses were not significant. Variation in dietary protein level had significant ($P<0.001$) effects on feed intake, weight gain and feed conversion efficiency (FCE). Similarly, final body weight declined ($P<0.001$) with a reduction in E:P ratios but this occurred only at dietary energy levels of 12 and 13 MJ/kg. The effects of increasing protein content (i.e. decreasing E:P ratios) beyond 250 g CP/kg on body weight gain were similar over all energy levels assessed. Body weight gain also responded ($P<0.05$) positively to increasing dietary energy level between 11 and 13 MJ/kg. FCE generally increased ($P<0.001$) with increase in dietary energy content.

The weight of the proventriculus and gizzard declined ($P<0.05$) with a decrease in E:P ratios below the range 44 to 56 MJ ME/kg protein, especially at the higher dietary energy levels, 13 and 14 MJ/kg (Table 7.2).

Table 7.2 Effects of energy and excess dietary protein at varying dietary energy to protein (E:P) ratios on empty weights of visceral organs (g/100 g body weight) of chicks aged 10 to 24 d.

Energy (MJ/kg)	Dietary Protein (g/kg)	E:P ratio (MJ ME/ kg protein)							
			Gizzard ¹	Duodenum	Jejunum	Ileum	Caeca	Pancreas	Liver
11	500	22.0	3.5 ^{ab}	1.1 ^a	1.5	1.3	0.7	0.4 ^{bc}	4.5
	400	27.5	4.0 ^a	0.9 ^{ab}	1.2	1.0	0.7	0.5 ^{ab}	4.1
	250	44.0	3.7 ^a	0.8 ^b	1.2	1.0	0.5	0.4 ^{bc}	3.9
		Mean	3.7	0.9	1.3	1.1	0.6	0.4	4.2
12	500	24.0	3.7 ^a	1.0 ^{ab}	1.4	1.3	0.7	0.6 ^a	4.2
	400	30.0	3.8 ^a	0.8 ^b	1.2	0.9	0.6	0.5 ^{ab}	4.1
	250	48.0	3.9 ^a	1.0 ^{ab}	1.3	0.9	0.5	0.4 ^{bc}	3.9
		Mean	3.8	0.9	1.3	1.0	0.6	0.5	4.1
13	500	26.0	3.2 ^b	0.8 ^b	1.1	1.0	0.5	0.4 ^{bc}	3.9
	400	32.5	3.1 ^b	0.9 ^{ab}	1.2	1.1	0.6	0.4 ^{bc}	3.8
	250	52.0	3.7 ^a	0.9 ^{ab}	1.3	1.1	0.6	0.3 ^c	4.1
		Mean	3.3	0.9	1.2	1.1	0.6	0.4	3.9
14	500	28.0	3.2 ^b	1.0 ^{ab}	1.6	1.2	0.7	0.4 ^{bc}	4.4
	400	35.0	3.8 ^a	0.8 ^b	1.3	1.1	0.6	0.4 ^{bc}	4.1
	250	56.0	3.8 ^a	0.8 ^b	1.3	1.0	0.5	0.4 ^{bc}	3.8
		Mean	3.6	0.9	1.4	1.1	0.6	0.4	4.1
		SEM	0.47	0.09	0.16	0.13	0.20	0.12	0.68
Source of variation									
Energy			*	NS	NS	NS	NS	*	NS
Protein			*	**	NS	NS	NS	***	*
Energy x protein			NS	**	NS	NS	NS	NS	NS

a, b, c - Mean values in the same column not sharing a superscript are significantly different (* $P<0.05$; ** $P<0.01$; *** $P<0.001$). SEM - standard error of difference between mean values.

¹. Including the proventriculus. Mean values of main effects are highlighted in **bold**.

The weight of the proventriculus and gizzard was also influenced ($P<0.05$) by dietary energy level, tending to decline with an increase in dietary energy. There was a significant ($P<0.01$) interaction between dietary protein and energy on the weight of the duodenum. Duodenal weight increased ($P<0.01$) with decreasing E: P ratios but this was only noticeable in birds fed diets in the 11 MJ/kg series. The weights of the jejunum, ileum and caeca were not affected by variation in dietary energy or protein. The weight of the pancreas increased ($P<0.001$) as dietary E: P ratios decreased. The mucosal protein content of the jejunum was affected ($P<0.01$) by the interactions between dietary energy and protein (Table 7.3).

Table 7.3: Effects of energy and excess dietary protein at varying energy to protein (E:P) ratios on mucosal protein, brush-border membrane protein (mg/g tissue) and specific activities of membrane-bound enzymes (μ mole product/mg protein/minute) in the jejunum of chicks aged 10 to 24 d.

Energy (MJ/kg)	Dietary Protein (g/kg)	E:P ratio (MJ ME/kg protein)	Mucosal protein	Membrane protein	Maltase	Sucrase	Protease (Units)	AP
11	500	22.0	63.9 ^{ab}	1.03 ^a	10.2 ^{bc}	0.44 ^c	9.9	7.2 ^{bc}
	400	27.5	69.1 ^a	1.10 ^a	14.6 ^a	0.63 ^{abc}	10.0	9.5 ^{bc}
	250	44.0	47.9 ^c	0.79 ^{abc}	10.5 ^{bc}	0.65 ^{ab}	10.0	9.8 ^{ab}
		Mean	60.3	0.97	11.8	0.57	9.9	8.8
12	500	24.0	63.3 ^{ab}	0.99 ^a	11.1 ^{bc}	0.48 ^{bc}	9.7	7.2 ^{bc}
	400	30.0	66.5 ^{ab}	0.74 ^{abc}	14.8 ^a	0.69 ^{ab}	9.9	12.1 ^{ab}
	250	48.0	54.4 ^b	0.81 ^{abc}	12.6 ^{abc}	0.75 ^a	10.7	15.1 ^a
		Mean	61.4	0.85	12.8	0.64	10.1	11.5
13	500	26.0	55.0 ^{bc}	0.55 ^c	13.5 ^{ab}	0.43 ^c	10.0	8.1 ^{bc}
	400	32.5	73.7 ^a	0.78 ^{abc}	10.6 ^{bc}	0.67 ^{ab}	10.0	8.5 ^{bc}
	250	52.0	51.8 ^{bc}	0.65 ^{bc}	11.6 ^{abc}	0.78 ^a	10.1	10.0 ^{abc}
		Mean	60.2	0.66	11.9	0.63	10.03	8.9
14	500	28.0	51.2 ^c	0.97 ^a	9.6 ^c	0.43 ^c	9.9	6.2 ^e
	400	35.0	58.0 ^{abc}	0.88 ^{abc}	9.7 ^c	0.48 ^{bc}	9.4	6.3 ^c
	250	56.0	60.9 ^{abc}	0.88 ^{abc}	9.9 ^c	0.54 ^{bc}	9.3	7.4 ^{bc}
		Mean	56.7	0.91	9.7	0.48	9.53	6.6
		SEM	5.18	0.15	1.62	0.08	0.42	1.9
Source of variation								
Energy			NS	**	*	**	NS	**
Protein			**	NS	NS	***	NS	NS
Energy x protein			**	NS	*	***	NS	*

a, b, c - Mean values in the same column not sharing a superscript are significantly different (* $P<0.05$; ** $P<0.01$; *** $P<0.001$). SEM - standard error of difference between mean values. Mean values of main effects are highlighted in bold

At a dietary energy content of 11 MJ ME/kg, mucosal protein tended to rise with decreases in E:P ratios while the reverse was the case at a dietary energy level of 14 MJ ME/kg. The activity of maltase, sucrase and AP (but not protease) increased slightly for birds fed diets in

the 11 to 12 MJ ME series and thereafter showed a declining trend with the lowest activity being recorded on the 14 MJ ME series. The interaction between energy and protein significantly ($P<0.05$) influenced the activity of jejunal maltase. Maltase activity was lowest ($P<0.05$) in chicks on the 14 MJ ME/kg diets, regardless of the dietary E: P ratios or crude protein content. There was no impact of dietary energy on the activity of sucrase at the lowest dietary E:P ratios within each ME series. However, sucrase activity was influenced by variation in dietary energy at other protein levels. Similarly, sucrase activity varied ($P<0.001$) with dietary E: P ratios for birds fed diets between the 11 and 13 MJ ME series, and the effects of energy x protein were significant ($P<0.001$). There was also a significant ($P<0.05$) interaction between dietary protein and energy on the activity of AP in the jejunum. The activity of AP increased with increasing E: P ratios within each ME series. In general, mucosal protein, the activity of maltase, sucrase and AP declined with an increase in dietary energy content from 12 to 14 ME series but this was dependent on the dietary protein content.

DISCUSSION

Relatively large biological responses were obtained with diets with E:P ratios of between 52 and 56 MJ AME/kg protein (13 to 14 MJ/kg, at 250 g CP/kg). At present, it is recommended that a diet with 250 g protein/kg and 13 MJ ME/kg be provided for a broiler in the starter period (NRC, 1994). The ME:CP ratio of this diet is 52 MJ AME/kg protein, which suggests that the recommendation by the NRC for broilers in the starter period is close to the optimum E:P ratio to ensure maximum efficiency of utilization of dietary protein. However, as genetic selection for growth rate (leanness) continues, so the protein requirement of the bird increases (Faulkner, 1993). The poor performance of birds fed diets at the lowest E:P ratios may be due to a shortfall in energy required for protein metabolism. These findings are in agreement with reports that protein utilization will be lowered if the E:P ratio is too narrow (Morris *et al.*, 1999). In a summary of experiments in which chicks were offered *ad lib.* access to diets containing 12-13 MJ ME/kg, Morris *et al.* (1999) indicated that the energy consumption of birds fed diets in the high protein series (i.e. 280 to 300 g CP/kg) was inadequate to ensure the maximum protein deposition rate. Furthermore, the activity of numerous catabolic enzymes such as threonine-serine dehydratase in the liver is increased in animals that are fed an excess of well-balanced protein mixtures (Moundras *et al.*, 1993). This results in some of the limiting amino acids being lost and protein utilization is lowered. Moundras *et al.* (1993) indicated that the high rates of gluconeogenesis in the liver affect the supply of some amino acids to the peripheral tissues. These authors reported significantly lower growth rates for rats

fed a 600 g casein /kg protein diet; while those fed 300 g casein /kg grew normally. In both groups plasma concentration of the essential amino acids other than threonine were similar to that of rats fed diets containing 150 g casein /kg. It is possible that the undersupply of threonine to the body tissues due the high rates of gluconeogenesis in the liver might provide a possible explanation for the lower growth rate that was observed in rats fed high protein diets. It is speculated that a similar mechanism may account for the lowered growth rate in broiler chickens fed diets containing protein in excess of their requirement for growth.

The small intestine of birds exhibits considerable variation in length and probably weight (per unit body weight), depending on the type of feed the bird eats (Low and Zebrowska, 1986). Results of the present experiment show that dietary energy and protein contents did exhibit a clear effect on GIT weights. Large amounts of nutrients are used at the intestinal level for self-sustenance and renewal. These requirements have not been established for poultry but in cattle, sheep and pigs, over 20 % of the whole-body consumption of oxygen occurs at the level of the GIT (Webster, 1980; Reynolds *et al.*, 1991). Most of the energy utilized by the GIT is channelled towards protein synthesis. In recent studies, Iji *et al.* (2001a) observed large changes in villus height and mucosal cell turnover in broiler chickens between hatch and 21 days of age. These changes may be supported by large amounts of nutrients, including protein.

It is not certain why there was a reduction in the weight of pancreas with increase in dietary energy. The pancreas is a major source of digestive enzymes although pancreatic enzymes were not assessed in the current study. The intestinal enzymes that were tested generally responded to variation in dietary E:P ratios. Normal development of digestive function is stimulated by the introduction of feed (Baranyiova and Holman, 1976; Moran, 1985) and enzyme activities generally respond to the presence of target substrates. The pattern of development in broiler chickens, in response to variation in dietary energy to protein contents (i.e. E:P ratios) has not been reported. There have been more extensive studies on the effects of age on intestinal enzyme activities in other strains of broiler chickens (Uni *et al.*, 1995a,b; Iji *et al.*, 2001b). Takahashi and Akiba (1996) reported on the effects of varying dietary protein on the activities of hepatic malic enzyme and fatty acid synthetase in male broiler chicks. The activities of these enzymes were higher in chicks on a low protein diet than in chicks on high protein. The responses of digestive enzymes in the GIT may be more directly related to the concentrations of their substrates and may differ from those of the liver, which are mostly involved in synthesis or detoxification. The findings are in agreement with the

results of Iji *et al.* (2001b) in which the specific activity of maltase was found to be higher than that of other enzymes.

The overall poor performance of birds on some diets (i.e. 22 to 28 MJ ME/kg protein or 500g CP/kg) could be partly ascribed to an underdeveloped GIT development. Dietary E:P ratios within the range 22 to 32 MJ ME/kg stimulated the deposition of protein in the intestinal mucosa, whilst higher E:P ratios within the range of 44 to 52 MJ ME/kg protein resulted in a decline in the concentration of mucosal protein. Intestinal mucosal protein content is a rough estimate of cell size (Waterlow *et al.*, 1978), therefore the higher the amount of mucosal protein the greater may be the bird's digestive ability and absorptive capacity, although most of the mucosal protein may be present as structural, rather than functional protein. The exact effects of the two factors on digestive enzymes may be a better indication of their effects on digestion. In the present study, the major changes in digestive enzyme activities were due to changes in both energy and protein.

The nutritional interactions of ME and CP for mucosal protein, maltase, sucrase and AP have an impact on poultry nutrition, yet their biochemical or physiological basis are not very well known. The increase in mucosal protein with increasing energy to protein contents is important. This is because a large amount of protein is made available for mucosal maintenance and replenishment of worn out cells. The partitioning of dietary energy among the birds physiological functions is likely to be dependent upon the PQ, DFA or the amount of the protein mixture fed. An adequate dietary protein content may support mucosal growth but this is likely to be dependent on the amount of energy that is available to be expended in protein synthesis. Similar relationships may exist for the membrane bound enzymes studied. Enzymes are protein in nature and therefore it is not surprising that an increase in ME and CP leads to an increase in the activity of enzymes. Maltase and sucrase may not be necessarily responding to their substrate.

CONCLUSIONS

The effects of feeding an excess of a well balanced protein (400 and 500 g CP/kg diet) at varying energy contents on the development of the gastrointestinal tract and biological performance of broiler chickens (10-24d) were evaluated. Changes in dietary protein level significantly ($P<0.001$) influenced feed intake, body weight gains and feed conversion efficiency. Performance decreased ($P<0.001$) as dietary E:P ratios decreased (i.e. 500 g

CP/kg). The weights of some visceral organs were also affected by dietary treatment. These included a reduction ($P<0.05$) in the weight of the proventriculus and gizzard with an increase in dietary protein and energy contents. The jejunal protein content was affected ($P<0.01$) by dietary protein level but the response was dependent on dietary energy. Mucosal protein was lowest at the highest dietary E:P ratios within the 11, 12 and 13 MJ ME series. Maltase activity in the jejunum was influenced ($P<0.05$) by both dietary energy and protein, being lowest ($P<0.05$) in chicks that were fed diets containing 14 MJ ME/kg and 500 g CP/kg. An increase in dietary E:P ratio resulted in an increase in the activity of sucrase and AP for birds fed diets in the 11, 12 and 13 MJ ME series. Overall, these findings suggest that the differences in biological performance of chicks fed diets varying in energy and protein contents may be due to a lack of energy for metabolic functions. The higher the amount of mucosal protein the greater may be the bird's digestive ability and absorptive capacity. There may also be changes in intestinal growth and function but these would require further evaluations. Determining the basis of nutritional interactions will offer the best chance of identifying the factors involved and developing practical approaches to exploiting them if they are beneficial. This will enable new nutritional approaches to increasing the efficiency of protein utilisation in broiler chickens.

CHAPTER 8:

GENERAL DISCUSSION:

A large amount of work has been reported on dietary energy and protein requirements to maximize the biological performance of broiler chickens in terms of weight gain and food conversion efficiency (Jackson *et al.*, 1982; Leeson *et al.*, 1996). There is also an enormous quantity of work showing the influence of dietary protein and energy levels on the composition of the broiler carcass, since Fraps (1943) showed that the manipulation of dietary protein affects broiler performance. For a broiler to achieve its 'potential' growth rate, it must consume sufficient energy for the efficient utilisation of the protein that is available. Without sufficient energy intake, potential protein growth will not be obtained. The concept of an ideal E:P ratio becomes important not only at high E:P ratios but also at low E:P ratios (i.e. when excess protein is fed). High protein diets are fed to meet minimum requirements for essential amino acids (EAA's) when poor quality protein sources are abundant and synthetic amino acids are unavailable or the cost is prohibitive. It is also desirable to feed higher-than-normal protein contents to offset the decreased protein intake related to heat induced anorexia.

Vegetable protein sources, when fed at high concentrations, lead to sub-optimal bird performance (Morris *et al.*, 1999). As the protein resource base becomes more limiting, there needs to be an evaluation of how the limited protein feedstuffs can be used to complement each other in order to maximize the efficiency of protein utilisation. The looming shortages of good protein sources that have become prevalent in certain parts of Africa also poses serious problems with regard to the efficient utilisation of the available protein resources. There is a paucity of information on the effects of consuming diets containing protein in excess of the broiler chickens needs on the efficiency of protein utilisation. An important question raised by these observations is why, when dietary protein content is increased beyond that required to meet the AA requirement of a broiler, the efficiency of protein utilisation is suboptimal. It must be noted that excess intake of protein will not increase protein retention if it is already at a maximum.

With this in mind a series of experiments were conducted to determine responses to diets containing excess dietary protein. A number of dietary factors (i.e. protein balance, daily food allocation, E:P ratio) were combined and some important traits were measured, both at

the whole animal level (i.e. biological performance and efficiency of protein utilisation) as well as at the organ level (some digestive tract characteristics). The first part of the following discussion focuses on a series of four studies conducted to determine the responses to diets containing excess protein and varying in E: P ratios, daily food allocation and protein balance. The responses were determined for two age ranges viz: 10 to 24 and 23 to 37 d. The second part of the discussion focuses on two studies on the structural and functional development of the gastrointestinal tract (GIT) of broiler chickens in response to dietary treatments.

Protein balance

(a) Effects of dietary treatments on biological performance and carcass composition:

The results of the first two studies showed that the utilization of large quantities of poor quality protein did not support maximum weight gain (ADG) nor food conversion efficiency (FCE) at any level of protein in excess of the birds requirement, despite there being similar protein contents in the unbalanced and balanced diets. It was observed that the intakes of diets containing AA's in excess of the bird's requirement presented a distinct disadvantage in the promotion of efficient utilization of protein by broiler chickens. This is because excess dietary protein is deaminated and a portion excreted as urea. This process has an energy cost associated with it (van Lunen and Cole, 1996) as well as an increased demand for choline methyl groups to support uric acid synthesis. The overall effect is a decreased growth rate and efficiency of protein utilisation. Thus in order to maximise PR and minimise LR, diets must be formulated in which there is an appropriate balance between protein and energy contents. These studies also illustrate that in practical least cost diet formulation, attention should be paid to the surplus intact amino acids (AA's) absorbed from the intestinal tract after the ingestion of a diet. The main effects of each protein series showed that the BPS enhanced performance in comparison to the UPS, regardless of the growth period. This confirmed the principle that diets supplying an array of nutrients that closely parallel the bird's nutrient requirements give rise to a better biological performance.

Morris *et al.* (1999) had suggested that in order to maximize growth and feed efficiency, the proportion of the critical AA's lysine (lys), tryptophan and methionine should be at least 0.057, 0.012 and 0.025, respectively, of the dietary crude protein concentration. Supplementation of diets whose lys/CP ratio was below the 0.057 recommended, with a synthetic lysine would ensure that birds fed these diets would maximize their growth

performances. However this was outside the scope of the series of experiments reported in this thesis.

If only one dietary protein source were available, this would have to be included at more than the minimum level to meet the amino acid requirements. Consequently the diet on offer would be imbalanced. Assuming the levels of vitamins and minerals are adequate, chick performance would still be sub-optimal. Apart from the AA supplementation of such high protein diets, the experimental evidence points to the fact that maximum growth performance may be obtained with increases in the dietary energy content to ensure that sufficient utilization of the excess dietary protein occurs.

Daily food allocation

(a) Effects on biological performance

It is evident from these studies that daily food allocation can have important effects on biological performance. The amount of feed allocated showed a close association with the rate of growth and thus also with the rate of protein deposition when diets with excess protein were fed. Restriction at 0.8 and 0.6 of *ad lib.* reduced biological performance (i.e. weight gain and FCE). For instance weight gain for birds fed the BPS and the UPS were reduced by 0.32 and 0.43, respectively, when daily food allocation was reduced to 0.75 of *ad lib.* intake. The negative effects of restricted feed intake on body weight gain was observed for both growth periods. The impairment of the growth rate due to feed restriction has been attributed to reductions in thyroxine, insulin-like growth factors (IGF) and growth hormone (Gonzales *et al.*, 1998). Another conceivable explanation for the reduced performance is simply that a lack of food hinders growth.

(b) Effects on carcass composition

The results of the first two trials showed that birds allocated food at a lower proportion of the previous *ad lib.* intake (i.e. 0.8, 0.75, 0.6 x *ad lib.*) were leaner at the end of the restrictive period (they contained more protein in relation to fat). Birds aged 23 to 37 days and fed diets allocated at *ad lib.*, 0.8 of *ad lib.* and 0.6 of *ad lib.* intake in the balanced protein series retained less energy (kJ/d) as lipid (viz: 341, 219 and 109) in comparison to those fed the unbalanced protein mixtures. However, birds fed diets in the unbalanced protein series retained less energy (kJ/d) as protein (viz: 173, 133 and 106) in comparison to those fed the balanced protein mixtures (viz: 226, 177 and 145) at the three levels of feed allocation. Thus at a low

level of food allocation, birds utilised almost all the energy available for protein synthesis, with very little energy being retained as fat. When birds are offered diets that provide adequate amounts of nutrients, they retain fat and protein according to their genetic potential. This enables them to achieve a body composition that is characteristic of their age and weight. Thus a period of restrictive feeding in which there is an inadequate supply of nutrients, influences body composition. The composition of growth was found to be dependent on the dietary protein balance, daily food allocation and energy to protein ratio. Increasing the level of daily feed allocation, and therefore energy intake increased the rate of LR. –birds aged 23 to 37 days of age fed diets in the BPS and UPS at the three levels of DFA increased their LR by 50 and 36%, 38 and 40% respectively as food allocation was increased from 0.6 to 0.8 and 0.8 to *ad lib* food intake. Birds severely restricted (0.6 of *ad lib*.) continued to gain protein but at the expense of LR. Regardless of dietary protein quality, decreasing daily feed allocation to 0.6 of the previous *ad lib*. intake, resulted in a reduction in LR.

Energy to protein (E:P) ratio

In the first study in this thesis the model of Kyriazakis and Emmans (1992 a, b), relating e_p to dietary E: P ratio, was confirmed in broiler chickens. The relationship is important and can be used in (i) predicting the growth of carcass components of protein and fat and (ii) the formulation of cost-effective diets that maximize the potential protein growth of the bird. The results of the first two trials indicated that there was a reduction in performance when high levels of protein were fed, so subsequent experiments were designed to find out the cause of this problem. To test whether there was insufficient energy in the feed a range of diets varying in E: P ratio- all with high CP contents, were fed to broilers and the biological performance and efficiency of protein utilisation were measured. The results of these trials leave little room for doubt that inadequate amounts of energy depressed growth and e_p . The E: P ratios and lysine concentrations of the diets ranged from 22 to 56 MJ ME/kg protein and 9.7 to 27 g/kg respectively. The dietary energy levels ranged from 11 to 14 MJ ME at excess dietary crude protein contents ranging from 250 to 500 g CP/kg.

(a) Effects on biological performance

The high-energy diets reduced feed intake, improved ADG, FCE and protein retention (PR). The improved FCE can be ascribed to a reduced food intake. For instance, food intakes and ADG of birds fed 11, 12, 13 and 14 MJ ME series at 10 to 24 d of age were 54.90, 54.48,

54.31, 53.01 and 30.51, 30.87, 33.66, 34.55 respectively. Figure 8.1 illustrates the improved average daily weight gain with increasing E: P ratios for birds fed diets containing excess protein.

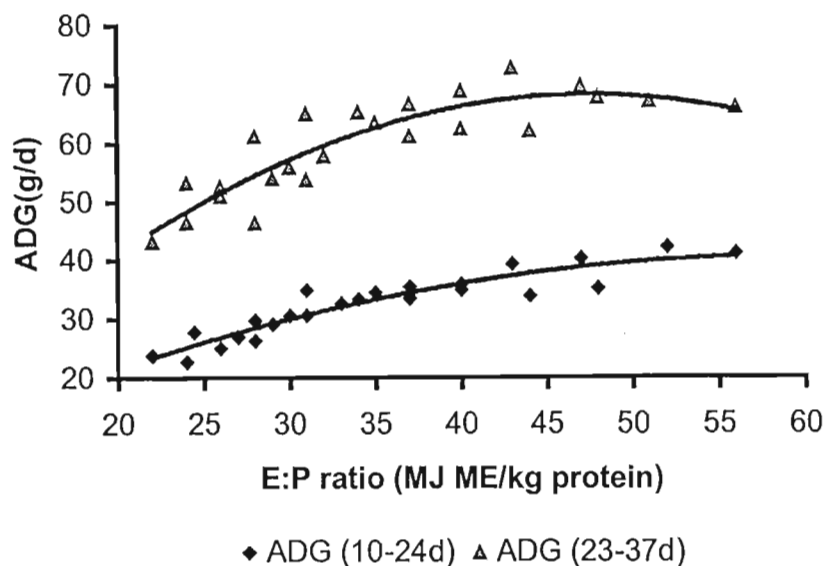


Figure 8.1 The effect of E:P ratio on average daily weight gain (ADG, g/d) of chicks in the starter (10 to 24 d) and finisher (23 to 37 d) periods of growth

The results showed that the broiler chicken is not tolerant of a low E:P ratio in both growth periods. The poorer performance observed for birds fed diets in the lower E: P ratios suggests that when the dietary protein content is increased beyond that required to meet the amino acid requirement of a broiler, the energy content should also be increased to ensure that sufficient energy is available for the efficient utilisation of dietary protein.

(b) Effects on carcass composition

Birds fed diets of high E: P ratios directed more of the excess energy into lipid deposition (LR) and heat production (H), whilst PR was higher than for birds fed low E: P ratios within the range 22-31 MJ ME/kg protein. With the exception of the beneficial effect of a reduced LR, negative effects on performance were observed when diets containing extremely high protein levels (i.e. 450 to 500 g CP/kg or low E: P ratios) were fed. The data indicate that low energy diets were associated with a lower LR and this effect persisted regardless of the age of the bird. The reduction in carcass fat suggests that dietary energy was utilised for the catabolism of excess protein instead of being deposited as fat. The catabolism of excess protein instead of it being used for PR implies that excess protein was used less efficiently for

PR at these low E:P ratios in the BPS. The reduced LR was associated with a smaller sized bird, and a reduced rate and efficiency of liveweight gain (i.e. reduced FCE). It is suggested that the feeding of high protein diets presents a means of modifying broiler carcass quality but an appraisal (i.e. biological, physiological, economic and environmental) needs to be carried out before adopting such a proposition. Caution must also be exercised when feeding high protein diets since these diets have been reported to increase nitrogen excretion and may be metabolically demanding to the bird as it attempts to accommodate excessive protein degradation (Wiseman, 2001).

For birds (23 to 37d) fed diets in the UPS, PR declined with increasing E: P ratio (or decreasing lysine content) while energy retention increased. Conversely PR, for birds fed BPS diets, increased with increasing E: P ratios (increasing lysine contents). The data from the series of studies indicated that the composition of growth is dependent on dietary protein balance, energy contents, the daily food allocation and E: P ratios.

The net efficiency of protein utilisation above maintenance

The series of studies in this thesis also examined the influence of (1) protein quality, E: P ratio and daily food allocation and, (2) different metabolizable energy contents on the efficiency of protein utilisation. A knowledge of the relationship between the efficiency of protein utilisation (e_p) and the dietary E: P ratio is essential to accurately predict the growth response of broiler chickens to a change in nutrient input (energy to protein content).

(a) The effect of dietary ME content and protein balance

The poorer performance observed for birds fed the BPS diets in the lower energy to protein (E: P) ratios suggests that when the dietary protein content is increased beyond that required to meet the AA requirements of a broiler, the energy content should also be increased to ensure that sufficient energy is available for the efficient utilisation of dietary protein. Thus when the dietary protein supply is equal to or above requirement, the efficiency of protein utilisation is influenced by the level of energy intake. Growth rate and e_p exhibited an improvement to increases in dietary ME over the range tested. For instance weight gain, FCE and e_p for broiler chickens in the growth period 10 to 24 d improved by 1.2, 8.3 and 2.6%; 1.6, 8.7 and 5.0%; 2.2, 8.2 and 5.8% respectively, as dietary energy levels were increased from 11 to 12, 12 to 13 and 13 to 14 MJ ME/kg respectively. With the exception of e_p there were no significant interactions between dietary protein and energy levels. Broiler chickens

fed on lowest energy diet (11 MJ ME/kg) could not compensate for the lower dietary energy content by increasing their food intake sufficiently, and therefore the supply of dietary energy in relation to available protein was inferior to that of birds fed the high energy diets (14 MJ ME/kg). This may account for the lower e_p of 0.44 for birds (10 to 24 days of age) fed diets of the lowest dietary energy contents in comparison to 0.52 for birds fed the 14 MJ ME/kg diets (Figure 8.2.).

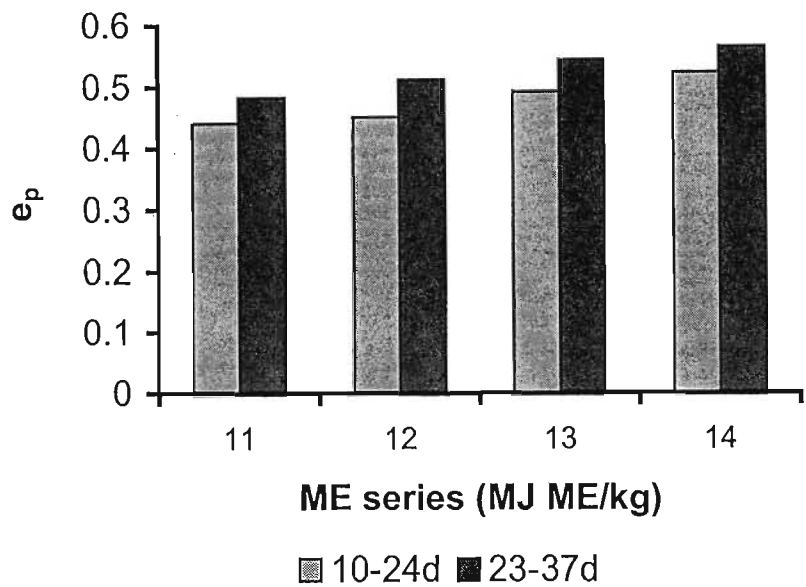


Figure 8.2 *Effect of dietary ME series on the efficiency of protein utilisation (e_p) in broiler chickens for the periods 10 to 24 and 23 to 37 days of age.*

The energy contents in the feeds may thus have been insufficient to allow the birds to grow at their potential. Birds on the lowest dietary energy intakes (11 MJ ME/kg), thus consumed insufficient energy to meet the amount required for the full utilisation of protein, PR was therefore lower (6.41 g/d) than the mean maximum value (6.94 g/d) that was achieved by birds offered diets of a higher energy content (14 MJ ME/kg). It must be noted that the effect of energy level was studied with diets that contained adequate protein (250 to 500 g CP/kg) that met or exceeded the requirements of the broiler chicken. When broiler chickens are fed diets within this range, the basic parameter, which determines the biological performance (ADG, FCE) and the efficiency of protein utilisation, is feed (energy) intake. As dietary ME increases, feed intake declines but energy intake increases thus increasing ADG and e_p . There were also interactions between dietary protein content (lysine) and energy content; the high lysine diets gave the highest ADG. Traditionally broiler chickens are offered high-energy

diets, because of these improvements. However, it is important to control the amount of energy intake because it also has potentially negative effects on carcass characteristics (Jackson *et al.*, 1982). These effects are of direct relevance for broiler chicken production. It is therefore worthwhile to consider the cost effectiveness in terms of feed cost per kilogram of broiler meat produced before any beneficial relationship between energy and protein can be applied profitably in attempts to improve growth by nutritional manipulations.

Several other factors apart from dietary energy are required to support maximal growth. Among these factors are DFA, AA availability (the proportion of the AA supply that is fully utilised for the synthesis of protein at the cellular level) and balance. The response to increasing energy content tended to be lower with birds fed protein mixtures of poor quality (UPS) than with well-balanced protein mixtures (BPS). The minimum lysine: CP ratio for broiler chickens fed diets in the BPS (0.061 to 0.062) were greater than that recommended by Morris *et al.* (1999), but the ratios for birds fed the UPS diets (0.015 to 0.031) were lower than the minimum requirement of 0.058 recommended. This accounted for the growth depression of birds fed UPS diets. An increase in dietary lysine content together with dietary energy to protein contents in balanced protein mixtures resulted in an improvement in bird performance.

A considerable body of experimental evidence has been presented to show that a large proportion of ingested protein for birds fed the UPS was not deposited but was utilised for purposes other than PR, resulting in poor biological performance. Consequently, e_p declined in birds fed UPS diets. The lowering of e_p could be partly due to:

- (i) the preferential oxidation of AA's to meet metabolic energy needs at limiting dietary energy intakes or at extremely limiting protein free energy intakes during which AA's are oxidized to provide energy and substrate for a minimum LR per unit of PR (Gerrits, 1996).
- (ii) AA imbalance and the consequent oxidation of AA's that are present in excess of that required for the bird to attain its maximum possible growth rate. To support this statement, Mc Donald *et al.* (1991) underlined that the synthesis of protein begins with an amino acid, with successive amino acids being added to form a polypeptide chain. There is therefore no protein synthesis if balanced amounts of amino acids are not present at the right time for protein synthesis. The AA's present in excess of the

requirements for protein tissue synthesis are removed and catabolised resulting in a wastage of amino acids and reduction of e_p .

- (iii) The use of AA's in non-protein deposition pathways. For example the precursor AA, lysine may be utilised in such a pathway resulting in the production of carnitine, which is not recycled back into protein metabolism. It is expected that when these metabolites are provided in the diet, this will spare the need for the AA from which they are derived and improve the efficiency of protein utilisation. For example, Scott *et al.* (1976) reported a growth improvement from the addition of creatine to chick diets that were deficient in arginine.

In general the efficiency of utilisation is less for protein mixtures than for synthetic AA's due to heat losses attributable to energy used in digestion. In practical situations where unbalanced protein sources are abundant and inexpensive (i.e. copra cake, palm kernel meal, groundnut oil cake), a lower dietary E: P ratio than that required for a fully balanced diet would be desirable. This will however result in a lowered protein utilisation.

The main effects of protein quality (balanced vs unbalanced) and feed allocated at various proportions of *ad lib.* intakes are illustrated in Figure 8.3. Birds fed balanced diets had a better efficiency of protein utilisation in comparison to bird's fed unbalanced diets. Protein efficiency of birds offered unrestricted access to starter diets in the BPS was not significantly different from that of birds fed 0.6 of the proportion of the *ad lib.* intake. Birds allocated 0.75 of the *ad lib.* intake in the growth period 10 to 24 d had a lower e_p than birds (23 to 37d) fed diets in the BPS at 0.8 of the *ad lib.* intake. The older birds may be less sensitive to the effects of severe feed restriction when fed excesses of protein. Apart from them having a lower requirement, older birds may have relatively greater protein reserves with which to buffer the effects of poor dietary AA patterns (Boorman, 1980).

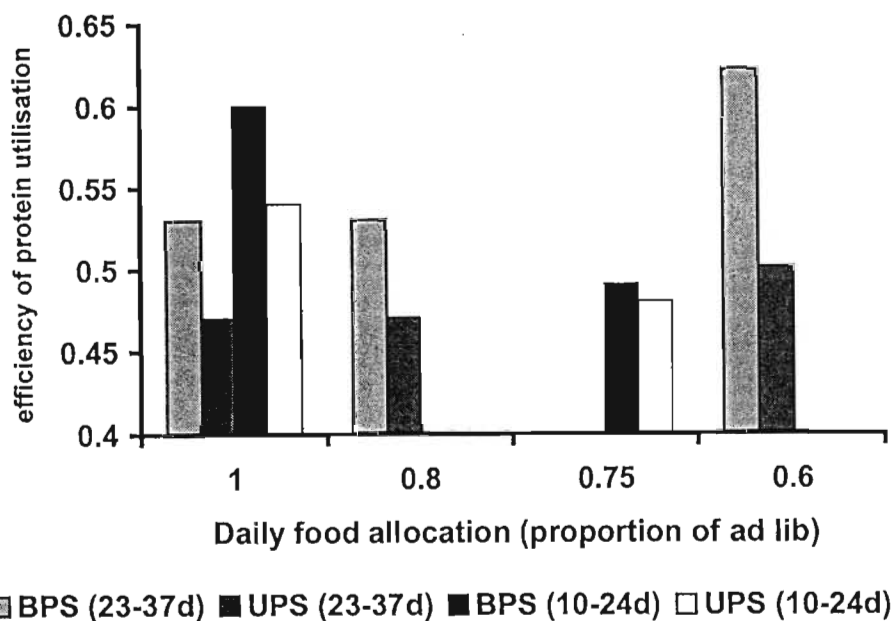


Figure 8.3. *Effect of daily feed allocation on the efficiency of protein utilisation in broiler chickens from 10 to 24 d and 23 to 37days of age fed the balanced (BPS) and unbalanced (UPS) protein diets*

(b) *The effect of dietary E:P ratio on e_p*

The relationship between e_p and E: P ratio for broiler chickens aged 10 to 24 days was best described by a linear model of the form:

$$e_p = 0.01309 (\pm 0.00015) \times \text{E: P ratio} \text{ (Adjusted } R^2 = 0.749 \pm 0.0604).$$

The e_p decreased gradually with increasing excesses of dietary protein or decreasing E:P ratio and improved with increasing dietary energy content. The efficiency of protein utilisation exhibited an improvement to increases in dietary ME for birds in both growth periods. When the data for all growth periods were combined (10 to 24 and 23 to 37 days of age), the relationship is adequately represented by the following equation:

$$e_p = 0.013 (\pm 0.0002) \times \text{E:P}, \text{ (Adjusted } R^2 = 0.7298).$$

The relationship between dietary E:P ratios and the efficiency of protein utilisation (e_p) for birds fed diets varying in ME contents in the two growth periods is shown in Figure 8.4.

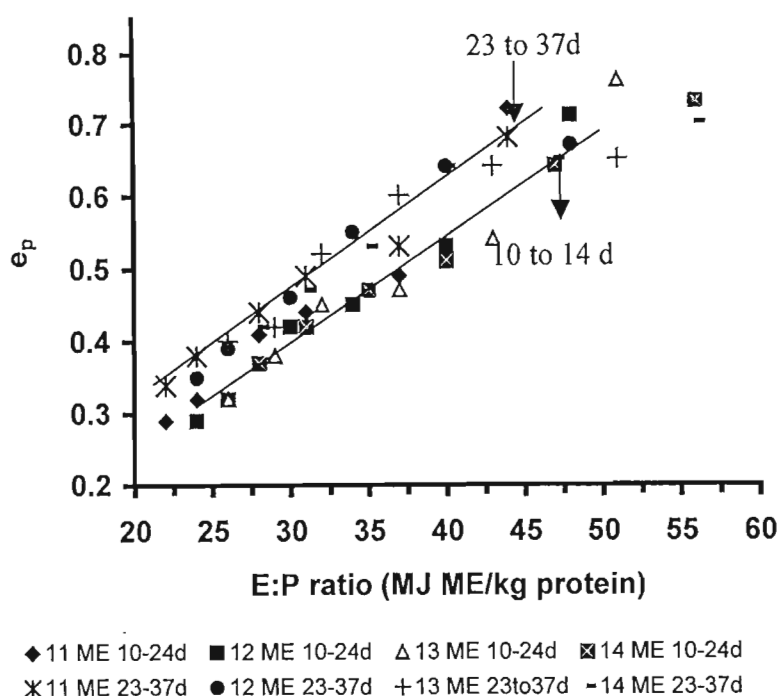


Figure 8.4 The relationship between dietary E:P ratios and the efficiency of protein utilisation (e_p) for birds fed diets varying in ME contents in the two growth periods.

There were no significant differences between slopes for broilers fed the 11 and 12 MJ ME diets for the two growth periods (10 to 24 d and 23 to 37 d). However the age of the bird significantly affected the slopes for birds fed the 13 and 14 MJ ME diets. The results show that, in the case of the overall comparison between the slopes for both growth periods, the age of the bird significantly affected the slope.

There were no significant differences between the slopes for the relationship between e_p and E:P ratio for birds fed the BPS and the UPS. The equation describing the overall relationship (combined data for all E:P ratios in the BPS and UPS) between E: P and e_p is:

$$e_p = 0.012009 (\pm 0.00029) \text{ E: P.}$$

For birds aged 23-37d fed diets in the BPS, increasing the dietary E: P ratios (reducing protein contents) in isoenergetic diets increased e_p . In spite of the similar protein intakes of birds fed diets in the UPS and BPS (31.4 versus 30.8 g/d), birds fed diets in the UPS at *ad lib.* intake recorded significantly ($p < 0.01$) lower protein gains (9.4 versus 12.3 g/d) than those fed the BPS. The combined slope for birds fed the BPS and the UPS were 0.0121 ± 0.001101 and

0.0106 $\pm$ 0.00049 respectively. This slope was not significantly different from that for birds fed the BPS at *ad lib.* and 0.8 of the *ad lib.* intake. The slope for birds (23-37d) fed the BPS at 0.6 of *ad lib.* intake was significantly greater ($P < 0.05$) than that for birds fed diets in the UPS. The proportion of total protein digested, which can be incorporated into body protein 'v' for the UPS, was low (i.e. 0.68 to 0.57) for E: P ratios in the range 46.3 to 65 MJ ME/kg protein. Birds fed diets in the UPS require less dietary energy than those offered BPS in order to maximize protein growth.

Birds restrictively fed had a limited energy intake and efficiently utilised any protein ingested resulting in a larger value for the slope regardless of PQ. The e_p for birds fed diets in the BPS and the UPS, with similar AA contents of 12.5 and 12.1 g lysine/kg at 32.5 MJ ME/kg protein was 0.38 and 0.32, respectively. The similarity in e_p implies that the content of the limiting AA exerts a similar effect on e_p irrespective of the balance of AA.

The equations obtained in these experiments can be used to estimate the efficiencies of protein use for broilers when fed diets of a given E: P ratio. These efficiency estimates are valuable for the development of simulation models to better predict the performance of broiler chickens. Consequently, this calls for more research into identifying pathways contributing to the inefficiency with an object of improving e_p . It must be emphasized that these equations should be used with caution (1) when substituting average E:P values outside the range used in these series of experiments (2) for flocks of birds reared under environmental conditions that are markedly different from those encountered in these experiments (3) for flocks fed diets in which the AA concentrations differ markedly from those used in this series of experiments.

Practical implications of results in feeding broilers:

In commercial practice a 200-g protein /kg and a 13.4 MJ ME/kg diet is recommended for broilers in the finisher period (3 to 6wks of age) yielding a ratio of 66.9 MJ ME/kg protein (NRC, 1994). The E: P ratios for the birds fed the BPS suggest that these recommendations for broilers fed *ad lib.* in the finisher period are close to the optimum for lean tissue accretion. The calculated value of 66.9 MJ ME/kg protein for commercially fed birds in the finisher period was higher than the average of 52.39 MJ ME/kg protein determined in the second study. The inflection point (i.e. 52.39 MJ ME/kg protein) is however closer to that determined for the starter stage of commercially reared broiler chickens between 0 to 3 weeks

of age fed a 230 g/kg protein diet at 13.38 MJ ME/kg protein giving rise to an E: P ratio of 58.17 MJ ME/kg protein. The calculated value of 58.17 MJ ME/kg protein for commercially fed birds in the starter period was slightly lower than the average of 61.08 MJ ME/kg protein determined for birds aged 10 to 24 d. There is thus the possibility of increasing the dietary energy content further by a small margin to take advantage of further improvements in nutrient utilisation for the starter period.

The availability of raw materials for the feed industry depends on quantities available after competing industries, that pay a premium price (i.e. human consumption), have been satisfied. In situations where the imports of protein sources is not adequate to meet the shortfall in local requirements, the price of protein raw ingredients will be very high relative to energy sources. In general, cereal products (i.e. maize) are over-produced and some exported while some oilseeds are under produced and imported leading to exorbitant prices. Consequently, diets of a higher E: P ratio (i.e. low CP, high ME content) are compounded for feeding broiler chickens. This results in birds being fatter than desirable because of the extra dietary energy intake. Lowering the dietary crude protein content results in fat deposition even if the total essential amino acids (TEAA) are minimised. Fat deposition is undesirable in terms of energy costs, consumer health concerns and carcass quality (i.e. partitioning and further processing). This situation is commonly observed in commercial practice with broilers processed at a market age of between 37 to 42 days. Unless agricultural production of alternative protein sources is increased and less reliance placed on the imports of protein seed products, chickens will continue to be fed low dietary protein diets since this is the most profitable option if the broiler industry is to survive in South Africa.

With the above scenarios, feeding programmes are based on variations in nutrient density, quality and the local economic conditions. In contrast, specialized markets with a prime consideration for a lean product place much more emphasis on carcass composition or quality. Results from the current studies show that this may be attainable by moderately increasing dietary protein (AA) levels whilst maintaining the standard ME levels. At higher dietary CP levels, the ME level needs also to be increased to maintain an adequate balance between dietary CP contents and ME. Mild feed restriction or lighting programmes that reduce feed intake may also be suitable to attain the desired carcass characteristics.

The structural and functional development of the gastrointestinal tract (GIT) of broiler chickens (10 to 14 d) in response to diets containing excess dietary protein.

It is expected that the efficiency of nutrient utilisation could be increased by nutrient manipulations that increase gastrointestinal (GIT) development and enzyme activity. Iji *et al.* (2001a, b) indicated that the digestive capacity of birds might be increased by an increase in total enzyme activity that is facilitated by an increased GIT surface area. Protein retention in broiler chickens is a process that requires a large amount of energy and is to some extent dependent on bird-related factors such as the development of the GIT. Apart from the commonly assessed effects of dietary energy to protein contents or E:P ratio on the biological performance of broiler chickens, the study of causal connections at the GIT/organ level has been largely ignored or underestimated. Measures aimed at improving the efficiency of nutrient utilisation would depend on an understanding of the role of nutritional, genetic and environmental factors in the partitioning of nutrients towards various physiological processes. Therefore, studies on the GIT and enzyme response to dietary treatments containing excess protein were conducted using birds between 10-24d of age.

(a) Development of the gastrointestinal tract and biological performance

The effects of consuming excess protein on intestinal function are not well understood. It is generally assumed that the changes in productivity observed are moderated by nutrient metabolism at the internal body tissues and alteration to the processing of energy in relation to other nutrients, including protein. However, nutrient processing by the gastrointestinal tract (GIT) determines the amount of nutrient that is available to the internal tissues for metabolism. It is suggested that nutrient availability improves with the growth of digestive organs and the increase in enzyme activities. Consequently, optimising growth will require the utilization of balanced amounts of energy to nutrient ratios to allow the GIT to reach its optimum capacity.

The overall poor performance of birds on diets containing different energy and protein levels could be partly ascribed to the lack of energy for conducting the various functions related to protein digestion and metabolism and an underdeveloped GIT. The poor performance of birds could also be due to a lack of intake of adequate amounts of nutrients to meet the needs for cell renewal, growth of the GIT and the other cells of the body. This is substantiated by the results that mucosal and membrane protein rose with an increase in dietary ME intake. It

must be noted that intestinal mucosal protein content is a rough estimate of cell size. Cell size is approximately the amount of tissue protein in relation to tissue DNA content (Waterlow *et al.*, 1978). Dietary protein contents of up to 400 g/kg also stimulated the deposition of protein in the intestinal mucosa as ME levels increased. Beyond 400 g CP/kg diet the concentration of mucosal protein declined. Thus, if the protein content of the feed is increased beyond that required to meet the amino acid requirement of the broiler, the energy content should likewise be increased so as ensure that there is sufficient energy available for the efficient utilization of dietary protein. Experimental evidence shows that the higher the amount of mucosal protein the greater will be the bird's digestive function and absorptive capacity. This is in spite of the fact that most of the mucosal protein may be present as structural, rather than functional protein.

(b) Visceral organ weights and intestinal mucosal morphometry

In broiler chickens the development of the GIT is most rapid within the first 10 days of life (Iji *et al.*, 2001a). The data indicates that the small intestine of birds exhibits considerable variation in length and probably weight (per unit body weight), depending on the amount and type of dietary nutrients ingested. The present findings show that dietary energy and protein contents did exhibit an effect on GIT weights. Intestinal development seemed to be greater for birds that were fed diets in the higher ME series or E: P ratios (44 and 56 MJ ME/kg protein). This is probably due to the large amounts of nutrients used at the intestinal level for self-sustenance and renewal. However, most of the energy utilized by the GIT is channelled towards protein synthesis. The large changes in villus height and mucosal cell turnover in broiler chickens needs to be supported by intakes of large amounts of balanced quantities of protein mixtures and increasing amounts of dietary energy.

The results of this study showed that the response to nutrient intake differs between organs and the type of nutrients in the diets on offer to broiler chickens. Thus, organs involved in the digestion and absorption of nutrients (i.e. intestines) showed a comparatively larger increase in weight with increasing energy intake. It remains uncertain why there was a reduction in the weight of pancreas with an increase in dietary energy. The pancreas is a major site of digestive enzyme synthesis although pancreatic enzymes were not assessed in the current study. Pancreatic weight increased with an increase in dietary protein level for birds fed restricted amounts of BPS. The increase in the weight of the pancreas in chicks that were reared on high protein diets may be due to the need for increased secretion of pancreatic

proteases that target protein. There are controversies with regard to the effects of feed restrictions on the development of visceral organs. Organs associated with nutrient derivation may not respond to poor nutrition due to the fact that such organs are preferentially developed in early life. In some instances, feed restriction was observed to lead to an increase in the weights of visceral organs. This is probably as a result of birds trying to increase their potential for digestion and absorption.

The crypt depth of chicks on *ad lib.* feeding regime was higher on the BPS than on the UPS. The results indicate a negative effect of feed restriction on the development of the crypt in the jejunum, the region associated with renewal of the mucosa, both structurally and functionally. The effect of feed restriction on the crypt did not, however, result into shorter villi, except at the lowest level of dietary protein. The protein content of the jejunal mucosa was higher for birds fed *ad lib.* on UPS than on the BPS. Feeding level also had a significant effect on jejunal protein content in birds that received the BPS, this being reduced in birds on restricted feeding. This investigation thus provides evidence linking the effects of dietary nutrients and especially that of dietary protein quality/level and daily food allocation to the development of the GIT. The lack of energy for conducting the various functions related to protein metabolism and deposition may also be partly responsible for the poor biological performance of birds fed diets of a lower energy to protein content. In the present study low E:P ratios resulted in low weights of the GIT, and increase in maltase and sucrase activity, which were directly related to an increase in the biological performance (ADG, FCE).

(c) Activities of digestive enzymes

The key enzymes assessed in the present study were maltase and sucrase. The total protease assessed represents a large group of enzymes. This makes it very difficult to accurately define its response to dietary treatments. The exact physiological role of AP is also unknown but the enzyme is commonly used as a marker of brush border membranes (Iji *et al.*, 2001b). The effects of the dietary treatments on digestive enzyme activities appear to be dependent on the nature of the enzyme. The intestinal enzymes that were tested responded to variation in dietary energy at excess protein contents. The response was complex and dependent on the dietary E:P ratio. For example at 11 and 12 ME MJ/kg maltase activity increased with the crude protein content increasing from 200 to 400 g CP/kg. Whereas at 14 MJ ME/kg, maltase activity decreased gradually as CP increased from 250 to 500 g CP/kg. Digestive function is known to be stimulated by the introduction of feed, with enzyme activity responding to the

presence of target substrates. The pattern of development of the GIT in broiler chickens, in response to variation in dietary energy at excess protein contents, is not clear. In this study the activities of these enzymes were higher in chicks fed the 200 g CP/kg diet than in chicks on the higher protein diets at the same dietary energy content. The responses of digestive enzymes in the GIT may be more directly related to the concentrations of their substrates and may differ from those of the liver, which are mostly involved in synthesis or detoxification. Poultry lack amylase in the saliva (Low and Zebrowska, 1986), thus the intestinal carbohydrases exert a significant effect on the digestion of sugars. This is in agreement with the results of Iji *et al.* (2001b) who reported a generally high activity of disaccharides (i.e. maltase).

Maltase activity in the jejunum was influenced by dietary energy, but this was dependent on the protein content. Maltase and sucrase activities were significantly lower in chicks offered *ad lib.* access to UPS. An increase in dietary energy content led to a decline in the activity of alkaline phosphatase (AP), in chicks raised on the 400 g CP/kg diets. At high excess dietary crude protein (CP) content, protein quality affected the specific activity of alkaline phosphatase; this tending to be lower in chickens on the UPS than in those fed the BPS. The activities of sucrase and AP were dependent on dietary energy. In the rat, the activity of aminopeptidase, a protease was low on low protein diets while there were no effects of protein level on the activities of sucrase and AP (Sonoyama, *et al.*, 1994). In the present study, the major changes in digestive enzyme activities were due to a significant interaction with protein. This effect may be due to the fact that both maltase and sucrase are involved in carbohydrate digestion. This work indicates that the differences in performance of chicks on diets varying in energy at excess protein contents may be traceable to the lack of energy for carrying out various functions related to protein digestion and metabolism. There may also be changes in intestinal growth and function but these would require further evaluations. More quantitative work on nutrition and metabolism of the broiler chicken will need to place some emphasis on the GIT's responses to nutritional manipulations.

Indicators from the series of investigations are that it is essential to pay attention to biological, economic as well as physiological requirements of the broiler chicken in assessing the efficiency with which dietary nutrients are utilized for growth. The reasons being that formulated diets that may be nutritionally adequate for broilers at a particular age may not always be physiologically adequate or economically cost-effective and vice versa. Further work should have as its goal quantitative estimates of the biological and physiological

responses of broiler chickens to diets varying in E: P ratios and under varied environmental regimes. With this information it may be possible to predict the performance of a broiler chicken in a particular environment. Several factors and interactions between factors affecting e_p are known to exist. The observation of these multifactorial aspects makes it necessary to combine results of various investigations into a simulation model to be able to adequately describe or predict e_p .

CONCLUSIONS

It is concluded that although e_p is influenced by excess dietary crude protein, it is the ratio of energy to protein in the diet that is important and not the actual amounts of each that are consumed. The superior performance of birds fed the higher ME series and high E: P ratios suggests that if the dietary protein content is increased beyond the needs of the broiler chicken, the energy content should also be increased to ensure an efficient utilisation of dietary protein. Some of the effects of low E: P ratios on the biological performance are partly explained by the lack of energy for conducting the various functions related to protein digestion and metabolism. An enhanced GIT performance is partly due to an increase in the mucosal and membrane protein with an increase in dietary ME intake. This may also be due to the increased capacity to meet their nutrient requirements for protein digestion and metabolism from the food on offer. The changes in the activities of some of the digestive enzymes also explain the effects of protein quality and feed supply on body growth. The link between intestinal structure and function and biological performance in relation to dietary energy to protein contents, protein quality and level of feeding is an area that warrants further investigations. The series of studies confirmed the principle that diets supplying an array of nutrients that closely parallel the bird's nutrient needs give rise to a better biological performance.

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