THE USE OF CHOICE FEEDING AND MIXTURE EXPERIMENTS TO EVALUATE PROTEIN SOURCES USED IN BROILER FEEDS

HARRY KOFI SWATSON
B.Sc. (HONS) AGRIC. UST GHANA

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I HEREBY CERTIFY THAT THIS RESEARCH IS THE RESULT OF MY OWN INVESTIGATION. WHERE USE WAS MADE OF THE WORK OF OTHERS IT HAS BEEN DULY ACKNOWLEDGED IN THE TEXT.

Harry Swatson.

HARRY KOFI SWATSON

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CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

The high cost of feed ingredients in many African countries has caused many poultry farmers to abandon the industry (Onyenokwe, 1994). Feed costs in South Africa represent more than 80% of the total cost of growing broilers. Poultry producers will have to use a combination of feed ingredients in which relatively large savings in feed costs can be obtained to avoid further deterioration in profitability. The combination used must ensure that the best possible balance is maintained between the amino acids making up the protein, thereby ensuring that the protein sources are utilized maximally.

Choice feeding is a system that may enable the determination of the combination of feed components chosen by birds that will maximise their biological performance whilst minimizing excesses of amino acids (AA). This system involves the feeding of two or more feed ingredients to broilers and allowing them to choose a feed blend that meets their requirement for maximum growth. If this is done for various protein feed sources, it may be possible to find out *a priori* whether the feeding of similar proportions would be suitable for the birds or whether no combination of the feeds would enable them to make the correct choice.

In order to determine the response surfaces in biologically important variables (e.g. weight gain) and whether broiler chickens have the ability to choose from various protein sources such that they will maximise their biological efficiency, use was made of mixture experiments. Mixture experiments enable the blending surface to be modelled by means of a mathematical equation in order to be able to predict empirically the biological responses for any mixture or combination of protein feed sources.

The purpose of using mixture experiments in conjunction with choice feeding experiments will be to compare the combination of components that maximises biological performance with the combination chosen by birds given *ad libitum* access to the components. This will provide a severe test of the ability of the broiler chicken to make the right choice.

1.1 CHOICE FEEDING

The basic principle of choice feeding is that, in a population of birds, each individual has different requirements, for temperature, light, protein and energy and is able to select a blend between two or more formulated feeds to suit its daily needs for protein, energy (Belyavin, 1993) and possibly other specific nutrients such as amino acids or minerals (Forbes, 1995).

The optimum nutrient requirements of birds vary with strain, age, sex and environmental factors (Michie, 1977), making it impossible to meet the requirements for every individual in a broiler flock, under every possible environmental and physiological condition, by offering a single compounded feed. It has been successfully demonstrated that it is possible for birds to make the correct choice if they are given two feeds which, in some proportion, meet their requirements, as illustrated in experiments with growing turkeys (Rose and Michie, 1982), laying hens (Kiiskinen, 1987) and growing broilers (Sinurat and Balnave, 1986). In contrast, several authors failed to show that choice fed birds have a superior productive performance (McDonald and Emmans, 1980; Blake, Mather and Gleaves, 1984 and Robinson, 1985). However, Rose and Kyriazakis (1991) indicated that, in situations of choice feeding, there must be free access to all food by birds. In addition the feeds should not contain any anti-nutritive factors. Birds are unable to make the correct choice if they are given two feeds which, in some proportion, do not meet their requirements for one or more nutrients (Holcombe, Roland and Harms, 1976; Savory, 1979). Some workers have attributed the poor results of some choice feeding experiments to poor experimental design or the inability to meet the principles and conditions of diet selection (Cumming and Mastika, 1987; Emmans, 1991 and Forbes and Covasa, 1995). In most cases, choice fed birds compared with birds offered a single conventional feed perform as well or better than complete fed birds (Rose and Kyriazakis, 1991 and Belyavin, 1993).

The aim of this chapter is to provide a review of choice feeding and the possibility that such a technique could be used in research.

1.1.1 THE PRINCIPLES UNDERLYING CHOICE FEEDING IN POULTRY

Animals are known to have exact physiological and nutritional needs. They are able to make the correct choice from a wide range of feeds which, in some proportion, will meet their needs for growth, production and maintenance (Hughes, 1984).

Rozin (1976) proposed a classification of animals into two groups.

(a) The "specialists" which detect and recognise each feed with the aid of some genetic control. These animals consume a small range of feedstuffs.

(b) The "generalists" which first sample feedstuffs to find out if they are nutritious or palatable before continuing to feed on them. This group includes chickens. Hughes (1984) observed that the role of learning and experience is necessary for the "generalists" like chickens.

Experiments by Hogan (1973) showed that chicks offered food pecked initially at these freely, and ingested them later. However, on ingestion of a pleasantly tasting food, there is a marked preference for more food within about an hour of feeding. A possible explanation for this, according to Hogan (1973), lies in the post ingestional feedback, with the ingestion of food reinforcing the learning process.

Forbes (1995) also suggested that learned associations between the foods on offer and its hedonic properties are mainly responsible for the choice of food on offer rather than immediate feedbacks. Thus, in order to maintain preferences for a particular type of feed, its ingestion should lead to nutrient intake. However, animals tend to become malnourished or ill on ingestion of an imbalanced feed and this affects feed intake. Animals tend to avoid harmful feedstuffs and this phenomenon is important in allowing the animal to select a suitable diet that meets its physiological and nutritional requirements whilst avoiding diets containing toxic substances.

The animal, its habitat and the food it eats have been noted by Emmans (1991) as important variables in the theory of diet selection. The main factors that affect the level of feed intake are its composition, palatability and variety (Hughes, 1984). These factors have to be considered in experimental work for choice feeding studies to be successful.

1.1.2 THE BASIC PRINCIPLE UNDERLYING CHOICE FEEDING OF BIRDS

The basic principle of choice feeding of birds is that a population or flock of birds is made up of individuals with different physiological and nutritional requirements, and that each individual animal theoretically can choose a blend between two feeds which, in some proportion, meet its unique requirements for protein, energy and possibly other nutrients (Belyavin, 1993). Experimental evidence has been presented which indicates the ability of poultry to select a balanced diet from two or more feeds to meet their nutritional and physiological needs. In most choice feeding work, two formulated feeds are offered to birds and the birds balance intakes of the diets to satisfy their changing needs as they grow. However, the provision of a third feed will add an extra dimension to the ability of the broiler chicken to make the right choice.

1.1.3 DIET SELECTION THEORY

Poultry rations commonly consist of a balance of proteins (amino acids), energy, mineral salts and vitamins. Emmans (1991) described the composition of a diet using a three component mixture of dimensions protein, energy and minerals with amounts of each represented by foods A_1, A_2, B_1, B_2 and B_3 (Fig 1.1). Each nutrient is represented by a simple point in space. Foods A_1, A_2 within the circled subspace represent feedstuffs which, when eaten in some proportion, will meet the animal's physiological and nutritional requirements. Feeds B_1, B_2 and B_3 are outside the subspace for adequacy. An individual bird when allowed free access to all feedstuffs, chooses feeds which, when joined by a line, pass through the adequate subspace. Such a combination of feeds will optimize growth of the bird. These two feeds may be one with a higher protein, vitamin and mineral content and the other higher in energy level but lower in protein. The combinations of feedstuffs which when taken in some proportion, will meet the animals requirements are A_1 and A_2, and A_1 and B_1. Feeds B_2 and B_1 are nutritionally inadequate when offered alone, thus any selection of a mixture of these two feeds does not make a diet of balanced composition (i.e. balanced amino acid profile) to meet the animal's nutritional requirements.

A combination of feeds which are below the animal's needs for one or more nutrients will influence dietary selection (Holcombe *et al.*, 1976; Savory, 1979; Kyriazakis, Emmans and Whitemore, 1990). A line between B_1 and B_3 passes through the adequate subspace and any appropriate mixture of these two might meet the animal's nutritional and physiological needs. For the animal to meet its requirements for growth when offered a choice between either A_1 or A_2 and B_1, B_2 or B_3, it must consume either A_1 or A_2 in comparatively greater amounts.

Forbes and Shariatmadari (1994) indicated that in order to determine whether birds can make the correct choice if they are given two feeds, the composition of at least one of the test feeds must be in the adequate subspace. Secondly, these workers noted that defining nutrient requirements for birds necessitates the carrying out of adequate experiments with single feeds of a varied composition. The performance of the birds on the feeds of this diluted series of diets can then be measured over a period of time. Such an observation of an appropriate change in the proportions of the different feeds selected over a long period of time is preferable than at a given time (Emmans, 1991; Emmans, personal communication 1996). If an adequate blend of feeds is selected at different stages of growth, this confirms that the animals are selecting the feeds on offer with nutritional purpose (Forbes, 1995).

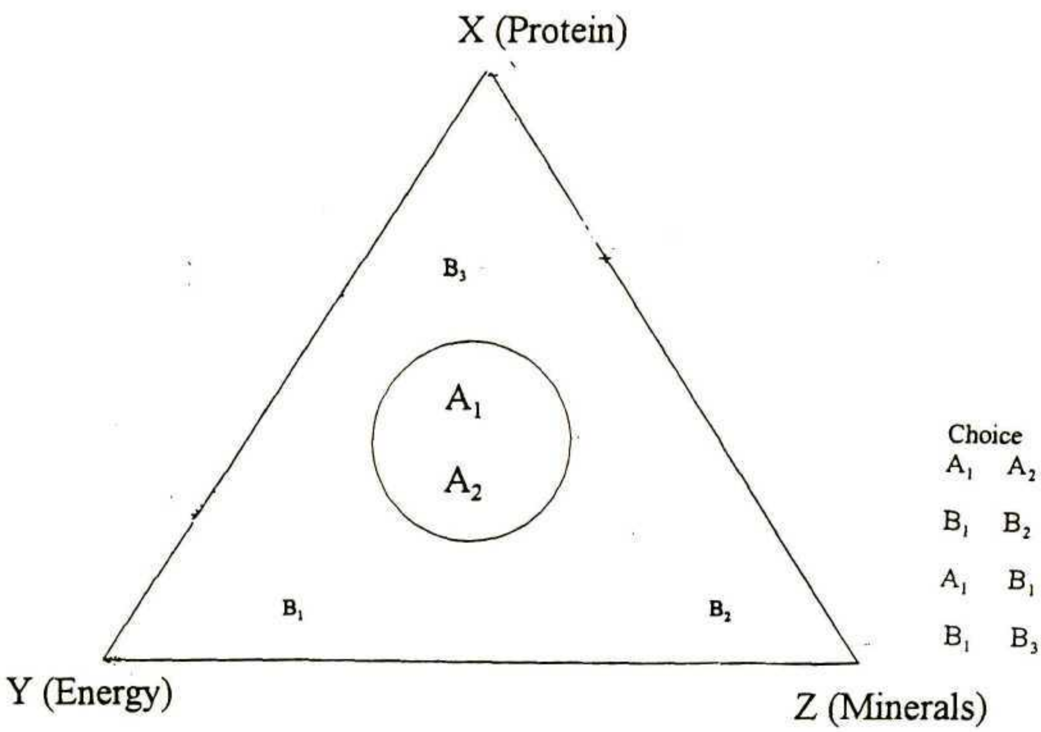


Figure 1.1. *Graphical representation of feed mixtures that need to be composed to meet an animals requirements for three nutrients in a three component space (source : Emmans, 1991).*

The nutrients enclosed within a circle will meet the animals requirements, whilst positions occupied by A_1-B_3 are representative of the contents of the three nutrients.

1.1.4 SOME FACTORS AFFECTING THE SELECTION OF A DIET BY POULTRY

A number of factors that determine the pattern of dietary selection of poultry have been summarized by Hughes (1984).

These include

(i) The metabolic and nutritional requirements of the bird.

(ii) The relative palatability of the diets offered.

(iii) The dietary composition, form, texture and flavour.

(iv) Learning and previous experience of the birds.

Additionally, an association between the sensory properties of each food with its nutrient content or metabolic effects is necessary for the animal to develop an appetite for the food. It has also been suggested that certain pathways (e.g. via the blood to the piriform cortex of the brain) may exist that enable the transmission of information about the'metabolic effects of a food (its amino acid levels) to the brain (Firman and Keunzel, 1988) and therefore control the ability of the birds to choice feed. The exact physiological and metabolic changes that take place and determine the diet selection ability of birds are however not accurately known (Covasa and Forbes, 1995).

Other limitations to diet selection in animals include the presence of toxic substances in the feed giving rise to an aversive taste in feeds and animals refusing to eat novel feeds because familiar feeds are nutritionally adequate. In contrast to other studies, when both feeds on offer are lower in crude protein content than the animals requirement there is a greater intake of the LP in preference to the HP resulting in a protein deficiency in broilers (Forbes and Shariatmadari, 1994) and laying hens (Holcombe Roland and Harms, 1976; Forbes 1995). Based on these observations, Forbes (1995) suggested that animals try to discriminate against feeds with excessive protein contents in a bid to avoid the toxic effects of the products resulting from the deamination of excess amino acids.

1.1.5 PRIOR EXPERIENCE AND TRAINING OF BIRDS

Published research indicates that animals have the ability to select foods that meet their nutrient requirements when given the chance to learn the differences between two foods on offer (Rose and Kyriazakis, 1991). The ability of poultry to learn to "choice feed" is improved if there is a period of prior exposure to the choice-fed diets. This allows them to learn the nutritional characteristics and the metabolic effects of the diets on offer (Cumming, 1987). If this period of learning occupies a substantial part of the experimental period, then feed intake during this stage will lead to a reduction in the overall productive performance of the choice-fed birds (Rose and Kyriazakis, 1991). This explains the poor results obtained in a few choice feeding experiments (Forbes and Shariatmadari, 1994). Mastika and Cumming (1987) fed three-week old broiler chicks a choice of whole sorghum and a high-protein food and observed that broilers which were previously choice-fed selected a higher energy:protein ratio. The choice-fed birds seemed to be more efficient with regard to protein utilisation. Choice feeding studies with growing pigs by Gous, Bradford and Kobus (1989) reveal that it is desirable to quicken the learning period by providing sole access to only one of each of the two feeds on alternate days at the start of the feeding trial. In line with the above concept, Shariatmadari and Forbes (1993) used a six day training period during which chickens were provided with high protein or low protein foods. The limitation to this method is that birds tend to avoid eating the cereal by starving until the normal food is provided (Rose *et al* ., 1994) or learning to eat only at a fixed time of the day (Pinchasov *et al*., 1985).

Covasa and Forbes (1994) performed experiments to determine whether a period of training is required before birds are introduced to cereals grains or conventional food. Their results suggested it is very important for birds to learn to choice-feed at an early age, so that they may not suffer from the few adverse effects later on in growth due to lack of prior preparation. Furthermore, birds introduced to the choice feeding system get used to the appearance of the food and its metabolic effects (Cumming,1987).

It is well documented from choice feeding studies that the selection of feed and performance of choice fed birds is greatly influenced by the type of feed to which they have been previously exposed (Covasa and Forbes, 1993a; Covasa and Forbes, 1993b). Covasa and Forbes (1993a) performed experiments with 48 two-week old female broiler chickens to determine the effect of time of exposure and the type of feed on diet selection. In the experiment, broiler chickens from two to four weeks of age, were either deprived of feed for two hours or not deprived and had access to whole wheat for either two or six-hours. For the rest of the experimental period, chickens had unrestricted access to commercial starter crumbles. In the second part of the experiment, from week five to seven, all birds were provided with a choice between wheat and a commercially pelleted feed. The length of the feeding time did not affect feed intake but the type of feed significantly affected choice feeding. Chickens previously exposed to whole wheat ate substantially more wheat in the growing phase. Feed intake of chickens with prior exposure to wheat was 13.9 g $bird^{-1}$ of wheat in contrast with 4.0g eaten by chickens exposed to the mixture. In other studies on prior exposure to whole wheat at different ages, and the subsequent choice of broiler chickens, Covasa and Forbes (1993b), observed that prior exposure is related to the age of the birds at training. Secondly, learning seems to be an important process in making nutritionally appropriate choices once choice feeding is initiated.

It is interesting to note that when birds are offered two feeds *viz* : a feed with a protein content greater than the birds requirements and another with an even greater protein content, they eat mainly from the former whilst still sampling the feed with an even greater protein content on a daily basis (Shariatmadari and Forbes, 1990).

1.1.6 THE EFFECT ON DIET SELECTION OF FEED TROUGH DESIGN AND POSITION

The position of feed troughs in choice feeding experiments may have an effect on the overall performance of choice-fed animals. Bradford (1989) in experiments with pigs to find out whether growing pigs have the ability to choice feed, used two feed bins for choice-fed pigs. The poor results obtained initially were attributed to the placement of the two feed bins on the opposite sides of the pen. It was suggested that the experimental animals may have developed positional preferences earlier on and did not discover the advantages of the other feed bin. When bins were placed side by side, this possibility was eliminated with an improved performance being obtained.

In investigations of dietary self selection, Lesson and Summers (1978) changed the positions of high-protein and low-protein feeds at three-day intervals and observed poor results of the birds. The reversal of the positions of the feed troughs was done with the view of avoiding nutritional bias. However, a change in feed position confused the birds since they had learnt the difference between the two feeds mainly by their position. A change in position meant that birds had to relearn to associate the new position with the nutrient content of the feed. This results in more time being spent to learn about the feed and results in decreased biological performance.

These experiments suggest that, under experimental conditions, certain non-nutritional factors such as feed trough position could have a considerable effect on diet selection of choice-fed birds. Rose and Kyriazakis (1991) suggested that in using choice feeding, there must be a free continuous and undisturbed access to all foods by birds. Hughes (1984) indicated that apart from behavioural responses concerned with the relative palatability of the diets offered, trough position may affect the results of a study, hence the poor results of some choice feeding experiments. In choice feeding experiments, it is necessary for different feeds to be offered in the same feed trough or identical and adjacent troughs during the learning period to enable the bird to balance its intake of nutrients efficiently (Cumming, 1994).

1.1.7 CHOICE FEEDING EXPERIMENTS IN BROILERS

Animals are able to show nutritional wisdom when offered a choice of foods. Kaufman, Collier and Squibb (1978) gave broiler chickens free access to foods containing 456 and 86 g CP kg^{-1} respectively. The birds selected proportions which gave them similar growth rates to the controls. Furthermore the proportion of the high protein food eaten dropped from 0.25 at 15 days of age to 0.15 at 50 days. Similar results from experiments by Shariatmadari and Forbes (1991) show that, if birds are given access to diets of various protein levels, they are able to choose the correct balance to meet their nutritional needs as they grow.

In experiments using male and female broilers, Cowan and Michie (1978) noted that males tend to select higher protein diets than females. This is probably due to the higher growth potential of males. Other studies conducted by Leeson and Summers (1978) with growing turkeys, show that when a low-protein, high-energy feed and a high-protein, low-energy feed are fed to male and female turkeys they chose a diet of similar energy concentration. However males with a higher growth potential selected a higher concentration of dietary protein than females. Brody, Chery and Siegel (1984) chose two chicken lines with either slow or fast growth potentials and provided them with the choice of a low-protein, high-energy food and a high-protein, low-energy food. They observed that the fast live-weight gain lines, with a higher protein requirement, ate considerably more protein in their diet. Males require a higher protein content in their diet than female broilers.

Emmans (1978) presented evidence suggesting that the nutrient requirements vary between individuals within a flock of birds primarily due to differences in growth potential. He suggested that it is practically impossible to meet the individual needs of each bird in a flock by using a single compounded ration. In order to meet the individual needs of each bird in a flock, a feeding system based on the choice feeding principle may be useful. This will allow birds to make the correct choice if they are given free access to feedstuffs which, in some proportion would meet their physiological and nutritional requirements. However, this is not possible if two feeds are incorrectly balanced in nutrients, as observed in experiments by Leclercq and Guy (1991). These authors, using broilers of a fat strain and a lean strain, offered a choice between a high protein (269 g CP kg^{-1}) and low protein (145 g CP kg^{1}) feed. Birds with a potential for gaining fat selected diets with a mean protein content of 179 g CP kg^{-1}, while the lean strain selected 200g CP kg^{-1}. Free choice fed birds were fatter and were less efficient in performance compared to birds fed the conventional diet. It was suggested that the inferior amino acid profile of the choice fed ration compared to the single compound feed may be the reason for the poor performance of choice-fed birds.

1.1.8 FUTURE RESEARCH INTO CHOICE FEEDING

The review of literature suggests that individual birds within a flock on a choice feeding regime can choose diets appropriate to their needs. Various factors affect the feed intake of choice-fed chickens apart from its metabolic and physiological state, these include :- inhibition of absorption due to nutrient imbalances, behavioural responses in relation to the palatability of the diets offered, the learning rate and previous experience of the birds, the age of breed, strain and sex of birds, genetic factors, management and the position of the feeding trough. All these factors *must* be considered when formulating diets for choice feeding experiments, and a failure to take these factors into account results in the inability of the broiler chicken to maximise its biological efficiency on diets on offer.

Future areas of research could dwell on the development of a technique that will enable a poultry producer to find out *a priori*, whether feeding two feeds would be suitable for the birds or whether no combination of the two would enable them to make the correct choice. Also an investigation on how two or more feeds could be blended before putting them into the feeding troughs rather than offering the two feeds simultaneously to the chickens could be carried out.

CHAPTER 1.2

THE EFFECTS OF INTAKES OF DISPROPORTIONATE AMOUNTS OF AMINO ACIDS IN BROILER NUTRITION.

1.2.1 INTRODUCTION

The proportions of amino acids in the diet of an organism normally vary from the proportions in which they are needed by the organism. The variation from the dietary pattern of amino acid that is suitable for growth and tissue protein synthesis is termed disproportion (Harper, Benevenga and Wohlhueter, 1970). Intake of a diet containing essential amino acids (EAA) and non-essential amino acids that are absorbed in quantities and patterns markedly different from the animals needs for maximum tissue utilization results in adverse effects (Harper *et al.*, 1970; Boorman, 1979; D'Mello, 1994; Forbes, 1995). Following the consumption of a diet with an amino acid profile deviating from an ideal balance, there are adverse effects varying from a lowered growth rate, food intake and nutrient utilization to acute neurological abnormalities and low survival rates (Harper *et al.*, 1970; D'Mello, 1994; Forbes, 1995). The severity of the adverse effects varies not only with the nature and degree of amino acid disproportion but also with the nutritional adequacy of the diet as a whole and with the age and the physiologic state of the animal (Harper *et al.*, 1970; D'Mello, 1994). The most severe adverse effects (i.e. food intake depressions and clear-cut toxic reactions) have been observed in young growing animals that are fed a low-protein diet containing an inordinately large amount of an individual amino acid. Well fed, healthy animals can withstand a considerable amount of disproportion without showing any adverse effects (Harper *et al.*, 1970).

The review and classification established in rats (Harper 1964; Harper *et al.*, 1970) indicates that the deleterious amino acid profiles due to the ingestion of diets containing disproportionate amounts of amino acids can be categorized as : amino acid toxicities, antagonisms and imbalances. These categories are now used as a basis for the classification of adverse effects of amino acids in farm livestock (D'Mello, 1994). It has been widely agreed that if the biological performance (i.e. efficiency of protein utilization) of animals is to be optimized then dietary disproportions of amino acids should be avoided. Waldroup, Mitchell, Payne and Hazen (1976) demonstrated that diets that provided adequate quantities of amino acids with minimum imbalances or excesses of others, were more efficiently utilized for growth at a lower level of total protein in the broiler chick. Morris, Al-Azzawi, Gous and Simpson (1987) showed that the requirement of the chick for the first limiting amino acid is increased with dietary protein content, which is contrary to the reports of D'Mello (1990).

The review by Harper *et al.* (1970) indicate the existence of well established interactions between related amino acids such that when the protein content is increased or the nutritional quality of the protein is improved (i.e.supplementation with the limiting amino acid) the deleterious effects of dietary disproportions of amino acids can be alleviated. In addition animals are known to adapt over a period of time to moderate disproportionate intakes of amino acids due to an increase in their capacity for amino acid catabolism (Harper *et al.*, 1970, D'Mello, 1994). Harper *et al.*(1970) indicated that, the effects of dietary intakes of unbalanced amounts of amino acids (i.e reduced food intake) seem to be either normal homeostatic reactions that may prevent extreme changes in plasma amino acid concentrations or effects of exceeding the homeostatic capacity of the organism to remove greater than required quantities of amino acids that are themselves toxic in very large amounts or that give rise to toxic products of metabolism. Thus it is important to provide animals with a well-balanced diet containing the correct amounts of amino acids that can be available at the same time for protein synthesis.

In this chapter the issue of amino acid imbalances, antagonisms and toxicities are reviewed.

1.2.2 THE EFFECTS OF THE EXCESSIVE INTAKES OF INDIVIDUAL AMINO ACIDS.

It has been well documented that animals tend to respond to intakes of disproportionate amounts of amino acids by decreasing their voluntary food intake. The three patterns of amino acids shown to influence this phenomenon are : amino acid toxicities, antagonisms and imbalances (Harper, 1964; Harper *et al.*, 1970).

1.2.2.1 Amino acid toxicity

The expression amino acid toxicity was used by Harper (1964) to describe excessive intakes of individual amino acids that could neither be categorized as amino acid imbalance nor amino acid antagonism. This deleterious category of amino acid profiles, precipitated on feeding large quantities of individual amino acids, results in a depression in food intake and growth rate similar to the effects of amino acid imbalance and antagonism (Nixey, 1989). Acute growth depressions due to amino acid toxicity may be accompanied by profound and specific lesions in tissues and organs (D'Mello, 1994). Baker (1989) provided experimental data that indicates that toxicities occur in non-ruminant livestock. He noted that the addition of 1% excess of any single amino acid depresses neither growth nor feed efficiency in pigs or chicks. However of all the essential amino acids, dietary supplementation with 40 g kg^{-1} methionine results in the most significant depression in growth, while 40 g kg^{-1} of either leucine, isoleucine and valine has no growth depressing effects in pigs or poultry. On the contrary, results from choice-feeding studies do indicate that diets containing 40 g kg^{-1} excess tryptophan are rejected to a greater extent than diets containing this level of excess methionine. Excess threonine is growth-depressing in poultry but not in pigs, whilst arginine is more toxic and growth-depressing in pigs than in poultry. Even though most of these essential amino acids have been shown to be toxic at very high levels of inclusion, this rarely occurs in conventional poultry diets.

1.2.2.2 Amino acid antagonism

Amino acid antagonisms are basically adverse effects resulting from the interaction between structurally similar amino acids with a resultant lowered growth rate and food intake which cannot be relieved by simply adding the amino acid that is limiting in the food on offer (Harper, 1964; D'Mello, 1994). Such effects can be alleviated by feeding a structurally or metabolically related amino acid. However, it has been clearly demonstrated that the amino acid required to be added to the diet is not necessarily the one most limiting in the diet (D`Mello and Lewis, 1970).

The most commonly encountered amino acid antagonisms in commercial diets are antagonisms among structurally similar branched chain amino acids (BCAA :leucine, isoleucine and valine) and between lysine and arginine. There is competition between these amino acids for certain enzymes, and for excretion and absorption sites, thus the occurrence of disproportionate amounts of one to the other creates a one-sided competition. The existence of inter-relationships between structurally similar branched-chain amino acids, leucine, isoleucine and valine has been shown to exist in chicks (D`Mello and Lewis, 1970; Burnham *et al.*, 1992).

Nixey (1989) has indicated that, the deleterious effects of leucine can be relieved mainly by the addition of valine, with a dietary supplementation of isoleucine being essential for a complete restoration of food intake and growth rate. Burnham *et al.* (1992) carried out experiments designed to determine the response of broiler chickens to dietary isoleucine and quantify the antagonistic effects of excess leucine and valine on this response. A summit diet was formulated to contain isoleucine, leucine and valine at 1.14, 1.76, 1.87 times the requirement respectively. In one experiment the proportions of leucine and valine were kept constant in all diets, whilst in the other experiment L-leucine and L-valine were added to diets either singly or in different combinations. These workers concluded that, although valine exerts no effect on the response to isoleucine, an increase in leucine to isoleucine ratio depresses food intake and weight gain. On the contrary, adequate amounts of isoleucine alleviate the growth depressing effects of comparatively large excesses of leucine, of valine, or both. In view of these interactions, they further cautioned that in practical broiler feeds in which maize or sorghum serve as the sole cereal source in place of wheat, the requirement for isoleucine should not be increased. Apart from maize by-products (i.e.maize gluten meal) and sorghum, bloodmeal also contains disproportionate amounts of these branched chain amino acids (D'Mello, 1994).

Lysine and arginine antagonism occurs in the chick (D`Mello and Lewis, 1970) and the turkey (D`Mello and Emmans, 1975). In experiments on lysine and arginine antagonism, D`Mello and Emmans (1975) showed that the arginine requirement of turkeys is increased from 1.00 to 1.75% as the amount of lysine in the diet is increased from 1.05% to 1.55% (Table 1.2.1). However excessive quantities of dietary lysine cause a decline in food intake and growth rate, with grossly elevated levels in plasma lysine concentration and a depressed plasma arginine concentration (Nixey, 1989). The condition in which arginine is present in excess (i.e. reverse antagonism) rarely occurs. With conventional ingredients it is not common for widely differing ratios of lysine to arginine to occur.

1.2.2.3 Amino acid imbalance

Harper (1964) suggested the term 'amino acid imbalance' for variations in the amino acid pattern of a diet that cause a decrease in growth rate and food intake which can be relieved by the addition of a small amount of the most limiting amino acid in the diet. When imbalances occur, most amino acids would have met their minimum requirements but a few could be in large excesses. An animal has a limited ability to store excesses of amino acids ingested above its requirements and those not utilized for protein synthesis are broken down, transformed into a dispensable amino acid required or used as an energy source (McDonald, Edwards and Greenhalgh, 1991).

The primary manifestation of a large excess of amino acids appears to be an effect on appetite, probably due to an increase in the heat of digestion or the influence of circulating blood levels. Apart from slowing the growth rate, an imbalanced mixture of amino acids increases the oxidation of all but the most limiting amino acid, resulting in an lowered utilization of dietary protein (Forbes, 1995). The efficiency of protein synthesis will depend on how closely these amino acid proportions resemble those of body proteins and the extent to which they can be modified (McDonald *et al.* 1991).

Table 1.2.1 The effect of different dietary lysine and arginine contents on the weight gain (g b day $^{-1}$) *of turkey poults from 7-21 days.*

Dietary arginine content (g kg $^{-1}$)	Dietary Lysine content (g kg $^{-1}$)		
	10.5	13.0	15.5
10.0	15.0	18.3	20.8
12.5	14.1	23.3	24.2
15.0	15.1	24.1	26.6
17.5	13.7	21.5	29.4

Wethli, Morris and Shresta (1975) conducted investigations into the growth responses of broiler chicks to increasing amounts of comparatively poor-quality proteins, supplemented and unsupplemented with their limiting amino acids. Groundnut-wheat-barley mixtures providing crude protein (CP) levels from 120 to 420 g kg^{-1} were supplemented and unsupplemented with methionine plus lysine. The control diets contained various proportions of herring meal providing CP levels from 120 to 240 g kg^{-1} of dietary treatment. It was assumed that when higher quantities of the protein sources are used the minimum amino acids requirement of the chicks will be met. The trial results showed that there was a gradual improvement in growth rate on the unsupplemented groundnut meal diets up to 360 g kg^{-1} but was comparatively lower than chicks receiving the low CP herring containing diets. There was however a progressive and efficient improvement in liveweight gains for chickens offered the methionine plus lysine supplemented groundnut meal diets of CP contents between 120 to 270 g kg^{-1} diet. At CP contents of 270 g kg^{-1}, chickens offered the methionine plus lysine supplemented groundnut meal diets had growth rates that were similar to that of chickens that maximised their growth on a 210 g CP kg^{-1} herring meal containing 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 was concluded from these trials that growth could not be maximised when soybean meal and groundnut meals are utilized as a supplement to cereal-based diets although very high dietary CP levels are used. Furthermore, they indicated that the amino acids in poorer quality oil-seed cakes occur in disproportion to the requirements of the chick such that the utilization of the first limiting amino acid is reduced. They also suggested that some of the improvement in growth was due to the amino acid imbalances being corrected with the use of pure supplements of the limiting amino acids.

In spite of these observations, several workers have suggested that amino acid excesses have no effect on the utilization of the first limiting amino acid (Harper *et al.*, 1970; Boorman and Ellis 1996). In explaining the reasons for poor performance on high intakes of poor-quality proteins, Boorman and Ellis (1996) argued that, apart from a small decrease 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 amino acid(s) from tissue protein.

There is considerable evidence to suggest that the intake of an imbalanced diet results in a rapid reduction in feed intake and growth rate. However there has been some controversy as to whether intake of an imbalanced diet firstly results in a reduction in growth rate or feed intake or *vice versa*. Convincing evidence indicates that there is a marked depression in feed intake within three to six hours after rats have been fed an imbalanced diet (Harper and Rogers, 1965). It was pointed out that the depression in feed intake firstly occurs and this is responsible for the growth depressing effects. When food intake of an imbalanced protein source is increased by insulin injections (Kumata and Harper, 1962), force-feeding (Leung, Rogers and Harper, 1968), cold exposure (Klain, Vaughan and Vaughan, 1962) and by the adjustments of dietary protein to energy ratios (Fisher and Shapiro, 1961), body weight gain was unaffected. Thus, if food intake of disproportionate amounts of amino acid(s) is increased, the animal will experience no deleterious effects.

Efforts have been made to explain the biochemical or physiological mechanisms responsible for the decrease in food intake due to dietary imbalances but the precise mechanisms are not yet known although theories such as the catabolic theory have been proposed (Harper and Rogers, 1965). These researchers hypothesized that synthesis or breakdown of protein in the liver is stimulated by the presence of surplus amino acids in the portal circulation after the absorption of a diet with an abnormal amino acid pattern, leading to a greater retention of the limiting amino acid in the liver. This causes a reduction in the amount of the most limiting amino acid in the plasma which results in an alteration of the body pools free amino acid pattern. This deranged pattern is detected by some appetite regulating receptors, most probably in the brain (Forbes, 1995), which respond by causing a reduction in feed intake. Since amino acids are primary constituents of structural, protective and soft tissues (i.e. muscles), a reduction in the supply of the most limiting amino acid due to a reduced food intake will either cause a reduction or cessation of growth.

1.2.3 SUMMARY

Amino acids, which are the building blocks of protein molecules, must be available in balanced amounts at the same time for adequate protein synthesis. These amino acids required for protein synthesis are mostly supplied by ingestion of a diet balanced in essential and nonessential amino acids but may also be withdrawn from the less vital body tissues. With the exception of animal protein sources (e.g. fishmeal), most plant sources fed as a sole source of protein are unable to provide a proper balance of amino acids for poultry.

In general, animals respond adversely to diets containing disproportionate amounts of amino acids by firstly reducing their food intake, probably due to an influence on appetite. Of the categories of amino acid balance, antagonisms and imbalances of amino acids are most likely to occur in conventional type diets, even though deficiencies also occur. The poor response of broiler chickens to high-protein diets based on poor-quality protein sources, due to a depressed utilization of the limiting amino acids, have been attributed to amino acid imbalances. Antagonisms occur in some commonly used feedstuffs (i.e. maize by-products, sorghum and bloodmeal) due the disproportionate ratios of the branched chain amino acids.

Chickens that are given feeds supplemented with amino acids tend to be more efficient in their utilization of dietary protein. If two or more natural protein sources can be blended in predetermined proportions and the limiting amino acids supplemented it is likely that excesses or deficiencies may be minimized and this may give rise to an improved biological performance. A major practical problem based on this review will be to be able to predict *a priori* what combinations of two or more protein sources in feed mixtures will maximize the biological performance of broiler chickens in terms of weight gain and food conversion efficiency (FCE) whilst at the same time minimizing amino acid excesses or deficiencies.

Hitherto the nutritional value of various combinations of two protein feeding-stuffs have been studied. However the response to such predetermined two-component mixtures or choice fed feeds may not take into account several dietary factors giving rise to the observed response. It is possible that by offering a three choice feeding regime to broiler chickens a three dimensional approach will be obtained with more factors taken into account to give a better biological response for any combination of protein feeding-stuffs.

By combining mixture experiments with choice feeding treatments, it is possible to determine, firstly, the response surface based on criteria such as weight gain and FCE, and secondly, whether broilers, given access to the components separately, will attempt to maximise either weight gain or FCE. This technique thus provides evidence of the importance of amino acid balance in the feed offered to broilers (by means of the mixture experiment) and of the ability or otherwise, of broilers to make a choice from two or three components such that weight gain or FCE is maximised (by means of the choice feeding experiments).

CHAPTER 2

CHOICE FEEDING EXPERIMENTS WITH BROILER CHICKENS AGED 18 TO 25 DAYS

2.1 INTRODUCTION

An important consideration in the successful formulation of diets for poultry depends upon the relative contributions of all essential nutrients at the disposal of the compounder. The choice of the relative proportion of each ingredient is dependent to a large degree not only on the price of each ingredient, but also on the natural variability of raw material sources and on the extent to which its nutrients are available to the chicken (Gous, personal communication 1996).

Although there is an accurate knowledge of the composition of each ingredient used in compounding rations, it has been shown that protein utilization by chickens is sometimes higher with certain mixtures or combinations of protein feeding-stuffs than when the individual components are fed alone (Woodham and Deans, 1977; McDonald *et al.*, 1991). This beneficial effect may be due to the mixture of indispensable amino acids being better balanced (Mc Donald *et al.*, 1991) and in a readily available ratio at the same time in protein mixtures thus meeting the animal's requirements for growth and protein synthesis (Forbes, 1995).

Woodham and Deans (1977) acknowledged the possibility of supplementary relationships between protein sources. These workers evaluated a large number of protein sources individually and combined in all possible pairs, as supplements to cereal based diets for broiler chickens. They concluded that the relative proportions of a number of amino acids determine the optimum combination of a mixture of proteins which will produce optimum performance in growing chickens.

Experiments on the nutritive value of mixtures of protein-containing ingredients for poultry feeding have hitherto been carried out with combinations of only two protein supplements, sometimes together with a cereal-based diet. However this poses the question " Can the amino acid requirements of the chicken, based on a maximal response in some criterion such as growth, feed utilization, improved carcass content or immune status of the broiler chicken, be met precisely with **only two** protein-containing ingredients in the diet ?" There are regrettably few protein feeding-stuffs which contain the correct ratios of all available amino acids and it is more likely that the amino acid requirement of the chicken may be precisely met by feeding more than two protein supplements in the diet.

The provision of an imbalanced mixture of amino acids slows growth, depresses food intake (Harper, *et al.*, 1970; Boorman, 1979; Gous, 1992; Forbes, 1995) and increases the oxidation of all but the most limiting amino acid resulting in inefficient utilization of dietary protein (Forbes, 1995). There is also the development of pathologic lesions and low survival rates (Harper *et al.*, 1970).

The adverse effect of deficiencies and excesses of amino acids on food intake and growth rate is of commercial importance due to a lowered profitability. Gous (1992) stated that feeds marginally deficient in an amino acid cause broilers to overconsume energy in trying to meet their requirement for the limiting resource, with the resultant deposition of energy as lipid. Furthermore, severe deficiencies cause protein growth to fall below the birds genetic potential.

Experiments with chickens by Waldroup, *et al.* (1976) indicate that diets formulated to minimize excesses of amino acids over the requirements of the chicks do improve the efficiency of protein and energy utilization. Thus offering broiler chickens a perfectly balanced feed will enable them to consume sufficient amounts that will provide adequate quantities of non-essential and essential amino acids that will maximize their biological efficiency.

There exists the possibility of substituting a considerable proportion of a high-quality protein feeding-stuff with an inferior and cheaper low-quality feeding-stuff at optimal ratios to attain an equivalent or even better performance than that of the superior individual supplement fed alone for a particular stage of growth (Woodham and Deans, 1977). Choice feeding experiments could be used in determining the optimum combination of those protein-containing ingredients that give a high profit margin and allow birds to attain their best biological performance.

The present experiment was designed to test the hypothesis that: broiler chickens, when provided with three separate protein feedstuffs on a free-choice basis, select a combination of three feeds that will meet their requirements for maximum growth and production and hence will maximize their biological efficiency.

Objectives :

The objectives of this study were to

(I) examine the effect on biological performance of providing broiler chickens between 18-25 days old with four feeds each based on a single protein source fed alone or in various three-choice combinations.

(ii) determine whether the choices that the chickens make are biologically sound on a theoretical basis. This was done by comparing the amino acid content chosen with the requirement as predicted by the EFG broiler simulation model and whether a different combination to that chosen by them could have produced a better amino acid balance.

2.2 MATERIALS AND METHODS

Pre-Trial:

Three thousand day-old commercial broiler chickens were floor-reared on a commercial starter mash fed *ad libitum* until 17 days of age before being placed on the test feeds. This commercial feed contained 220 g kg^{-1} protein, 50 g kg^{-1} fibre, 120.0 g kg^{-1} moisture, 8.0 g kg^{-1} calcium, 7.0 g kg^{-1} phosphorus, 12.0 g kg^{-1} total lysine. Lights were provided 23 h/day and the temperature maintained at approximately 32^{0}C. Water and feed was provided *ad libitum*. Gas heating with canopy brooders was used until two weeks of age in twelve pens each containing 250 unsexed chickens making a total of 3,000 chickens.

Experimental period

Animals and animal environment : To reduce experimental errors pens were made up at approximately 17-days. Broiler chickens from each of the 12 pens were subdivided and placed into the 24 experimental pens. Each pen measured 276cm by 354cm and contained 100 broiler chickens of a similar live weight. The 24 pens were divided into three blocks. The floor of the pens was covered with wood shavings which was kept unchanged for the duration of the experiment. Damp patches of litter were replaced when necessary.

From 18 days of age, the side curtains were used to control the broiler house environment within parameters of the natural environment. The experiment lasted for 7 days.

Experimental Design:

Eight feeding treatments (Table 2.1), replicated three times were randomly allocated within three blocks. Feed was provided in six tubular feeding troughs hung equidistant from each other, a pair of troughs providing feed for each free-choice feed. Each pen was provided with two water drinkers.

Dietary treatments:

Four protein feeding-stuffs namely, sunflower oilcake meal (SF), soybean oilcake meal (SY), maize gluten meal (MG) and fish meal (FM) were analysed for crude protein and available amino acids. These protein feeding-stuffs were then used in compounding four isonitrogenous experimental feeds. Dietary treatments (Table 2.1) consisted of three protein basal feeds presented on a free-choice basis in three pairs of separate feeding troughs (Treatments 1 to 4) and four individual protein basal feeds (Treatments 5 to 8). The composition of the protein feed sources and the four basal feeds are shown in Tables 2.2, and 2.3. The protein sources were analysed to determine the amino acid content following the methods described by Dennison and Gous (1980). Feeds were formulated on an "as-fed" basis. In order to compare only the amino acid balance between protein sources, crude protein, energy concentration (MJ ME kg^{-1}) and all major and minor vitamins and minerals were kept constant in all four basal feeds. The AME of all feeds was therefore fixed at 12.5 MJ AME kg^{-1}. Sugar and an inert filler (sand) were used to equalize feed energy level as amounts of protein feedstuffs were altered, whilst the amount of added sunflower oil was kept constant in all feeds. No attempt was made to balance the amino acids in the basal feeds. In order to ensure that an excess of protein would not obscure amino acid deficiencies in the protein feedstuff tested, a low protein content of 120g kg^{-1} crude protein was used in the feeding treatments.

Chickens were allocated to experimental feeds for an adaptation / acclimatization period. This entailed exposing chickens on the three free-choice protein diets to only one diet at a time for a period of six-hours. This was done only once. The purpose was for them to get used to the trough position, food form and its nutritive value.

Variables measured :

Accurate records of group feed supply, food intake and body weight changes were recorded per pen for the experimental period using a load cell. Feed wastage was minimized by adjusting trough height regularly. Feed in troughs was weighed and recorded on a short term basis, and food intake was based on the number of birds alive at the end of each three or four day recording period. The difference between the initial weight and the final weight of feed in troughs was taken as the food intake. The remaining feed was stirred, after which food was added to the trough which was then weighed again using the load cell.

Table 2.1 *Feeding Treatments and Pen Allocation.*

Treatment	Feeding regime	Pen Allocation		
1	FM-SF-SY (Choice)	1	9	20
2	SF-FM-MG (Choice)	5	16	24
3	FM-SY-MG (Choice)	2	15	19
4	SF-SY-MG (Choice)	6	10	23
5	Fishmeal basal (FM)	3	7	14
6	Sunflower basal (SF)	11	18	22
7	Soybean basal (SY)	4	8	12
8	Maize gluten basal (MG)	13	17	21

FM, fishmeal; SF, sunflower oilcake meal; MG, maize gluten meal;
SY, soybean oilcake meal.

Statistical analysis :

Data were analysed for statistical differences between treatments by ANOVA according to the General Linear Models procedure of the Minitab Statistical software (1994). The pen-means for each variable were analysed where applicable by the Fishers pairwise comparisons and the t-test.

Table 2.2 *Available amino acid content and nutrient composition (g kg^{-1}) of the protein sources used in the experiment.*

	Protein feed ingredient			
	FM	SY	MG	SF
Amino acid				
Arginine	7.5	8.0	3.5	8.6
Histidine	4.2	3.0	1.9	2.6
Isoleucine	5.0	5.4	4.2	4.9
Lysine	4.8	7.1	1.6	2.7
Leucine	8.8	8.8	17.0	6.7
Methionine	3.7	1.3	2.9	2.1
Phenylalanine	4.8	6.0	6.4	6.1
Proline	6.0	5.5	10.1	4.9
Serine	4.9	5.9	5.5	4.0
Threonine	5.1	4.5	3.5	3.5
Tryptophan	1.3	1.9	0.7	1.5
Valine	6.1	4.9	4.5	5.7
Tyrosine	3.6	3.6	5.7	2.3
TSAA	5.0	3.1	5.0	3.9
Proximate analysis:				
Crude protein	623.1	444.6	665.6	431.4
Calcium	54.4	7.7	6.1	8.5
Phosphorus	29.4	6.9	5.9	11.4
Fat	75.1	13.5	11.1	23.0
Available lipid	98.1	74.9	15.0	77.5
Ash	20.1	7.7	2.6	6.5
Moisture	89.5	95.3	73.3	79.8
MJ AME kg^{-1}	12.9	9.8	15.3	8.9

FM, fishmeal; SF, sunflower oilcake meal; MG, maize gluten meal; SY, soybean oilcake meal.

Table 2.3 *Composition ($g\ kg^{-1}$) and calculated analysis of basal feeds used in the experiment.*

	Basal Feeds			
Ingredient	FM	SF	SY	MG
Soybean oilcake meal			276.0	
Maize gluten meal				179.0
Sunflower oilcake meal		280.0		
Fishmeal	194.0			
Sugar	439.0	475.0	436.3	429.0
Sunflower oil	80.0	80.0	80.0	80.0
Monocalcium phosphate	3.3	18.0	18.9	19.0
Limestone	4.7	17.3	17.3	18.7
Salt	1.5	3.9	4.3	4.5
Filler (sand)	277.5	123.5	167.9	267.5
Vitamin +mineral premix	2.5	2.5	2.5	2.5
Calculated analysis:				
Crude Protein	120.0	120.0	120.0	120.0
AME($MJ\ kg^{-1}$)	125.0	125.0	125.0	125.0
Calcium	10.0	10.0	10.0	10.0
Phosphorus (available)	5.0	5.0	5.0	5.0
sodium	1.8	1.8	1.8	1.8

[1] The vitamin premix consisted of ($mg^{1}g$) : thiamin 35, riboflavin 23, pyridoxine 8, biotin 0.8, pteroylmonoglutamic acid 5.7, menaphthone 7, cyanacobalamin 0.03, nicotinic acid 213, ascorbic acid 354, retinol ($ug\ g^{-1}$) 212.7, cholecalciferol (2000 $ug\ g^{-1}$) 10.2, alpha-tocopherol (250 $mg\ g^{-1}$) 6, maize starch 125. The mineral premix consisted of ($mg\ g^{-1}$):KH_2PO_4 479, NaCl 365, ferric citrate 23, $MgSO_4.H_2O$ 9, KI 0.46, $CuSO_4$ 0.58, $ZnCO_3$ 9, $Na_2MoO_4.H_2O$ 0.46.

2.3 RESULTS

Group records of body mass and food intake of birds on all treatments were kept for the one-week trial period. The mean daily body mass gain (g bird d^{-1}), daily food intake (g bird d^{-1}) and feed conversion efficiency (FCE, g gain/kg food) were calculated from these data. The results of the experiment are presented in Table 2.5. The amino acids present in the blend chosen by the broiler chickens as a proportion of its requirements are shown in Appendix 2.2. This was based on the requirement for essential amino acids as suggested by Gous (1996) which is similar to the NRC (1994) specifications (Appendix 2.1). A comparison of the mean proportions of each dietary protein feedstuff chosen by the chickens given the choice of three feeds is presented in Table 2.4.

2.4 DISCUSSION

The results confirmed the hypothesis that broiler chickens when provided with three separate protein feedstuffs on a free-choice basis, select a combination of the feeds such that their requirements for growth are met.

An analysis of the performance data obtained for food intake, showed that broilers on T5 consumed significantly ($P<0.05$) more food than broilers on the choice feeding treatments T2 and T4. Food intake of broilers on T1, T2 and T3 were significantly ($p< 0.05$) higher than food intake of broilers on T4, T6, T7 and T8.

According to Mc Donald *et al.* (1991), adequate amounts of the major limiting amino acids *viz* : methionine, lysine and tryptophan in feeds will ensure that the feed will automatically provide optimum amounts of others. Thus, an examination of the amino acid profiles of the food consumed as a proportion of requirement given in Appendix 2.2 explains the superior performance of chickens on T3 and T1. Broiler chickens on these treatments consumed a blend of feeds that provided at least 50% of each essential amino acid needed as a proportion of requirement.

Table 2.4 *Basal feeds chosen by the birds as a proportion of the total food consumed.*

Treatment	Basal Feed	Mean Proportion	SD	P-Value	
1	FM	0.3933	0.0833	0.3200	n.s
1	SY	0.2567	0.0611	0.1700	n.s
1	SF	0.3500	0.0400	0.4800	n.s.
2	FM	0.4300	0.0200	0.0130	*
2	MG	0.0833	0.0153	0.0013	*
2	SF	0.4967	0.0058	0.0005	*
3	FM	0.5467	0.0493	0.0170	*
3	SY	0.2800	0.0436	0.1900	n.s.
3	MG	0.1667	0.0116	0.0017	*
4	SY	0.3767	0.0764	0.0400	n.s.
4	MG	0.1067	0.0208	0.0029	n.s.
4	SY	0.5133	0.0643	0.0390	*

Hypothesis Ho: Mean = 0.333

Mean ≠ 0.333

n.s. not significantly different from 0.333

otherwise significantly different from 0.333 at P<5% (*).

Table 2.5 *Response of broiler chickens to feeding treatments from 18 to 25 days of age.*

Treatment		Food Intake ($g\ bird\ d^{-1}$)	Weight gain ($g\ bird\ d^{-1}$)	FCE[1] g gain/kg food intake
1	FM-SF-SY	48.17^{ab}	115.28^{ab}	420^{a}
2	SF-FM-MG	47.07^{ab}	111.06^{bc}	420^{a}
3	FM-SY-MG	49.51^{ab}	118.20^{ab}	420^{a}
4	SF-SY-MG	44.83^{ab}	96.90^{d}	467^{a}
5	FM	55.39^{a}	123.74^{a}	450^{a}
6	SF	30.72^{c}	86.24e	356^{a}
7	SY	40.22^{b}	105.00^{c}	383^{a}
8	MG	12.61^{d}	61.00^{f}	206^{b}
SD		2.60	2.95	34.35

^{a,b,c,d,e,f} Means within a column with different superscripts are significantly different ($p<0.05$);
FCE[1] Food conversion efficiency

Broilers on T4, T6 and T8 consumed less food and were unable to meet at least 40% of their lysine requirement from the food consumed. Methionine was also limiting in the food consumed by birds on T7. The main effect of such a poorly balanced amino acid profile in food consumed is a decrease in food intake (Harper, *et al.*, 1970; Forbes, 1995; NRC, 1994 and Gous, 1992). Forbes (1995) suggested that imbalanced feeds results in metabolic disturbances and a reduction in food intake which is directly proportional to the degree of amino acid deficiency or imbalance. This explains why intake was lower on maize gluten (T8) and was selected against (Table 2.4), this being the most badly balanced amino acid containing mixture offered to the broilers in the trial. Similar effects were observed by Haper *et al.* (1970). Furthermore, Forbes (1995) suggested that the metabolic cost of deaminating the excess amino acids which are not utilized for protein synthesis may be responsible for the lowered food intake of such feeds.

There were no significant differences ($p < 0.05$) in weight gain (g bird d^{-1}) among birds on T5, T3, T1, T2 and T4. Live weight gain was maximal on the fishmeal feed and broilers did not attain their maximum on the combinations. Growth was not impaired by the free-choice regimes and was superior to that of birds fed sunflower and maize gluten basal feeds individually. Nixey (1989) indicated that lysine is important for body weight gain. Thus the improvement in growth of birds allowed to select their food, may be due to an overall increase in the blend of feeds chosen which was nutritionally adequate in lysine. This confirms work by Woodham and Deans (1977) who illustrated that the performance of broiler chickens offered a mixture of protein feedstuffs was superior than would be expected from the results of feeding the components singly. This is due to an improvement in the amino acid composition resulting from mixing complementary protein sources together.

The results of this experiment are in line with the evidence that chickens do select an adequate diet from a choice of two or more foods which are individually inadequate (Cowan and Michie, 1978; Mastika and Cumming 1987; Belyavin, 1993; Forbes and Shariatmadari, 1994).

Broilers on T8 had significantly ($P < 0.05$) lower weight gains in comparison to broilers on other feeds. Broilers offered SB only as a main protein feedstuff, were found not to grow as well as those offered a mixture of SB and other protein sources. This confirms observations by Irish and Balnave (1993). The low weight gains of birds on the SF (T6) and MG (T8) are to be expected on the basis of the amino acids present in the single protein sources (Table 2.2). These results support the view that an inadequate consumption of essential amino acids leads to a lowering or cessation of growth (National Research Council,1994). Furthermore, there is the withdrawal of protein from the less vital body tissues to maintain the proper functioning of the more vital tissues. This explains why birds on T8, which consumed very little food, had very low weight gains.

The feed conversion efficiency (FCE) of broilers fed T1 was not significantly ($p < 0.05$) different from broilers on treatments T2, T3, T4, T5, T6 and T7. Broilers offered feeds on a free-choice basis did maximize their FCE on one of the combinations (T4). The significantly lower ($p < 0.05$) FCE obtained for birds on T8 (MG) was not surprising as these birds were slow growing, consumed very little food and put on very little weight.

2.5 CONCLUSION

In conclusion, maximum growth was obtained on the fishmeal feed, but this was not significantly greater than for the combination of feeds chosen although it was significantly greater than for the other protein sources fed alone. Broilers did maximize their FCE on one of the combinations (T4), and did improve their performance (weight gain and FCE) on each of the protein sources other than fishmeal when given a choice. In all cases broilers selected against maize gluten, this being the most badly balanced amino acid containing mixture offered to chickens in the trial.

CHAPTER 3

MIXTURE EXPERIMENTS USING FISHMEAL, SUNFLOWER OILCAKE MEAL, AND MAIZE GLUTEN MEAL WITH BROILERS AGED 7 T0 21 DAYS

3.1 INTRODUCTION

Mixture experiments are a broad class of response surface experiments in which factors are the ingredients or components of a mixture. The measured response such as weight gain, is dependent only on the proportion of the components present in the mixture and not on the total amount of this mixture (Khuri and Cornell, 1987; Minitab, 1994; Myers and Montgomery, 1995). Additionally, the number of components (q) in the mixture are normally referred to in terms of their proportion to the whole, which is taken as 1, with the levels of the factors being nonindependent in contrast to other response surface experiments.

Mixture experiments have been widely utilized in a number of different areas such as product formulation, in which the products being manufactured consist of a mixture of two or more ingredients or components (Snee, 1977; Khuri and Cornell 1987; 1994; Myers and Montgomery, 1995). Industrial products manufactured using mixtures of components include textiles, rocket fuel, fibre blends, paints, soaps and beverages. Stainless steel is a mixture of great tensile strength whose properties depends on the relative proportions of the components iron, nickel, copper and chromium present in the mixture. Gous (1993) suggested a similar blending of two feeds that are different in protein content, to maximise efficiency in broiler production.

Classical techniques for investigating the effects of independent variables, viz: factorial and response surface designs, are not appropriate for studying the response of a mixture system. This is because the proportion of each component in a mixture must lie between 0 and 1.0 and the sum of all the components expressed as fractions of the mixture must add up to 1.0. (Snee, 1971; Khuri and Cornell 1987; Minitab, 1994; Myers and Montgomery, 1995). Snee (1971) shows how only two points fit this constraint in a 2^2 factorial design, namely (1, 0) and (0, 1), and three points fit in a 2^3 factorial arrangement viz : (0, 0, 1), (0, 1, 0) and (1, 0, 0). The use of additional points in the design, making the number of points to exceed the number of parameters, eliminates the problem of measuring lack of fit of the model.

Whereas mixture experiments are of interest in their own right, they are used in the present series of experiments to determine response surfaces in biologically important variables such as weight gain and food conversion efficiency, the purpose being to compare the combination of components that maximises biological performance with the combination chosen by birds given *ad libitum* access to the three components simultaneously.

Objective:

An experiment was carried out to :

(i) determine the effect on the biological performance of providing broiler chickens between 7-21 days old with three basal feeds in which the only protein source in each basal was fishmeal (FM), sunflower oilcake meal (SF) or maize gluten meal (MG). These basal feeds were in fed *ad libitum* either alone in predetermined mixtures, or individually as two- or three free-choice components.

(ii) model the blending surface with a mathematical equation in order to be able to predict empirically the biological responses for any mixture or combination of protein feed sources.

3.2 MATERIALS AND METHODS:

Birds, housing and management.

A flock of 1000 day-old sexed broiler chickens, obtained from a commercial source, were reared from day-old to one week of age in electrically heated five-tier battery brooders. The birds were housed in the brooders in groups of 20 and under continuous artificial lighting using standard brooding practises. Each brooder was considered to be a block.

The ambient temperature was progressively decreased from about 32 °C to about 22 °C at three weeks of age. House temperature was monitored carefully with minimum and maximum temperature recorded daily throughout the duration of the trial. Temperature was maintained at a comfortable level.

Ventilation was achieved via the regulation of vent windows at the side of the experimental house.

At one week of age ten birds of almost uniform weight and of the same sex were group weighed and randomly assigned to feeding treatments. Each cage was equipped with its own feeder and two nipple drinkers with cups. Free access to food and water were allowed.

Masonite was used to divide the horizontal feeding troughs into two or three depending on the choice-feeding treatment offered. Metal sheets were fitted between the top and bottom tiers to prevent faecal contamination of the birds in the lower cages and to assist in the estimation of spilled feed.

Design of Experiment

(i) Description of feeding treatments:

A randomized block design with all the feed*sex treatments replicated twice was used. Broiler chickens were reared on a commercial starter mash fed *ad libitum* until seven days of age before being placed on the test feeds. This commercial feed contained 220 g kg^{-1} protein, 50 g kg^{-1} fibre, 120.0 g kg^{-1} moisture, 8.0 g kg^{-1} calcium, 7.0 g kg^{-1} phosphorus, 12.0 g kg^{-1} total lysine.

Three protein sources differing in amino acid (AA) composition, were used. The protein, amino acid and AME (MJ kg^{-1}) contents of fishmeal (FM), sunflower oilcake meal (SF) and maize gluten meal (MG) were determined as in Experiment 1, after which three basal feeds were formulated to contain 120g crude protein kg^{-1} and 12.5 MJ AME kg^{-1} (Table 3.1 and 3.2).

The formulation was based on the requirements for essential nutrients as suggested by Gous (1996) which is similar to the NRC (1994) specifications (Table 3.3). Only one protein source was used in each basal feed. The basal feeds were iso-energetic as well as iso-nitrogenous and were mixed at the onset of the experiment.

Thirteen predetermined feed mixtures were offered to the birds in addition to a second series of treatments, 14-17 which consisted of offering birds combinations of either two or three of the basal feeds as a choice (Table 3.4).

(ii) The augmented simplex lattice mixture experimental design:

If the number of basal feeds in the mixture and the proportion of the i^{th} feed in the mixture are represented by q and x_i respectively, then $x_i > 0, \quad i = 1, 2, 3, \ldots, q$ (2.1)

and $x_i = x_1 + x_2 + x_3 + \ldots + x_q = 1.$ (2.2)

The constraints in equations (2.1) and (2.2) imply that the levels of factors x_i are nonindependent and any change in the proportion of one basal feed in the mixture will result in a change in the proportion of at least one other basal feed in the experimental region. For this experiment with three basal feeds (q=3), the feasible experimental region is an equilateral triangle (Figure 3.1). The proportion of each basal feed chosen for the mixtures was based on the augmented simplex lattice design (degree 3) model available in the Minitab (1994) release 10 software. This design was selected because it is superior in studying the response of complete mixtures and can detect and model curvatures in the interior of the triangle that cannot be accounted for by terms in the full cubic model (Myers and Montgomery, 1995). Additionally, it has more power for determining the lack of fit than does the {3,3} lattice. The graphics in Figure 3.2 shows a general layout of points in the three component design using the simplex coordinate system and the thirteen feeding treatments (T1-T13) offered to the birds.

The range of design points included : vertices consisting of one-component or pure blends, edge trisectors consisting of 2/3:1/3 binary blends with no other components (i.e. the component labelled on the opposite vertex is absent), face centroid consisting of a mixture with equal proportions (1/3:1/3:1/3) from each of the components, a centre point and interior axial points. The interior points of the triangle represent mixtures where all the component proportions are non zero, implying that, $x1 > 0$, $x2 > 0$, $x3 > 0$.

Allocation of feeds:

Birds on the four choice feeding treatments were allotted experimental feeds for an adaptation period. This entailed exposing chicks to each feed for a period of six hours at a time in rotation in order for the chicks to get used to the trough position, food form and its nutritive value. All feeds were fed *ad libitum* to the sexed broiler chickens for a 14 day test period. Seventeen groups of male and female chickens, ten per pen were randomly allotted to 34 cages in a block and fed one of the seventeen experimental feeds.

Weighing and handling facilities : Adequate food storage, to allow uniform feeding over the experimental period, was provided. Feed was uniformly compounded to last for two weeks using the weighing, mixing and handling facilities at the farm. All raw materials for the experiment were purchased in bulk and samples of the protein feedstuffs were analysed. Feed wastage was minimized by not filling the trough more than half full. The food allocated to each pen of birds at the start of each week, and that remaining at the end of each week were accurately recorded, as was the weight of the chicks at the start and the end of each week. Chickens from each pen were weighed simultaneously and the number being weighed was recorded. The experiment was terminated only after the chicks had been weighed on the 21st day of age.

The different basal feeds offered as a choice in treatments 14 to 17 were weighed separately. The difference between the weight of food allocated and that remaining was taken as the feed intake of the group. This was divided by the average number of chicks per pen to determine the food intake per bird.

The variables measured

The criteria studied were body weight gain (g bird d^{-1}), food intake (g bird^{-1}d) and food conversion efficiency (g body weight gain/kg food intake).

Statistical analysis

The results were analysed for statistical differences between treatments using the Minitab statistical software (1994) to analyse the experimental Augmented Simplex Lattice Design 3. A mathematical model was used to fit the responses obtained from the experiment by using the MIXREG function. Modelling the blending surface with a mathematical equation enabled the empirical prediction and measurement of the mixtures influence on the biological response of each component singly or for any combination of feed protein sources. All final weight data were statistically treated by the analysis of variance (ANOVA). One-sample t-test computations were performed on the means of food intake for treatments 14 to 17 to determine whether the amount of each basal feed chosen by the birds as a proportion of the total differed significantly from 0.5 (in the two-component choice feeding treatments), or from 0.33 (in the three-component treatments). The complete data set was reduced to means and the degree of variation indicated by giving the standard deviation of the mean.

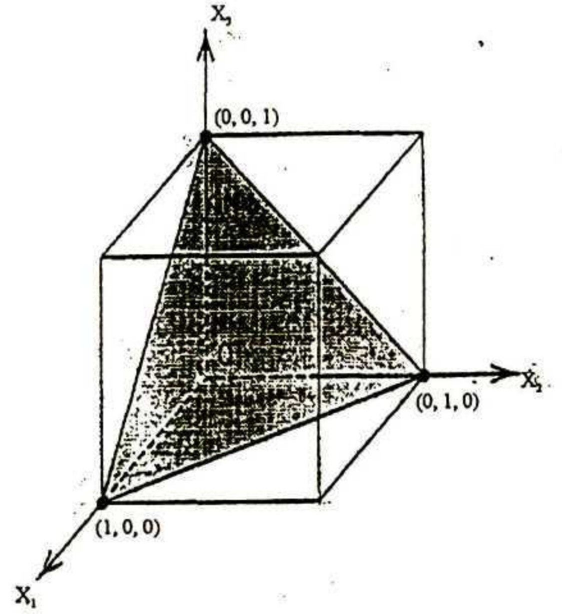


Figure 3.1. *Simplex factor space for mixtures with three components, where* $x_1 + x_2 + x_3 = 1$

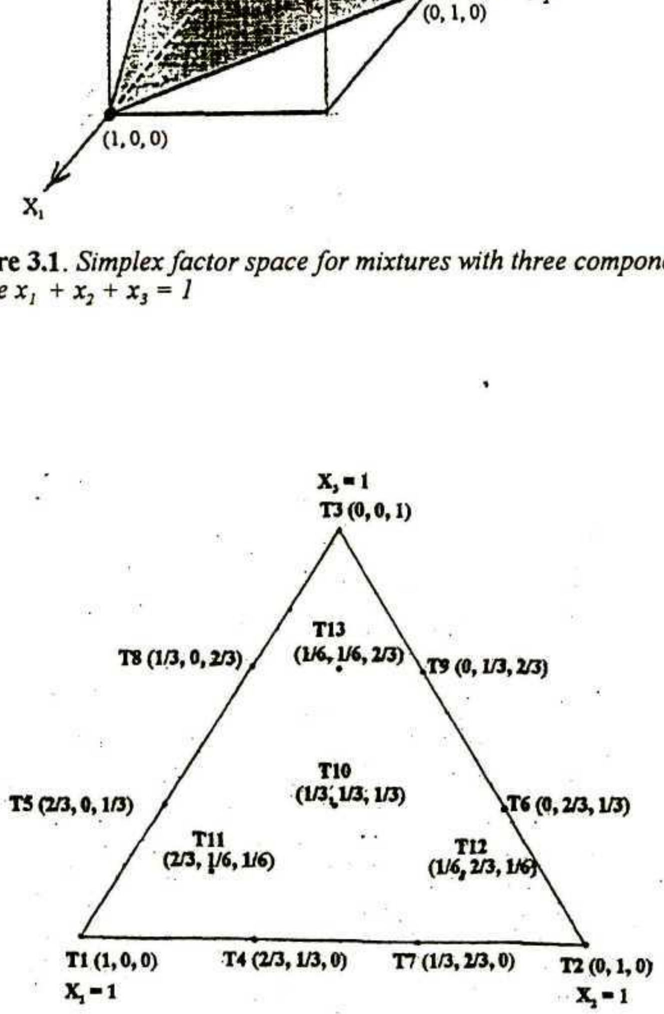


Figure 3.2. *The general layout of the 13-point {3.3} simplex lattice design augmented with three interior points and the assigned feeding treatments.*

The coordinates of the points on the figure are shown in (x_1 , x_2 , x_3) *order.*

Table 3.1 *Composition (g kg⁻¹) and calculated analysis of basal feeds used in the experiment.*

Ingredient	Basal Feeds FM	MG	SF
Maize Gluten		185.00	
Sunflower oilcake meal			329.00
Fishmeal	198.00		
Sugar	440.00	423.00	455.00
Sunflower oil	80.00	80.00	80.00
Monocalcium phosphate	2.90	19.00	17.80
Limestone	4.40	18.70	17.10
Salt	1.00	4.50	3.80
Vitamin + mineral premix [1]	2.50	2.50	2.50
Sand	270.50	267.50	95.30
Calculated analysis:			
Crude Protein	120.00	120.00	120.00
ME(MJ Kg^{-1})	12.50	12.50	12.50
Calcium	10.00	10.00	10.00
Phosphorus (available)	5.00	5.00	5.00

[1] The vitamin premix consisted of (mg g^{-1}) : thiamin 35, riboflavin 23, pyridoxine 8, biotin 0.8, pteroylmonoglutamic acid 5.7, menaphthone 7, cyanacobalamin 0.03, nicotinic acid 213, ascorbic acid 354, retinol (ug g^{-1}) 212.7, cholecalciferol (2000 ug g^{-1}) 10.2, alpha-tocopherol (250 mg g^{-1}) 6, maize starch 125. The mineral premix consisted of (mg g^{-1}):KH_2PO_4 479, NaCl 365, ferric citrate 23, $MgSO_4.H_2O$ 9, KI 0.46, $CuSO_4$ 0.58, $ZnCO_3$ 9, $Na_2MoO_4.H_2O$ 0.46.

Table 3.2 *Nutrient composition (g kg^{-1}) protein feed sources used in the experiment.*

	Protein Source		
Amino acid	Fish	Gluten	Sunflower
Arginine	38.9	19.8	30.6
Histidine	21.7	10.9	9.1
Isoleucine	25.8	23.6	17.6
Lysine	48.2	9.2	9.8
Leucine	45.4	95.1	24.1
Methionine	19.0	16.1	7.6
Phenylalanine	24.9	35.7	18.3
Proline	31.0	56.3	17.6
Threonine	26.3	19.6	12.5
Tryptophan	6.8	3.8	5.3
Valine	31.4	25.2	20.2
TSAA[1]	25.6	27.7	14.0
Proximate analysis (as is basis):			
Crude protein	605.4	648.6	364.9
Calcium	21.4	0.2	4.5
Phosphorus	16.8	4.9	9.9
Fat	75.1	11.1	23.0
Available lipid	98.2	15.0	77.5
Ash	20.1	2.6	6.6
Moisture	89.5	73.3	81.0
MJ AME kg^{-1}	12.9	9.8	8.9

TSAA[1], Total Sulphur Amino Acids.

Table 3.3 *The essential amino acid requirements (g kg^{-1} diet) of broiler chickens between 7 to 21 days of age according to various authors.*

Amino acid	Source Gous* (1996)	NRC (1994)	Leclercq *et al.*, (1987)
Arginine	12.00	12.50	13.40
Histidine	4.20	3.50	5.00
Isoleucine	7.20	8.00	9.60
Lysine	12.00	11.00	12.40
Leucine	12.60	12.00	17.30
Methionine	4.00	5.00	5.20
Phenylalanine	7.20	7.20	-
Proline	6.00	6.00	-
Threonine	7.60	8.00	7.40
Tryptophan	1.90	2.00	2.30
Valine	8.30	9.00	10.80
TSAA	7.40	9.00	9.30

*EFG Broiler simulation model

TSAA, Total Sulphur Amino Acids.

Table 3.4 *Proportions of the three basal feeds used.*

Treatment	Proportion of each basal feed		
	Fish	Gluten	Sunflower
1	1.00	0.00	0.00
2	0.00	1.00	0.00
3	0.00	0.00	1.00
4	0.67	0.33	0.00
5	0.67	0.00	0.33
6	0.00	0.67	0.33
7	0.33	0.67	0.00
8	0.33	0.00	0.67
9	0.00	0.33	0.67
10	0.33	0.33	0.33
11	0.66	0.17	0.17
12	0.17	0.66	0.17
13	0.17	0.17	0.66
14	+	-	+
15	-	+	+
16	+	+	-
17	+	+	+

3.3 RESULTS

The main effect of sex and the interaction of sex by feed were not significant ($p<0.05$) even though in most cases, the male broilers consumed more food and had higher weight gains than the females (Appendix 3.1). Because there were no statistically significant differences between the sexes the results were pooled across the sexes (Table 3.5). Maximal growth and FCE were obtained on the T5 (FM:SF, 67:33 feed mixture). The lowest response in all biological variables was obtained on T2. Birds offered T2 and T6 had significantly ($p<0.05$) lower weight gain and feed intake than other treatments.

The amino acids present in the feed as a proportion of the birds requirement for all feeding treatments is shown in Appendix 3.2. This was based on the requirement for essential amino acids as suggested by Gous (1996) which was similar to the NRC (1994) specifications. Figures 3.33.4, 3.5 and 3.6 show the amino acids present in the fishmeal, maize gluten meal, sunflower oilcake meal basal feeds and the FM:MG:SF (0.33:0.33:0.33) feed mixture as a proportion of the birds requirement.

It is difficult to interpret the responses to all the 13 mixture treatments without making use of some organizing system. Use was made of calculations based on an empirical model and response surface contour plots. Sequential F-tests indicated that the quadratic effects were highly significant ($P<0.05$). A quadratic model in three components was therefore fitted to the biological responses. This was based on the null hypothesis : the model provides an adequate description of the response. The output obtained from fitting the augmented simplex lattice design with a quadratic mixture model in three components for weight gain, food intake and FCE are shown in Appendices 3.3, 3.4, 3.5 and 3.6. Figures 3.7, 3.8 and 3.9 presents the response surface contour plots for the estimated response functions. These contours were plotted using the MATLAB (1995) software. The linear and special cubic fitted models were found to have a significant lack of fit at the 0.05 level for the biological responses. However, the quadratic model displays no significant ($P<0.05$) lack of fit for the responses and has a larger adjusted-R^2, whereas the other models do not. The quadratic model did give a better representation of the response surface of this mixture system within the experimental error of the data.

Table 3.5 *Response of broiler chickens to feeding treatments from 7 to 21 days of age.*

Treatment	Food intake (g bird d^{-1})	weight gain (g bird d^{-1})	FCE[1] (g gain /kg food)
1	34.18^{bcde}	11.27^{cd}	315.00^{bcde}
2	16.80^{f}	2.33^{f}	135.00^{f}
3	29.74^{cde}	10.51^{cde}	354.00^{abcd}
4	36.38^{bcd}	11.58^{cd}	322.00^{bcde}
5	46.67^{ab}	20.88^{a}	447.00^{a}
6	22.65^{ef}	5.44^{ef}	240.00^{e}
7	27.86^{de}	8.66^{cde}	310.00^{bcde}
8	40.02^{abc}	17.26ab	413.00^{ab}
9	27.46^{de}	7.24^{de}	272.00^{cde}
10	39.57^{abcd}	14.17^{bc}	362.00^{abc}
11	40.64^{abcd}	12.10^{dc}	297.00^{cde}
12	29.62^{cde}	7.48^{de}	256.00^{de}
13	35.10^{cde}	11.80^{cd}	335.00^{bcde}
14	49.40^{a}	18.41^{ab}	374.00^{abc}
15	34.38^{bcde}	8.84^{cde}	259.00^{cde}
16	44.01^{ab}	14.38^{bc}	319.00^{bcde}
17	51.58^{a}	18.52^{ab}	360.00^{abcd}

^{a,b,c,d,e,f} Means within a column with different superscripts are significantly different ($p<0.05$);

FCE[1] Food conversion efficiency

Table 3.6 *Comparison of basal feeds chosen by the birds as a proportion of the total.*

Treatment	Basal Feed	Mean Proportion	SD	P-Value	
14	FM	0.5975	0.1164	0.1900	n.s.
14	SF	0.4027	0.1166	0.1900	n.s.
15	MG	0.0463	0.0172	0.0000	*
15	SF	0.9538	0.0172	0.0000	n.s.
16	FM	0.9332	0.0347	0.0007	*
16	MG	0.0665	0.0343	0.0001	*
17	FM	0.6090	0.0533	0.0019	*
17	MG	0.0557	0.0172	0.0001	*
17	SF	0.3364	0.0615	0.9300	n.s.

Hypothesis for T14-T16

Mean = 0.500

Hypothesis for T17

Mean = 0.333

n.s. not significantly different from 0.500 (T14-T16) or 0.333 (T17)

otherwise significantly different from 0.500 (T14-T16) or 0.333 (T17) at P<5% (*)

The analysis of variance for the fitted quadratic models test the hypothesis that the response surface is a level plane above the simplex region.

This hypothesis is :

$H_o : \beta_1 = \beta_2 = \beta_3 = \beta, \beta_{12} = \beta_{13} = \beta_{23} = 0$

H_1 : At least one equality is false

The test statistic for weight gain, food intake and FCE are F = 26.15 15.47 and 7.20 respectively, each with a P = 0.00, thus the above hypothesis is rejected.

A quadratic model in three components was therefore fitted to all the biological responses since it did provide a better predicted description of the mixture system.

Because the regression coefficients (Appendix 3.6) $\beta_1 > \beta_3 > \beta_2$, it was observed that component 1(FM) produced the greatest weight gain and a better FCE when fed as a sole protein source as illustrated in the contour plots in Figs. 3.7-3.9.

The regression coefficients β_{12} and β_{13} are positive for weight gain, feed intake and FCE, whilst b_{23} is negative for weight gain and FCE. Blending components 1 (FM) and 2 (MG), 1 and 3 (SY), gave rise to a higher weight gain, feed intake and FCE than the predicted and observed biological responses of the pure blends. The binary quadratic blend term x_2*x_3 for FCE and weight gain have antagonistic blending effects because the coefficients are negative. Thus mixtures of SF and MG gave rise to lower weight gains than mixtures of SF and FM as illustrated in the response surface contour plots.

Choice-fed birds maximized their feed intake on T17 and had weight gain and FCE on T14 and T17 which was similar to that regarded as maximal for birds offered the T5 protein mixture. A comparison of the mean proportions of each dietary protein feedstuff chosen by the chickens given the choice of two or three components as a proportion of the total is presented in Table 3.6. The one-sample t-test computations on the means of food intake indicated that the amount of each basal feed chosen by the birds as a proportion of the total, differed significantly ($P<0.05$) from 0.5 (in the two-component choice feeding treatments) for FM and SF in T14 and from 0.33 (in the three-component treatment) for FM and MG in T17.

As the proportion of FM or SF in mixtures of these two protein basal feeds from 17 to 67%

improved the biological performance of birds above that obtained if either had been fed alone. However, improvement was greater for feeds containing a higher proportion of FM than of SF. As the proportion of MG in feeding mixtures increased from 17% to 67% of the feeding treatment, birds tended to discriminate against such feeds, and exhibited lower feed intakes and weight gains. Similar observations were made by Calet (1967) with chickens receiving increasing amounts of mixtures of FM and MG. In other experiments Gous, van Niekerk, Neil and Crichton (1995) offered a series of six experimental diets, ranging in maize gluten meal (MG) concentration :0, 3, 6, 9, 12 and 15 % MG to laying hens. They also observed a significant (p=0.019) downward trend relating to increasing levels of maize gluten in the feed

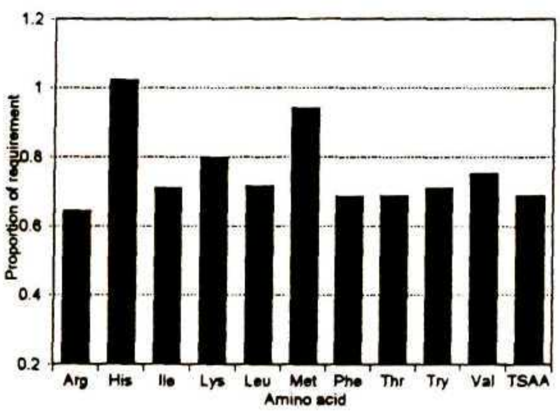


Fig. 3.3 *Amino acids present in fishmeal basal feed (FM) as a proprotion of requirement.*

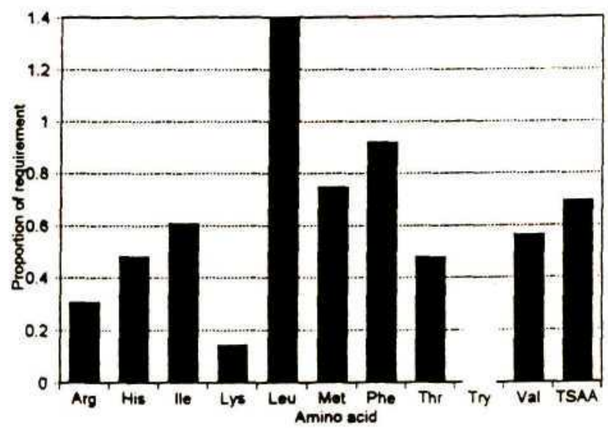


Fig. 3.4 *Amino acids present in maize gluten basal feed (MG) as a proprotion of requirement.*

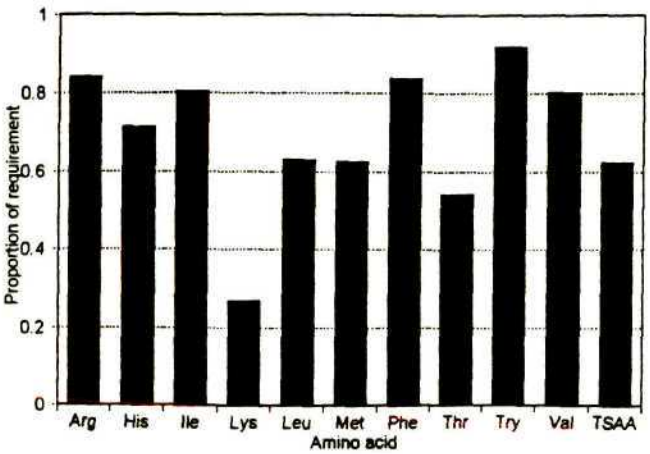


Fig. 3.5 *Amino acids present in sunflower oilcake meal basal feed (SF) as a proprotion of requirement*

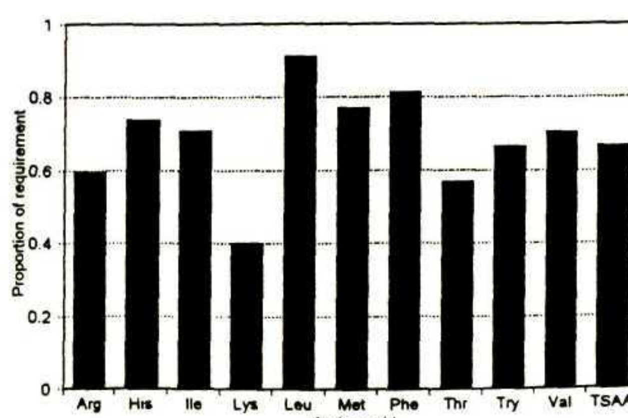


Fig. 3.6 *Amino acids present in FM:MG:SF (0.33:0.33:0.33) feed mixture as a proprotion of requirement*

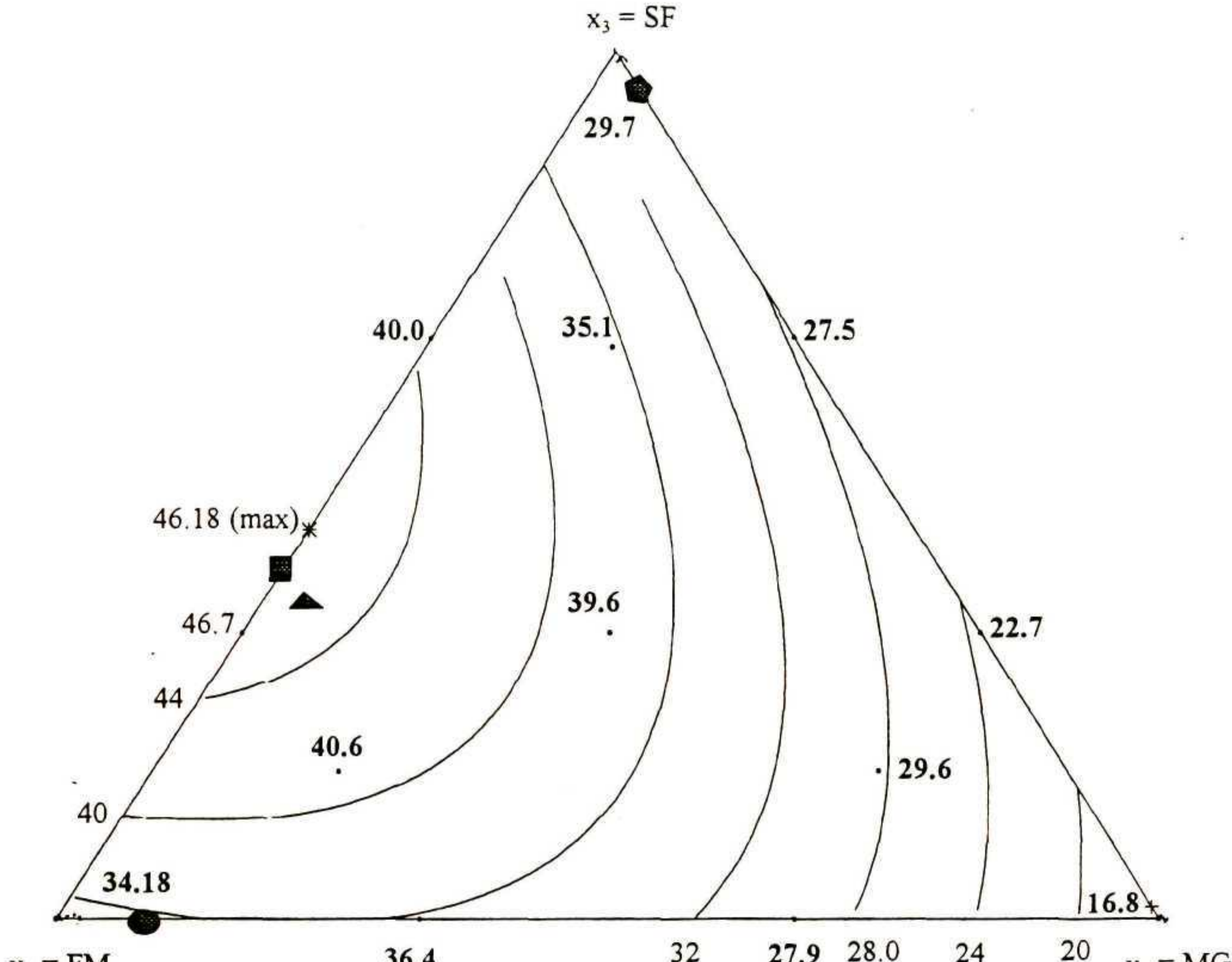


Figure 3.7. *Response surface contours plots for feed intake (g/bird d).*

The predicted maximum is 46.2 g/bird day, and would occur where $x_1 = 0.55$, $x_2 = 0.00$, $x_3 = 0.45$. The observed values are highlighted in bold.

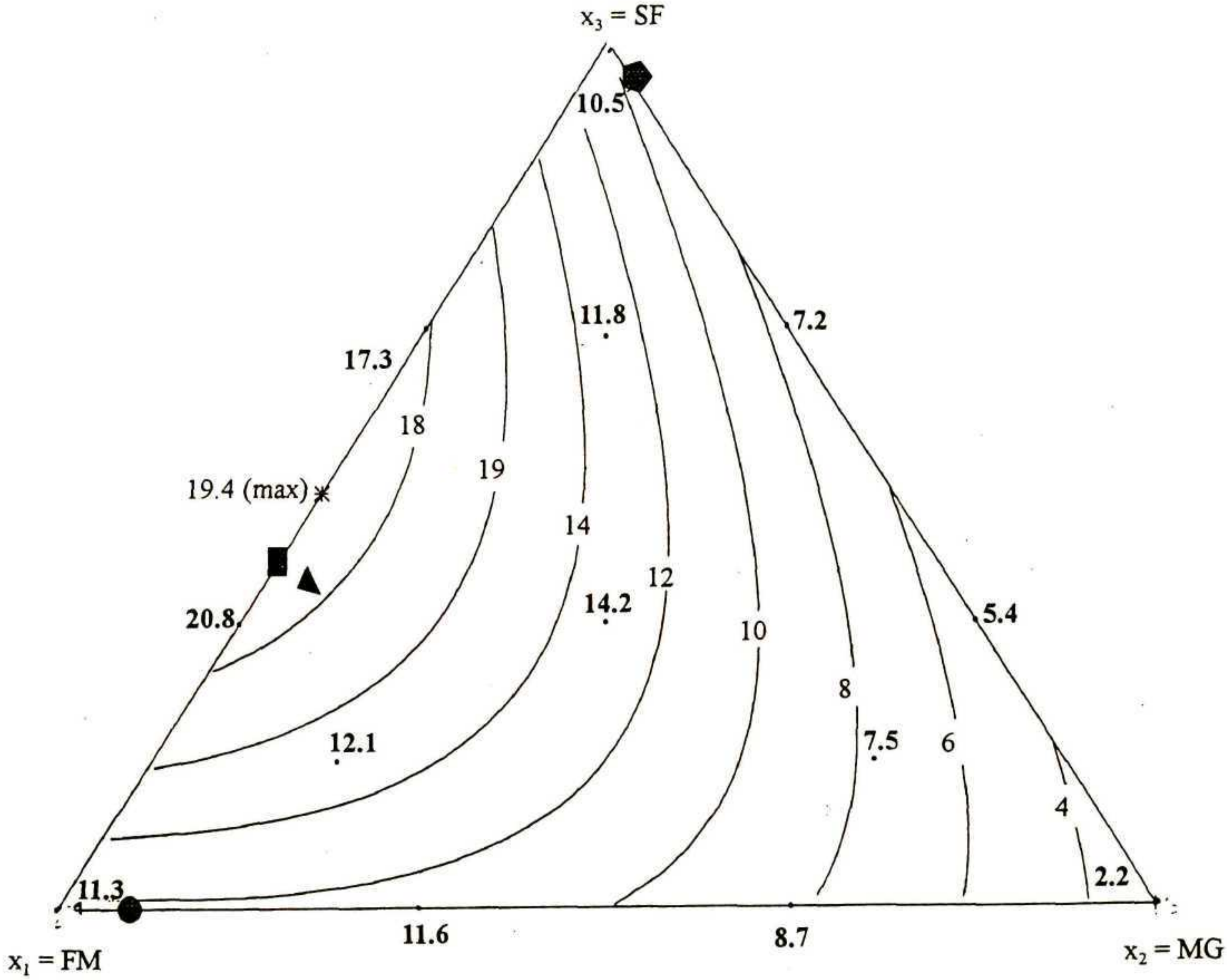


Figure 3.8. *Response surface contour plots for weight gain (g/bird day).*

The predicted maximum for weight gain is 19.4 g/bird day and occurs where $x_1 = 0.52$, $x_2 = 0.00$, and $x_3 = 0.48$

The observed values are highlighted in bold.

Choice

■ FM - SF (T14)

⬟ SF - MG (T15)

● MG - FM (T16)

▲ FM - MG - SF (T17)

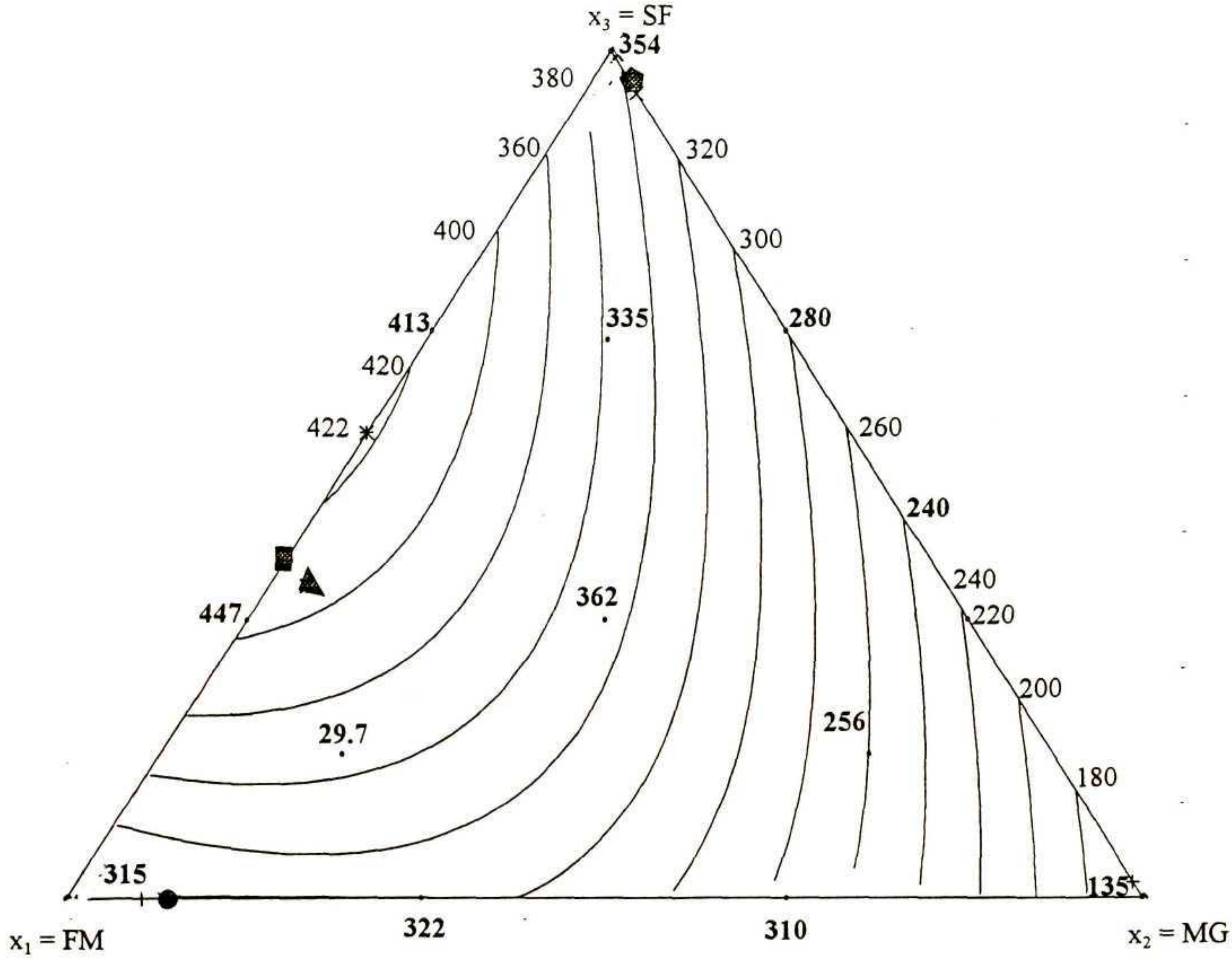


Figure 3.9. *Response surface contour plot for feed conversion efficiency (g gain/kg feed).*

The predicted maximum FCE is 422.8(g gain/kg feed) and occurs where $x_1 = 0.45$, $x_2 = 0.00$, $x_3 = 0.55$. The observed values have been highlighted in bold

Choice

- FM - SF (T14)
- SF - MG (T15)
- MG - FM (T16)
- FM - MG - SF (T17)

3.4 DISCUSSION

The results showed that mixtures of protein sources did enhance the biological performance of broilers than any of the protein sources offered as a sole source of protein. After a few days of feeding, birds offered T2 (MG) avoided this feed and had a reduced nutrient intake and growth. The aversion to MG may be a learned response, and thus palatability may not be responsible for the decrease in feed intake. A deficiency of lysine in T2, T3, T6, T9 and T12 and the high leucine levels in T2 (leucine at 1.40 times the requirement) (Appendix 3.2) may have been worsened by a general amino acid imbalance resulting in adverse biological performance on these feeds. The depression in chick growth confirms similar observations by Burham *et al.* (1992) that dietary leucine at 1.76 times the requirement depresses chick growth. D'Mello (1979) reported that excess leucine intake occurs widely because maize gluten meal is high in this amino acid.

An excess of leucine in T2 and MG-containing feeds may have enhanced isoleucine and valine requirements of the chicks (D'Mello and Lewis, 1970; D'Mello, 1994). Furthermore, excesses of amino acids are deaminated and nitrogen is excreted as uric acid in birds, this results in their inefficient utilization . The experimental results agree with reports that dietary deficiencies of indispensable amino acids or an amino acid imbalance affects food intake and performance (Harper, *et al.*, 1970; Gous, 1992; D'Mello, 1994; NRC, 1994; Forbes, 1995; Mc Donald *et al.*, 1991). Chicks fed diets that are deficient in any one amino acid had comparatively lower biological performance, probably due to their inability to make use of all the essential and non essential amino acid for protein synthesis. This could have resulted in unutilized amino acids. A notable feature of such a deficiency was a rapid reduction in feed intake.

An increase in the proportion of FM or SF in mixtures of these two protein basal feeds from 17 to 67% did improve the biological performance of birds above that obtained if either had been fed alone. The improvement was however greater for feeds containing a higher proportion of FM than SF. This confirms similar "synergistic" blending effects observed by Woodham and Deans (1977). On the contrary, as the proportion of MG in feeding mixtures increased from 17% to 67% of the feeding treatment, birds tended to discriminate against such feeds, and exhibited lower feed intakes and weight gains.

Besides amino acid imbalances, deficiencies and antagonisms, certain other factors may also account for the refusal of birds to consume large quantities of MG or MG-containing feeds. Factors such as the physical form of ingredients, composition of the food, the way in which feed protein ingredients are associated in the diet, and the non-protein portions of the feed are all likely to play a role in the control system enabling a bird to select suitable quantities of food to satisfy its nutritional requirements (Calet, 1967; D'Mello 1978; Forbes 1995 and Mc Donald *et al.*, 1991).

A comparison between birds offered a three-choice mixture of MG, SF, and FM (T17) and the two-choice regimes T14, T15, and T16 showed that weight gain and feed intake for the former was higher. Birds offered T17 had significantly ($p<0.05$) higher weight gain and food intake than those offered T11, T12 or T13, but this was not significantly different ($p<0.05$) from T10. These experimental observations prove that as the balance of amino acids in the feed consumed improved, the performance of birds on such feeds also improved (Woodham and Deans, 1977). Additionally, the birds were able to choose from two or three-feed choices a feed blend that was nutritionally adequate, thus confirming similar reports by Cowan and Michie (1978); Belyavin (1993); Forbes and Shariatmadari (1994) and Mc Donald *et al.*, (1991) and Forbes (1995).

In all cases the birds on the choice treatments chose the proportions that were predicted by the mixture experiment to maximise performance. Birds on T14 (FM-SF) and T17 (FM-SF-MG) chose proportions closest to the maximum predicted (0.52, 0.48, 0.00). Birds on T15 (SF-MG) chose SF almost exclusively whilst those on T16 (FM-MG) also chose FM almost exclusively. It can be deduced from these results that birds chose a higher proportion of the feed closer to adequacy whilst avoiding the MG feed which was inadequate to give the highest possible response (Table 3.6)

3.4 CONCLUSION

In this mixture trial, 83.8, 75.4, and 65.2% of the total variances in weight gain, feed intake and FCE respectively are explained by the quadratic models. In general, birds given *ad libitum* access to the FM and SF or to the three protein sources simultaneously as a choice maximized their performance, which was comparable to that predicted by the model. In this experiment, maize gluten meal was found to be inappropriate as a protein source for broiler growth. Broiler chickens demonstrated the ability to choose the combination of two- or three feeds that maximised performance (i.e. weight gain and FCE). When a feed is formulated (i.e. with the WinFeed formulation programme) using the three components with the objective of minimising the total essential amino acids (TEAA), it was found that the formulation that maximises broiler performance (51:44:5) of FM:SF:MG was close to the optimum chosen.

CHAPTER 4

MIXTURE EXPERIMENTS USING FISHMEAL, SUNFLOWER OILCAKE MEAL, AND SOYBEAN MEAL WITH BROILERS AGED 7 TO 21 DAYS

4.1 INTRODUCTION

In the previous experiments (1 & 2), maize gluten meal was selected against, being the most badly balanced amino-acid containing mixture offered to the broilers in these trials. Based on this actual evidence and in an attempt to use a different combination of ingredients, soybean meal was substituted for maize gluten meal in the experiment reported in this chapter.

The potential for a broiler chicken to attain optimum growth depends on its voluntary intake of feed, which is reduced if there is an accumulation of excess amino acids (Waldroup *et al.*, 1976). Therefore, limiting the excesses of essential amino acids, will increase feed intake and improve the biological performance of broiler chickens. Leclerq *et al.* (1987) also reported that excess quantities of essential amino acids will not reduce biological performance of broiler chickens as long as certain imbalances are avoided.

There is evidence that food intake is increased on marginal deficiencies, and that it is only when the deficiency is severe that food intake declines. Such a decline and invariably a deficiency of an amino acid, results in an impairment of body weight gain (D'Mello, 1994). This is due to a lowered efficiency of utilization of dietary protein.

Soybean meal is characterized by a low fibre content and a high degree of availability and digestibility of amino acids (Balloun, 1980). It is however deficient in methionine whilst sunflower oilcake is deficient in lysine (Saunders, 1974; Balloun, 1980). Most plant proteins widely used in poultry starter feeds contain only about half the natural lysine concentration of soybeans. Feeding very high levels of soybean meal may not always be practical and fishmeals are very expensive. In view of the abovementioned facts, partly replacing fishmeal with soybean meal and sunflower meal in broiler feeds, whilst ensuring a proper balance of amino acids along with minerals and vitamins, may be more beneficial to the biological performance of broilers than either of them fed alone. In fact, Woodham and Deans (1977) indicated that the performance of birds given a mixture of feeds is sometimes greater (i.e. they exhibit some synergistic effect) than would be predicted when the components are fed singly.

This experiment was carried out to determine the effect on the biological performance of providing three basal feeds in which the only protein source in each basal was fishmeal (FM), sunflower oilcake meal (SF) or soybean oilcake meal (SY). These basal feeds were fed *ad libitum* either alone, or as two- or three free-choice or predetermined mixtures. This was carried out to determine response surfaces in biologically important variables such as weight gain and food conversion efficiency, the purpose being to compare the combination of components that maximises biological performance with the combination chosen by birds given *ad libitum* access to the two or three components simultaneously.

4.2 MATERIALS AND METHODS:

This experiment was a repeat of the previous experiment with a few modifications listed. The protein, amino acid and AME (MJ kg^{-1}) contents of the protein sources (Appendix 4.1) were determined as in Experiment 1.

Three basal feeds viz : fishmeal (FM), sunflower oilcake meal (SF) and soybean meal (SB) were formulated to contain 120g crude protein kg^{-1} and 12.56 MJ AME kg^{-1}. The basal feeds were iso-energetic as well as iso-nitrogenous and were mixed at the onset of the experiment. The composition of each basal feed is shown in Table 4.1.

The variables measured

The criteria studied were body weight gain (g bird d^{-1}), food intake (g bird^{-1}d) and food conversion efficiency (g body weight gain/kg food intake).

Table 4.1 *Composition (g kg⁻¹) and calculated analysis of basal feeds used in the experiment.*

Ingredient	Basal Feeds		
	FM	SY	SF
Soybean meal		276.0	
Sunflower oilcake meal			315.0
Fishmeal	183.0		
Sugar	280.0	257.0	270.0
Starch	280.0	257.0	270.0
Sunflower oil	32.0	46.0	46.0
Monocalcium phosphate	4.0	19.0	18.0
Limestone	5.5	17.0	17.0
Salt	0.7	4.7	4.0
Filler (sand)	212.0	121.0	55.0
Vitamin + mineral premix [1]	2.50	2.5	2.5
Calculated analysis:			
Crude Protein	120.0	120.0	120.0
ME(MJ Kg^{-1})	12.6	12.6	12.6
Calcium	10.0	10.0	10.0
Phosphorus (avail.)	5.0	5.0	5.0

[1] The vitamin premix consisted of (mg g^{-1}) : thiamin 35, riboflavin 23, pyridoxine 8, biotin 0.8, pteroylmonoglutamic acid 5.7, menaphthone 7, cyanacobalamin 0.03, nicotinic acid 213, ascorbic acid 354, retinol (ug g^{-1}) 212.7, cholecalciferol (2000 ug g^{-1}) 10.2, alpha-tocopherol (250 mg g^{-1}) 6, maize starch 125. The mineral premix consisted of (mg g^{-1}):KH_2PO_4 479, NaCl 365, ferric citrate 23, $MgSO_4.H_2O$ 9, KI 0.46, $CuSO_4$ 0.58, $ZnCO_3$ 9, $Na_2MoO_4.H_2O$ 0.46.

Table 4.2. *Proportions of the three basal feeds used.*

Treatment	Proportion of each basal feed		
	Fish	Sunflower	Soybean
1.	1.00	0.00	0.00
2.	0.00	1.00	0.00
3.	0.00	0.00	1.00
4.	0.67	0.33	0.00
5.	0.67	0.00	0.33
6.	0.00	0.67	0.33
7.	0.33	0.67	0.00
8.	0.33	0.00	0.67
9.	0.00	0.33	0.67
10.	0.33	0.33	0.33
11.	0.66	0.17	0.17
12.	0.17	0.66	0.17
13.	0.17	0.17	0.66
14.	+	-	+
15.	-	+	+
16.	+	+	-
17.	+	+	+

Statistical analysis

The biological response data were analysed for statistical differences between treatments as in Experiment 2.

4.3 RESULTS

The main effect of sex and the interaction of sex by feed on the three variables measured (weight gain, feed intake and FCE) were not significant ($p<0.05$) (Appendix 4.2). Because there were no significant differences between the sexes the results were pooled across the sexes (Table 4.3)

The amino acids present in the feed blend as a proportion of the listed requirement (i.e. EFG broiler simulation model) are presented in Appendix 4.3. Of all the amino acids, lysine was the most limiting and provided less than 50% of the broiler chicken's requirements in treatments 2, 6, 7 and 12.

Results for the mixture experiment show that maximal growth and FCE was obtained on T11 but this was not significantly different from T10. Broilers maximized their feed intake on T2, but the difference was small and not significantly different from T1, T4, T5, T6, T8, T9, T10, T12 and T13 at the 5% level.

Growth improved with the use of mixtures of these protein sources but chicks fed higher levels of SF and SY, of up to 67% of the feed had lower weight gains than those fed similar levels of FM in feeding mixtures. As the proportion of FM in feeding mixtures increased from 16.7% to 66.7% of the feeding treatment, biological performance of birds does improve significantly.

A sequential model fitting exercise was carried out on the response data collected at points of the augmented {3,3} simplex lattice design. The analysis of variance when fitting a quadratic model to the biological response data (weight gain, feed intake, FCE) obtained when broilers were offered mixtures of three protein sources, are shown in Appendix 4.3, 4.4 and 4.5. The regression coefficients obtained by fitting a quadratic or full cubic model to the biological responses are shown in Appendix 4.6. The mathematical model fitted to the biological responses was based on the null hypothesis : the model provides an adequate description of the response or the response is a constant at all points in the simplex.

Table 4.3. *Response of broiler chickens to feeding treatments from 7 to 21 days of age .*

Treatment	Food Intake (g bird d^{-1})	Weight gain (g bird d^{-1})	FCE[1] g gain/kg food
1	45.34^{abc}	15.37^{cd}	339.1^{cdef}
2	51.63^{a}	7.28^{f}	141.0^{h}
3	43.30^{bc}	10.74^{e}	250.0^{g}
4	48.34^{ab}	16.78^{bcd}	348.5^{bcdef}
5	45.70^{abc}	16.18^{bcd}	354.5^{cdef}
6	48.54^{ab}	13.69^{d}	282.3^{fg}
7	40.56^{c}	15.74^{cd}	396.5^{abcd}
8	48.63^{ab}	14.73^{cd}	302.3^{efg}
9	46.82^{ab}	14.86^{cd}	317.2^{defg}
10	47.07^{ab}	17.94^{abc}	381.1^{abcde}
11	42.95^{bc}	19.16^{ab}	447.1^{a}
12	47.45^{ab}	13.99^{d}	294.9^{efg}
13	47.69^{ab}	14.27^{d}	299.7^{efg}
14	47.10^{ab}	20.57^{a}	436.7^{ab}
15	36.46^{d}	13.35^{d}	369.0^{abcdef}
16	42.84^{bc}	18.00^{abc}	419.4^{abc}
17	46.75^{ab}	19.17^{ab}	410.3^{abc}

^{a,b,c,d,e,f} Means within a column with different superscripts are significantly different ($p<0.05$);

FCE[1] Food conversion efficiency

Table 4.4 *Basal feeds chosen by the birds as a proportion of the total food consumed.*

Treatment	Basal Feed	Mean Proportion	SD	P-Value	
14	FM	0.7022	0.0676	0.0093	*
14	SF	0.2978	0.0676	0.0093	n.s.
15	SF	0.5339	0.0786	0.4500	n.s.
15	SY	0.4661	0.0786	0.4500	n.s.
16	FM	0.7942	0.0662	0.0030	*
16	SY	0.2058	0.0662	0.0030	*
17	FM	0.6049	0.0910	0.0094	*
17	SF	0.2792	0.0612	0.1800	n.s.
17	SY	0.1159	0.0515	0.0035	*

Hypothesis for T 14-T16

Mean = 0.500

Hypothesis for T17

Mean = 0.333

n.s. . not significantly different from 0.500 (T14-T16) or 0.333 (T17)

otherwise significantly different from 0.500 (T14-T16) or 0.333 (T17) at P<5% (*)

An F-Test for the quadratic model displays no significant lack of fit for weight gain and FCE (Appendix 4.3 and 4.5). Each of the linear, quadratic, and special cubic models had a lack of fit at the 0.05 level for feed intake. The full cubic model displays no significant lack of fit for food intake (Appendix 4.4) and has a larger adjusted R^2, whereas the other models do not. The analysis of variance for the quadratic model shown in Appendix 4.3 and 4.5 tests the hypothesis that the response surface is a level plane above the simplex region.

This hypothesis is :

H_o : $\beta_1 = \beta_2 = \beta_3 = \beta$, $\beta_{12} = \beta_{13} = \beta_{23} = 0$

H_1 : At least one equality is false

The test statistics for weight gain and FCE are F = 31.44 and 16.88 respectively, each with a P = 0.00, thus the above H_o hypothesis is rejected. The quadratic and full cubic effects were highly significant ($P<0.05$). On the basis of these statistics, these models gave a better representation of the response surface of this mixture system within the experimental error of the data.

The regression coefficients $\beta_1 > \beta_3 > \beta_2$, component 1 (FM) therefore produces the greatest weight gain and the best FCE in terms of single components fed alone. The regression coefficients β_{12} and β_{13} are positive for weight gain and FCE, whilst β_{13} and β_{23} are positive for feed intake. Thus blending components 1 (FM) and 2 (SF), 1 and 3 (SY), will be expected to give rise to a higher weight gain and FCE than would be expected from an average of the responses of the pure blends as observed from the actual biological responses.

The binary quadratic blend term $x_1 * x_2$ and the cubic model term $x_1 * x_3 * (x_1 - x_3)$ for feed intake have antagonistic blending effects because thier coefficients are negative. Blending these protein sources is not likely to appreciably improve the feed intake of broilers.

The response surface contour plots for weight gain, feed intake and FCE are shown in Figures 4.1, 4.2 and 4.3. It is worth noting that the predicted and observed results are similar. The predicted maximum in terms of pure components (protein basal feeds) for weight gain was 18.08 g bird d^{-1}, where $x_1 = 0.57$, $x_2 = 0.28$, $x_3 = 0.15$. Authenticating these results, a reasonably close weight gain of 19.16 g bird d^{-1} was observed for the FM:SF:SY (0.67, 0.17, 0.17) feed mixture.

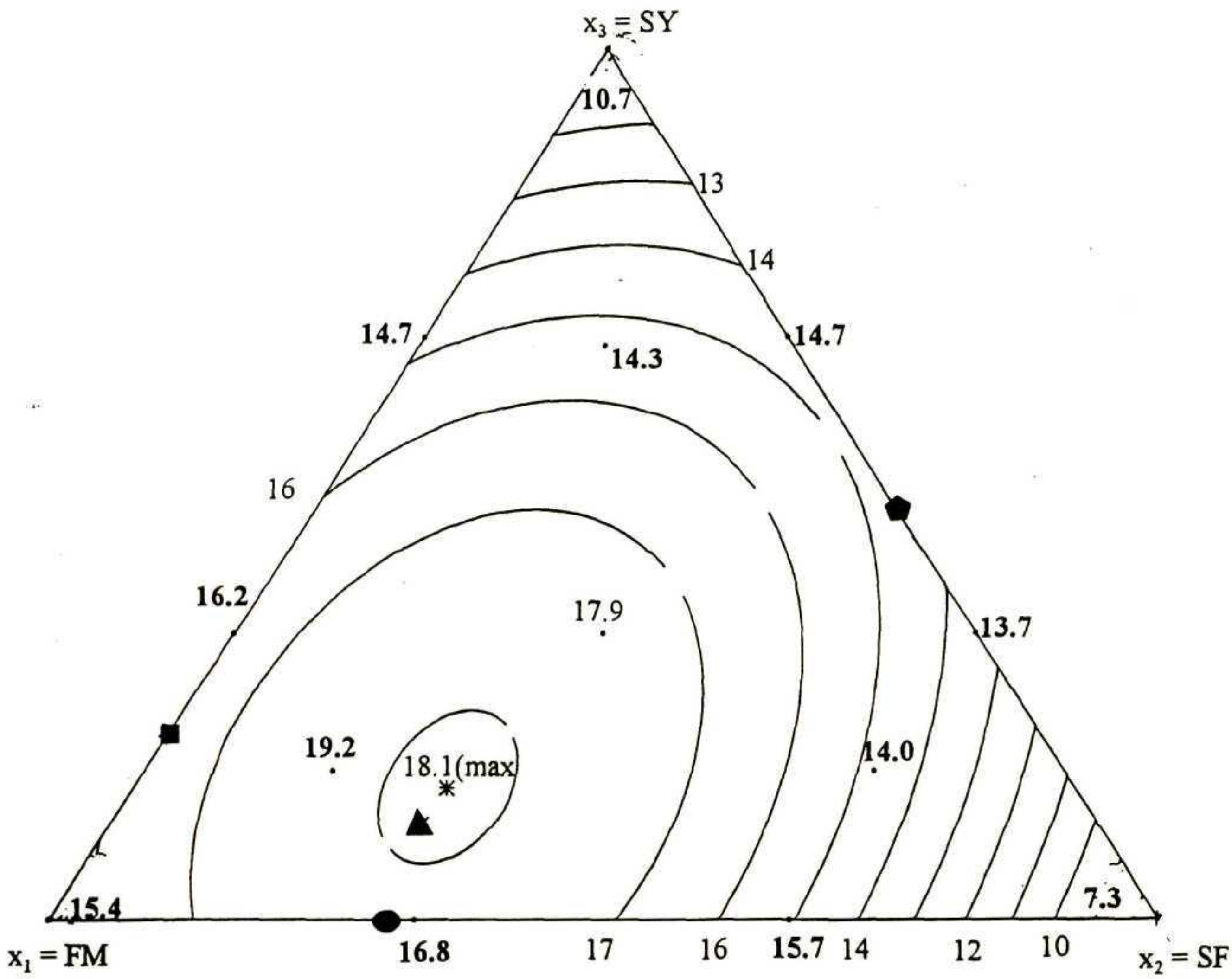


Figure 4.1. *Response surface contour plot for weight gain (g/bird d).*

The predicted maximum for weight gain is 18.1 g/bird d, and occurs where $x_1 = 0.57$, $x_2 = 0.28$, $x_3 = 0.15$.
The observed values are highlighted in bold.

Choice

■ FM - SY

⬟ SF - SY

⬤ FM - SF

▲ FM - SF - SY

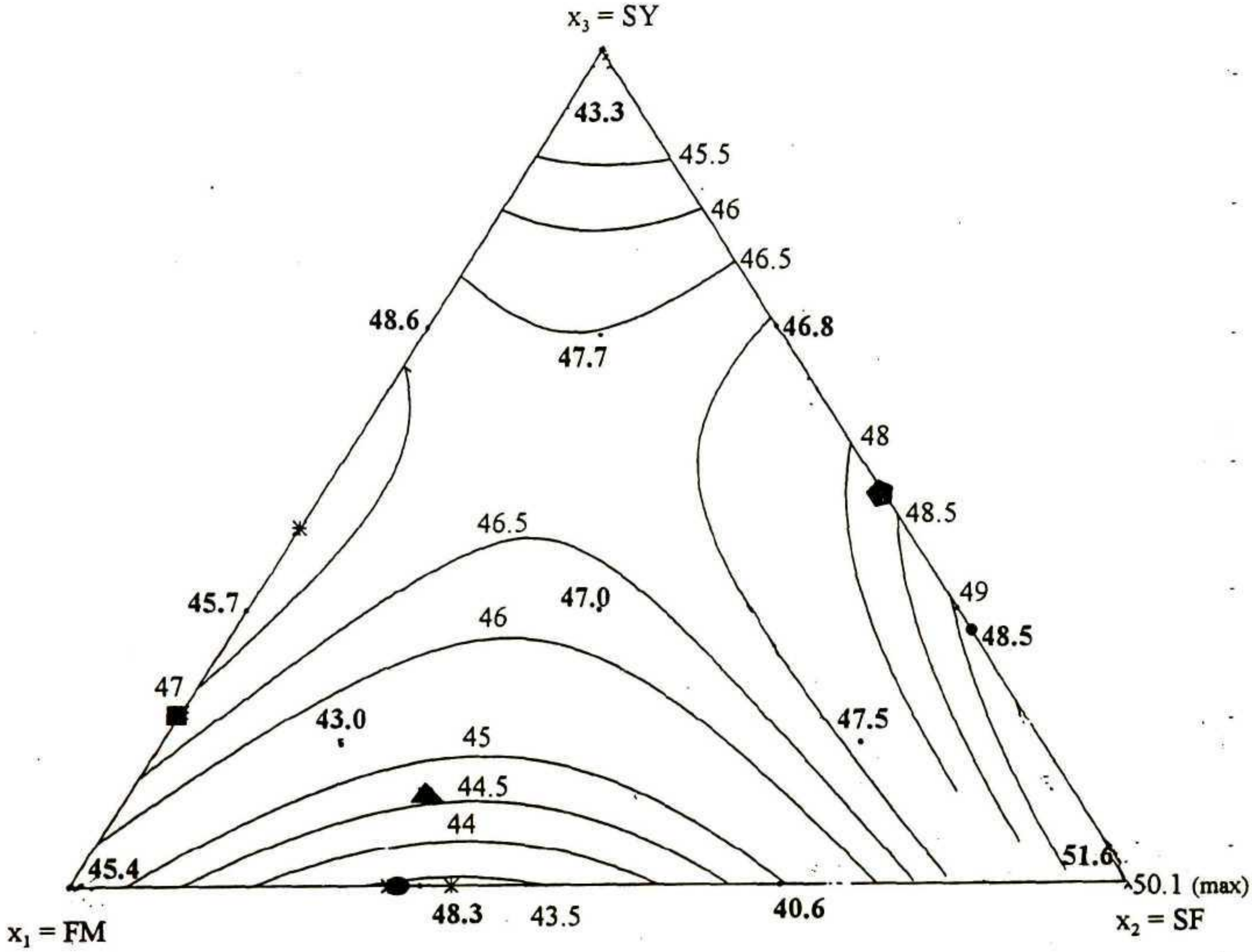


Figure 4.2. *Response surface contour plot for food intake (g/bird day).*

The predicted maximum for food intake is 50.1 g/bird day, and occurs where $x_1 = 0$, $x_2 = 1$, $x_3 = 0$
The observed values are highlighted in bold.

Choice

■ FM - SY

⬟ SF - SY

● FM - SF

▲ FM - SF - SY

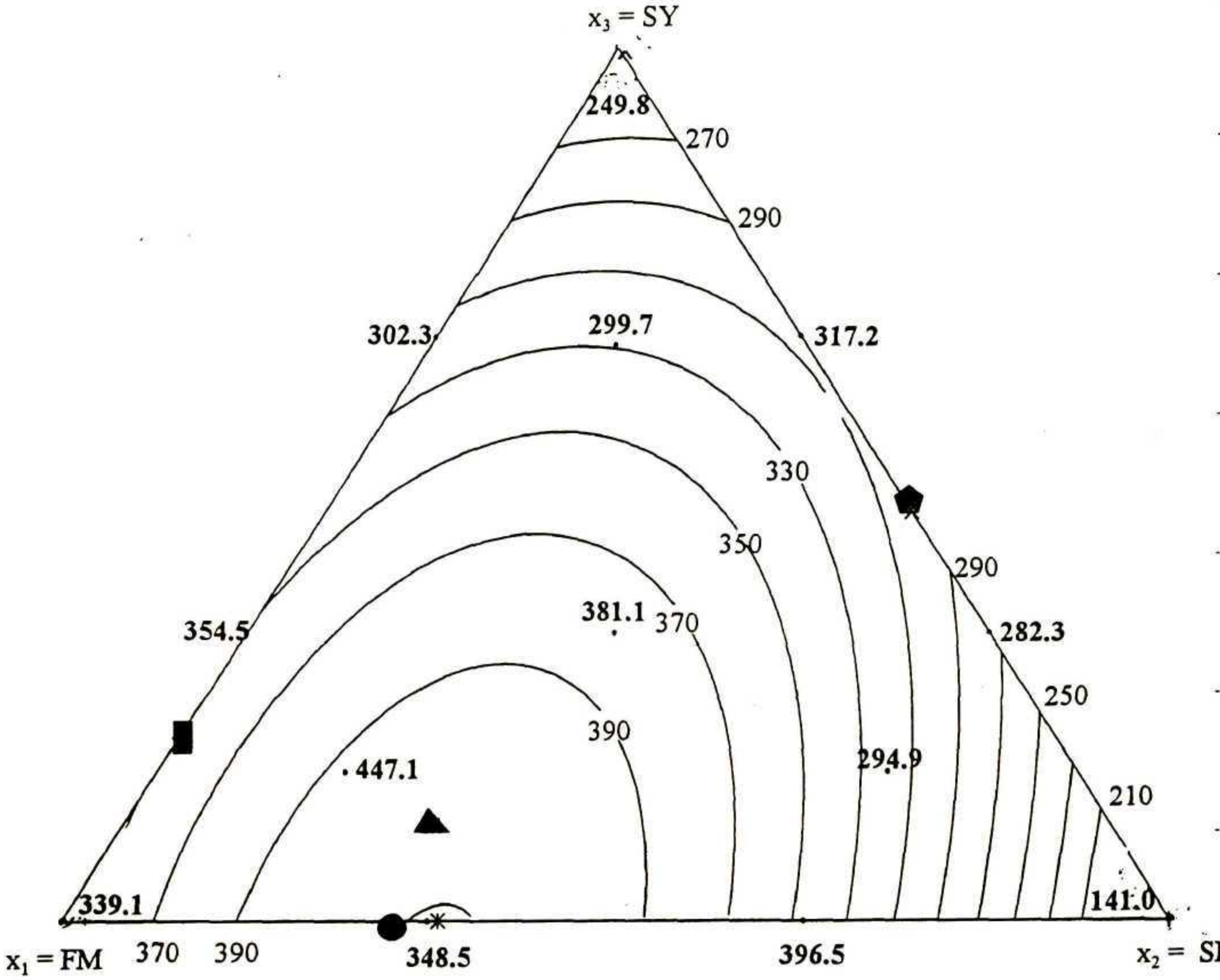
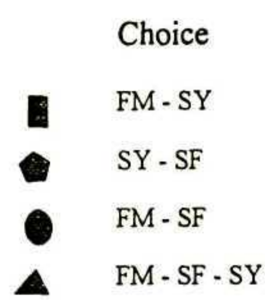


Figure 4.3. *Response surface contour plot for feed conversion efficiency (g gain/kg food).*

The predicted maximum FCE of 410.5 occurs where $x_1 = 0.66$, $x_2 = 0.34$, $x_3 = 0.00$.
The observed values are highlighted in bold.



When offered a choice between two or three feeds differing in amino acid balance broilers maximised growth on T14 (FM-SF choice). The data for the free-choice fed birds however showed no significant differences in either weight gain amongst birds offered T14, T16 and T17 or FCE amongst those fed T14, T15, T16, and T17. Birds offered T16 and T14 had significantly ($p<0.05$) lower food intake than T15 and T17.

The amount of each basal feed chosen by the birds as a proportion of the total food consumed for T14-T17, differed significantly ($P<0.05$) from 0.5 (in the two-component choice feeding treatments) for FM in T14 and FM and SY in T16 and from 0.33 (in the three-component treatment) for FM and SY in T17 (Table 4.4). The amount of FM chosen in all cases was greater than 60% of the total feed consumed, whilst SY chosen was less than 50%. Treatment 15 was the only choice fed treatment that was limiting in lysine (i.e. 48% of requirement). Broiler chickens were able to choose reasonably close proportions that were predicted by the mixture experiment to maximise weight gain and FCE (Table 4.5). Overall, choice fed birds maximized growth on T14 (FM-SF choice) whilst birds offered feed mixtures maximized thier FCE on the FM:SF:SY (0.67:0.33:0.33) feed mixture.

4.4 DISCUSSION

The results show that it is possible to predict the response of any mixture or provide some measure of the effect on each component singly or in combination with other components. In this mixture experiment, the measured response was noted to depend on the type and proportion of the protein basal feeds present in the mixture.

In general lysine was limiting in most diets that gave rise to poor broiler performance. Birds offered T2 consumed the largest amount of feed, but this did not provide sufficient quantities of digestible nutrients, particularly lysine as a proportion of its requirement to maintain rapid growth.

Table 4.5 *Comparison of the predicted and observed performance of broiler chickens.*

	Basal feed proportion		
	FM	SF	SY
Predicted proportions to maximise :			
Weight gain	0.57	0.28	0.15
FCE	0.66	0.34	0.00
Choice made to maximise :			
Weight gain (T17)	0.60	0.28	0.12
FCE (T14)	0.70	0.30	0.00

The adverse effects on growth were not restricted to SF alone but to SY fed alone. Wethli, *et al.* (1975) suggested that the amino acids in oil-seed cakeş occur in such disproportion to the requirements of the chick that the utilization of the first limiting amino acid is severely impaired. It could be speculated that a deficiency of lysine and methionine in SF (i.e. 29% of requirement) and SY (49% of requirement) respectively (Appendix 4.3.) may have been worsened by a general amino acid imbalance resulting in unfavourable biological performance on these feeds. These findings agree with reports that dietary deficiencies of indispensable amino acids or an amino acid imbalance affects animal performance (Harper, *et al.*, 1970; Wethli, *et al.*, 1975; Gous, 1992; NRC, 1994; Forbes, 1995; Mc Donald *et al.*, 1991). A moderate deficiency (i.e. as in T2) led to an increase in feed intake confirming reports by Gous (1992), Mc Donald *et al.*, (1991) and Forbes, (1995).

Examination of the results reveal that as the proportion of FM in the mixtures increased from 16.7% t0 66.7% of the total food on offer, the biological performance of the chickens improved.

This appeared to be caused primarily by the higher intakes of amino acids. The FM component contributed amino acid(s) lacking in the other basal feeds, thus improving the balance of amino acid(s) or other nutrients. Additionally, it may have minimized the levels of other amino acid(s), provided a more balanced ingested amino acid(s) ratio or profile as a proportion of its requirement. FM could also possibly have enhanced the acceptability of the feeds on offer and improved feed intake. This allowed the required amino acids to be available to the cell at the time protein synthesis is taking place thus improving the birds biological performance than if basal feeds had been fed singly. The observations authenticate those made by Woodham and Deans (1977) who indicated that the performance of birds given a mixture of protein feed sources is sometimes greater (i.e. they exhibit some synergistic effect) than would be predicted when the components are fed singly.

The results of this experiment agree with reports by Waldroup, Hillard and Mitchell (1970) who found that complete replacement of soybean meal with sunflower meal resulted in a decrease in chick performance. The results are similar to reports by Rojas, Lung and deGuzman (1969) and Avila and Balloun (1974) who reported that soybean oilcake meal could replace up to 80% and 70% respectively of fishmeal in broiler feeds with no differences in weight gain. On the contrary the results do not agree with reports of Avila and Balloun (1974) that weight gain and FCE of birds offered 100% SY was significantly greater than those offered 100% FM feed. In the present experiment, birds fed fishmeal only had significantly ($p<0.05$) greater weight gain and FCE than those fed SY only or SF only.

The present experiment was carried out to extend ealier observations by comparing the performance of broilers fed on protein mixtures with those given free choice between the protein components. This method allows broilers to select the amounts of protein feeds that maximises performance. The results suggest that when birds are given appropriate prior exposure to two- or three protein feed sources differing in amino acid(s) they are capable of composing an adequate diet that maximises performance. This proves that birds are able to regulate their intakes of two or more feeds which are individually inadequate, as far as the properties of the feeds allow, to meet their requirements for amino acid(s) and probably certain nutrients (Cowan and Michie, 1978; Mastika and Cumming 1987; Belyavin, 1993; Forbes and Shariatmadari, 1994; Mc Donald *et al.*, 1991). The results of these studies suggest that the broiler chicken can balance its intake of amino acid(s) well. The type of protein source however appears to be of importance to achieve the best balance of AA and therefore eliminate deficiencies. When given a choice between two- or three protein basal feeds broilers select a greater proportion of the feed closer to adequacy. Thus when offered a choice between FM and SF or SY broilers selected between 60 to 70% of the total feed consumed as FM. When provided with a choice between SF and SY they selected 54% of their intake from SF which is closer to adequacy.

4.5 CONCLUSION

In this mixture trial, 75.70% and 63.20% of the total variances in weight gain and feed conversion efficiency respectively are explained by the models. By modelling the blending surface with a mathematical equation, the prediction of the response of any mixture or some measure of the effect on each component singly or in combination with other components was obtained. The measurable response (weight gain, feed intake, FCE) was noted to depend on the proportions and type of the protein basal feeds present in the feed mixture. Performance seemed to be better on a feed mixture containing 67:17:17 of FM:SF:SY.

The biological performance resulting from the feeding of different combinations of the two- and three component mixtures of protein sources was used to determine the combination of ingredients that maximised performance. A comparison of the mixture results with that obtained when a choice of diets was offered showed that in all cases broilers did make appropriate choices to coincide with those combinations that maximised performance. Broilers were clearly able to differentiate between two feeds on offer on the basis of their amino acid(s) content and ate predominantly from the protein sources that matched their requirement.

CHAPTER 5

MIXTURE EXPERIMENTS USING FISHMEAL, SUNFLOWER OILCAKE MEAL AND SOYBEAN OILCAKE MEAL WITH BROILERS AGED 7 TO 21 DAYS

5.1 INTRODUCTION

In the previous experiments (1, 2 & 3), birds tended to eat more feed to offset the small deficiency of a limiting AA, whilst a severe deficiency or excess resulted in a decline in feed intake. A decrease in feed intake suggests that there will be a limited intake of some of the amino acids necessary for optimum biological performance. Results from the previous trial show that soybean oilcake meal basal feed (SY) was selected against, this being notably deficient in methionine but rich in lysine (Balloun, 1980, Mc Donald *et al.*, 1991).

Amino acids available to the chicken from the feed are required for protein synthesis. The most limiting essential amino acids in poultry feeds are methionine and lysine. These amino acids may become limiting due to low levels in feeds (eg. high corn-soybean meal feeds are lower in methionine) or from feed inhibitors (i.e. mycotoxins from cereal grains tend to lower the concentration of methionine and lysine in feeds resulting in weight loss/malabsorption syndrome) or from the lack of intake (Balloun, 1980; Elliot, 1995). In comparison, a lack of methionine induces a much greater decrease in body protein and weight gain than lack of lysine (Elliot, 1995). Supplementation of soybean with DL-methionine to augment the quality of the dietary protein is a conventional procedure particularly in poultry enterprises where it is almost impossible to formulate adequate grain-soya feeds without supplementary methionine. For a 23% crude protein broiler starter feed based on maize and soya, about 0.21% synthetic methionine is added to obtain a proper balance of amino acids that meets the birds requirements.

There is variation in the reported methionine requirements that would support optimum biological performance (Table 5.1). In the present experiment, methionine was kept at 4.10 g kg^{-1} by the supplementation of the soybean basal feeds with 0.25% DL-methionine, with the exception of treatment 18.

Supplementing feeds with methionine is known to have increased the biological performance in broilers (Askelson and Balloun, 1964; Ross and Harms, 1970; Ross, Damron and Harms, 1972; Soares, Nicholson, Bossard and Thomas, 1974; van Weerden, Schute and Sprietsma, 1976). The amino acid deficiencies of soybean were further examined in the present experiment.

An experiment was carried out to determine the effect on the biological performance of providing broiler chickens between 7-21 days of age with three protein basal feeds in which the only protein source in each basal was soybean oilcake meal supplemented with DL-methionine (SY+M), fishmeal (FM) and sunflower oilcake meal (SF) respectively. These basal feeds were fed *ad libitum* either alone, or as two- or three free-choice or predetermined mixtures. This was carried out to compare the combination of components that maximises biological performance with the combination chosen by birds given *ad libitum* access to the two- or three components simultaneously.

Table 4.1 *Summary of experimental evidence about the methionine requirements of broiler chickens between 7-21 days of age according to various authors.*

Estimated requirement ($g\ kg^{-1}$)	Age Period (days)	References
3.90	7-21	Hewitt and Lewis, 1972.
4.40	8-21	Robins and Baker, 1980
4.90	7-21	Thomas., Bossard, Farran and Tamplin, 1985
5.00	0-21	NRC, 1994.
4.00	0-21	Gous, 1996.

5.2 MATERIALS AND METHODS:

This experiment was a repeat of the previous experiment with the following modifications : three protein sources *viz* : fishmeal, sunflower oilcake meal and soybean meal oilcake meal differing in amino acid composition were used. Their protein and AME (MJ kg^{-1}) contents were determined as in Experiment 1 after which three basal feeds were formulated to contain 120g crude protein kg^{-1} and 12.56 MJ AME kg^{-1}. The basal feeds were iso-energetic as well as iso-nitrogenous. The SY basal feed was supplemented with 0.25% DL-methionine except for Treatment 18. The composition of each basal feed is shown in Table 5.2. The basal feeds were blended in appropriate proportions as indicated in Table 5.3 producing 13 feeding mixtures (T1-T13) whilst maintaining a constant crude protein and energy concentration. A second series of treatments, T14-T17 consisted of offering birds combinations of either two- or three of the basal feeds as a choice. A randomized block design with feed*sex treatments replicated twice was used. The experiment run for fourteen days.

The variables measured

The criteria studied were weight gain (g bird d^{-1}), feed intake (g bird d^{-1}.) and feed conversion efficiency (g body weight gain/kg feed).

Statistical analysis

The biological response data were analysed as in Experiment 2. Those response variables resulting in a significant F test were further analysed using the Fishers pairwise comparison (Minitab, 1994). A sequential model fitting was carried out on the biological performance data that was collected at the points of the {3,3} simplex lattice, augmented with three interior points as described by Myers and Montgomery (1995).

Table 5.2 *Composition (g kg^{-1}) and analysis of basal feeds and protein sources used in the experiment.*

Ingredient	Basal Feeds		
	FM	SY+M	SF
Soybean oilcake meal		271.00	
Sunflower oilcake meal			325.00
Fishmeal	183.30		
Sugar	280.00	257.00	270.00
Starch	280.00	257.00	270.00
Sunflower oil	32.00	46.00	46.00
Monocalcium phosphate	4.00	19.00	18.00
Limestone	5.50	17.00	17.00
Salt	0.70	4.70	4.00
Filler (sand)	212.00	122.80	55.00
Vitamin + mineral premix	2.50	2.50	2.50
Mineral premix			
DL-Methionine	-	2.50	-
Calculated analysis:			
Crude Protein	120.00	120.00	120.00
ME(MJ Kg^{-1})	12.56	12.56	12.56
Calcium	10.00	10.00	10.00
Phosphorus (avail.)	5.00	5.00	5.00
Proximate analysis (as is basis) of protein sources:			
	Fish	Soybean	Sunflower
Crude protein	670.02	451.00	383.30
Calcium	25.10	2.80	3.90
Phosphorus	14.30	6.40	8.40
Ash	124.30	73.80	64.40
Moisture	90.50	61.00	89.00
MJ AME Kg^{-1}	14.63	10.40	9.13

FM, Fishmeal basal feed; SF, Sunflower oilcake meal basal feed;

SY+M, Soybean oilcake meal basal feed supplemented with DL-methionine.

5.3 RESULTS

The main effect of sex and the interaction of sex by feed on the three variables measured (weight gain, feed intake and FCE) were not significant at the same 5% level and are shown in Appendix 5.1. Because there were no statistically significant differences between the sexes the growth responses were pooled across the sexes (Table 5.3).

Broiler chickens fed feed mixtures maximised their feed intake and weight gain on T10, whilst FCE was maximized on T11. Beneficial biological responses were obtained following the use of mixtures of these protein sources but chicks fed higher levels of SF of between 67 to 100% of the diet still had lower weight gains than those fed similar levels of FM or SY+M in feed mixtures. SF and SY basal feeds fed as a sole source of protein significantly ($p<0.05$) depressed growth rate and FCE, in spite of the fact that *ad libitum* access to feeds was allowed.

The amino acids in the feed blend consumed as a proportion of the broilers requirement (i.e. EFG broiler simulation model) are presented in Appendix 5.2. Of all the essential amino acids lysine was the most limiting and provided less than 0.5 of the broilers requirement in treatments 2, 6, 7, 9 and 12.

To the response data measured at points of the augmented {3,3} simplex lattice design, a sequential model fitting exercise was carried out. The analysis of variance when fitting a quadratic model to the biological response data obtained when broilers were offered mixtures of three protein sources are illustrated in Appendix 5.3, 5.4 and 5.5. The purpose of using the simplex lattice design was to model the blending surface with some form of mathematical equation. This enabled predictions of the response of any mixture or provided some measure of the effect on the response of each component singly or in combination with other components. An F-Test for the quadratic model warrants some discussion. The analysis of variance for the quadratic model shows no significant ($P<0.05$) lack of fit for weight gain and feed intake (Appendix 5.3 and 5.4), whilst for FCE the quadratic model displays no significant lack of fit at $P<0.01$ (Appendix 5.5). The quadratic models had a smaller error mean square, a larger adjusted R^2 and a highly significant effect. On the basis of these statistics, the quadratic models did give a better representation of the response surface of this mixture system within the experimental error of the biological response data.

Table 5.3 *The response of broiler chickens to feeding treatments from 7 to 21 days of age.*

Treatment	Proportion of basal feed FM	SF	SY+M	Food Intake (g bird d^{-1})	Weight gain (g bird d^{-1})	FCE[1] (g bird d^{-1}) /kg food
1	1.00	0.00	0.00	44.01^{ab}	16.52^{abc}	372.1^{bcd}
2	0.00	1.00	0.00	25.30^{d}	7.45^{f}	294.0^{e}
3	0.00	0.00	1.00	46.87^{a}	20.01^{a}	427.6^{abc}
4	0.67	0.33	0.00	45.89^{ab}	19.07^{ab}	415.5^{abc}
5	0.67	0.00	0.33	43.63^{ab}	18.48^{abc}	423.0^{abc}
6	0.00	0.67	0.33	34.37^{bdc}	12.10^{de}	355.3^{dc}
7	0.33	0.67	0.00	39.79^{abc}	14.40^{bd}	366.8^{bcd}
8	0.33	0.00	0.67	45.50^{ab}	19.64^{ab}	431.1^{abc}
9	0.00	0.33	0.67	44.15^{ab}	18.14^{abc}	411.0^{abc}
10	0.33	0.33	0.33	47.64^{a}	20.03^{a}	421.5^{abc}
11	0.66	0.17	0.17	43.99^{ab}	19.66^{ab}	446.7^{ab}
12	0.17	0.66	0.17	31.59^{dc}	13.79^{dc}	432.7^{abc}
13	0.17	0.17	0.66	41.72^{abc}	18.29^{abc}	438.3^{ab}
14	0.33	0.00	0.67	51.29^{a}	21.67^{a}	432.1^{abc}
15	0.00	0.15	0.85	41.37^{abc}	19.10^{ab}	460.8^{a}
16	0.70	0.30	0.00	44.41^{ab}	17.08^{abc}	392.1^{abc}
17	0.31	0.17	0.52	49.70^{a}	21.50^{a}	434.2^{abc}
	FM	SF	SY			
18	0.00	0.00	1.00	29.09^{d}	9.05^{ef}	310.8^{de}

^{a,b,c,d,e,f} Means within a column with different superscripts are significantly different ($p<0.05$);

FCE[1] Food conversion efficiency

Regression coefficients obtained when fitting a quadratic model to the biological responses are shown in Appendix 5.6. The regression coefficients are helpful in interpreting the results. An examination of the coefficients shows that $\beta_3 > \beta_1 > \beta_2$, thus it can be predicted that component 3 (SY+M) produces the greatest weight gain and the best FCE in terms of single components fed alone. A linear term only contributes to the model when $x_i > 0$; with a maximum contribution at $x_i = 1$. The quadratic terms which represent the excess response over the linear model have positive regression coefficients $\beta_{12} > \beta_{23} > \beta_{13}$ for weight gain and FCE (i.e. synergism due to nonlinear blending) whilst β_{13} is negative for feed intake (i.e. this combination shows antagonistic blending effects). Blending components 1 (FM) and 2 (SF) produces a higher weight gain and better FCE than would be expected from the additive effect of the responses of the pure blends and a better biological performance than the other binary blends.

Figures 5.1, 5.2 and 5.3 presents the response surface contour plots for the biological responses. These plots and regression coefficients (Appendix 5.6) show that very little increase in response is obtained when mixtures of FM and SY+M only are offered to broilers in comparison to SF-SY+M and SF-FM. The actual and predicted biological performance values were similar (Figs. 5.1, 5.2 and 5.3).

Weight gain and feed intake were maximised by broilers offered T14, although this was neither significantly ($P<0.05$) different from any of the other choice fed birds. The best FCE was recorded for birds offered T15, but there were no appreciable differences ($P<0.05$) in FCE between these broilers and those offered other choice feeding treatments.

The proportion of each basal feed chosen by broiler chickens offered free-choice feeding regimes are shown in Table 5.4. The proportions of the blend of basal feeds which gave a similar higher response varied amongst treatments. The three basal feeds had the same protein content (120 g kg^{-1}) but were not eaten in equal amounts or at random when offered as a two- or three choice feed. The amount of each basal feed chosen by the birds as a proportion of the total for T14-T17, differed significantly ($P<0.05$) from 0.5 (in the two-component choice feeding treatments) for FM and SY+M in T14, SF and SY+M in T15, FM and SF in T16 and from 0.33 (in the three-component treatment) for SF and SY+M in T17. Broilers given a choice between FM or SY+M and SF tended to choose predominantly from the feeds (i.e.FM or SY+M) with an amino acid composition closer to the requirement. When a choice between FM and SY+M was offered birds consumed approximately 67% SY+M and 33% FM to give a similar performance (weight gain) to that predicted by the model.

A comparison of the biological performance resulting from the feeding of different combinations of the two- or three- component mixtures with the choices made by chickens when offered the choice of two- or three- components individually showed that in most cases the choices made coincided with those combinations that maximised performance (Table 5.6, Figs.5.1, 5.2 and 5.3). Gradually decreasing the quantities of SF in the feed whilst increasing the amounts of either FM and SY+M or both improved the growth performance of the broilers.

When offered a choice between two- or three feeds weight gain and food intake were maximised by broilers offered T14 although this was not significantly different from broiler chickens offered T1, T3 and T10 at the 5% level. The best FCE was recorded for birds offered T15, but there were no appreciable differences between these and broiler chickens offered T1, T3 or T10.

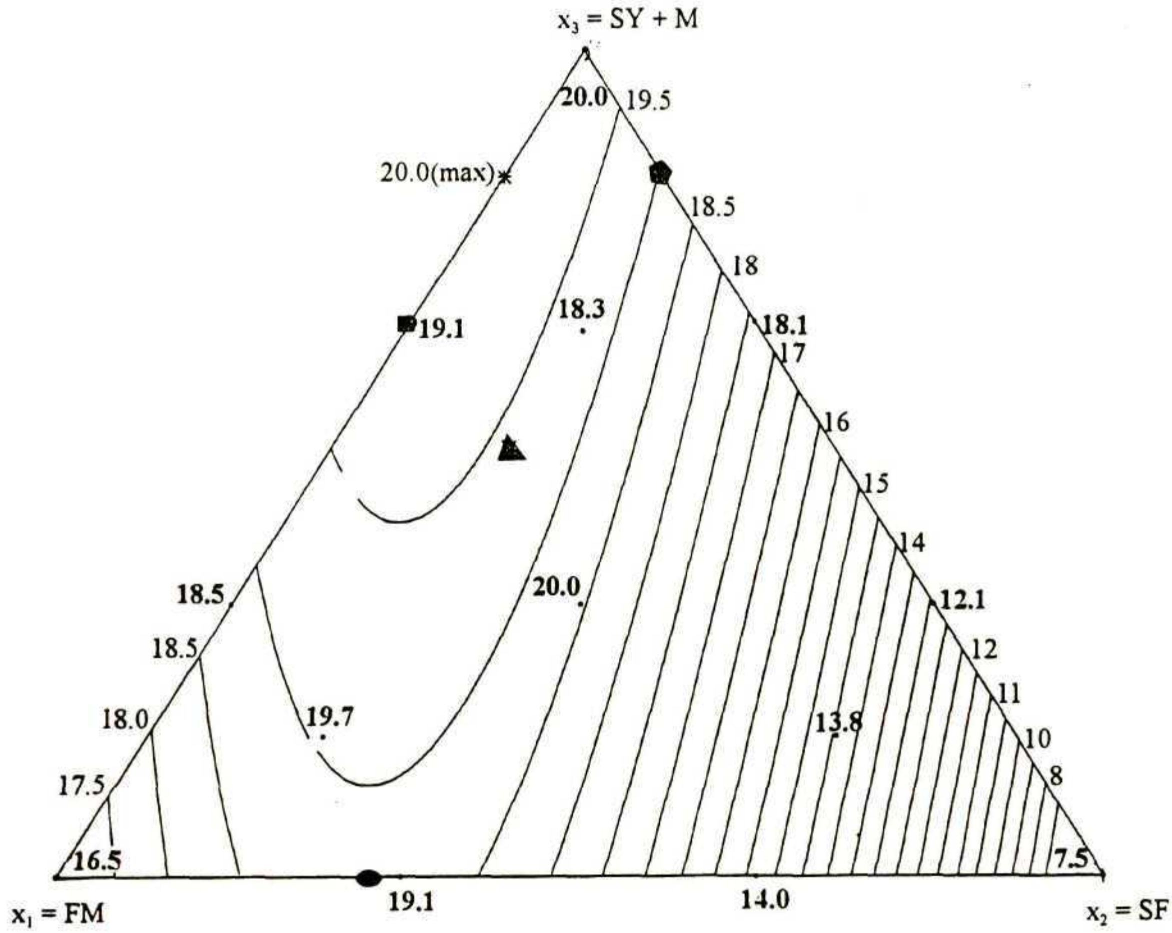


Figure 5.1. *Response surface contour plot for weight gain (g/b d)*

The predicted maximum weight gain is 20.0 g/b d, and occurs where $x_1 = 0.15$, $x_2 = 0.00$, $x_3 = 0.85$.
The observed values are highlighted in bold.

Choice

■ FM - SY + M

● FM - SF

⬟ SY + M - SF

▲ FM - SF - SY + M

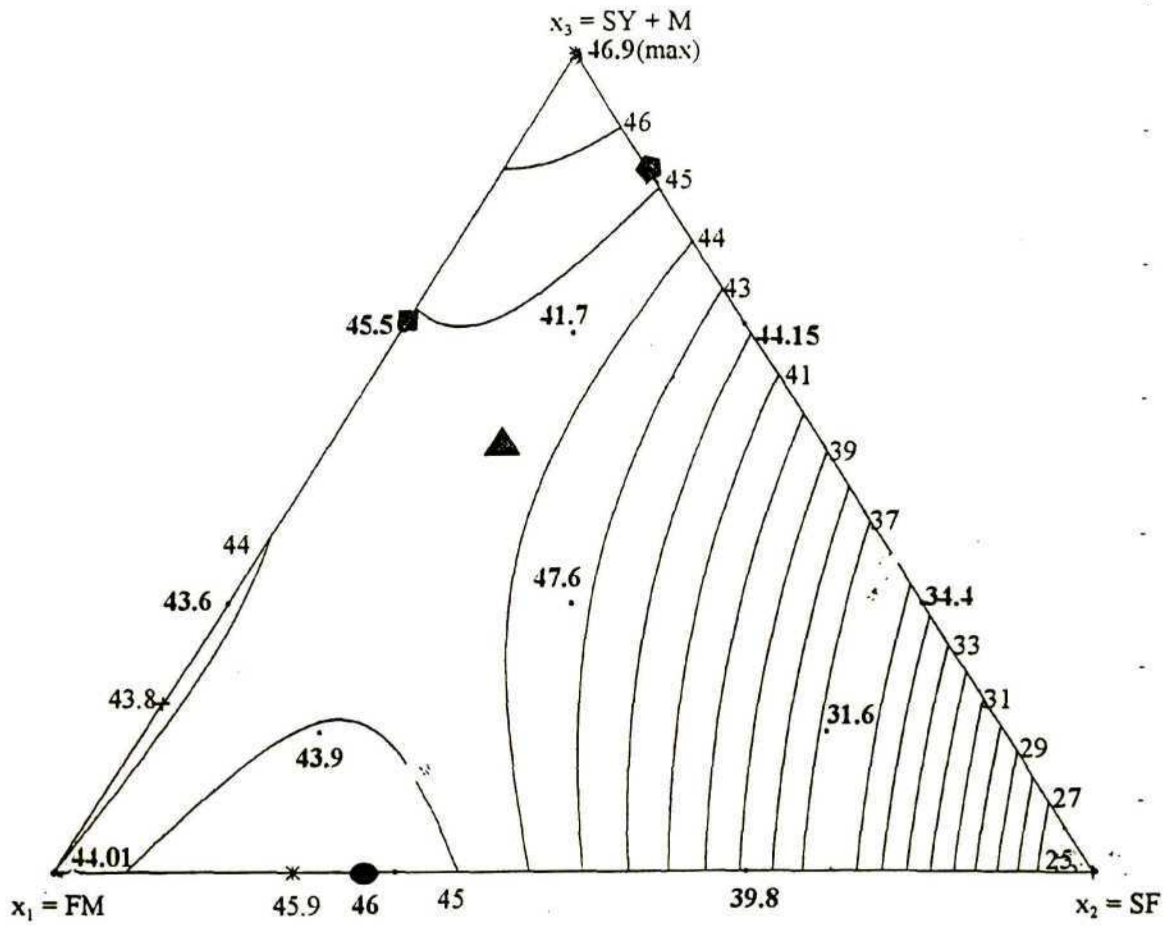
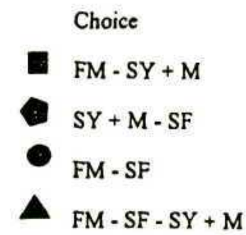


Figure 5.2. *Response surface contour plot for feed intake (g/bird day)*

The predicted maximum feed intake of 47.1 (g/bird day) occurs where $x_1 = 0$, $x_2 = 0$, $x_3 = 1$. The observed values are highlighted. in bold



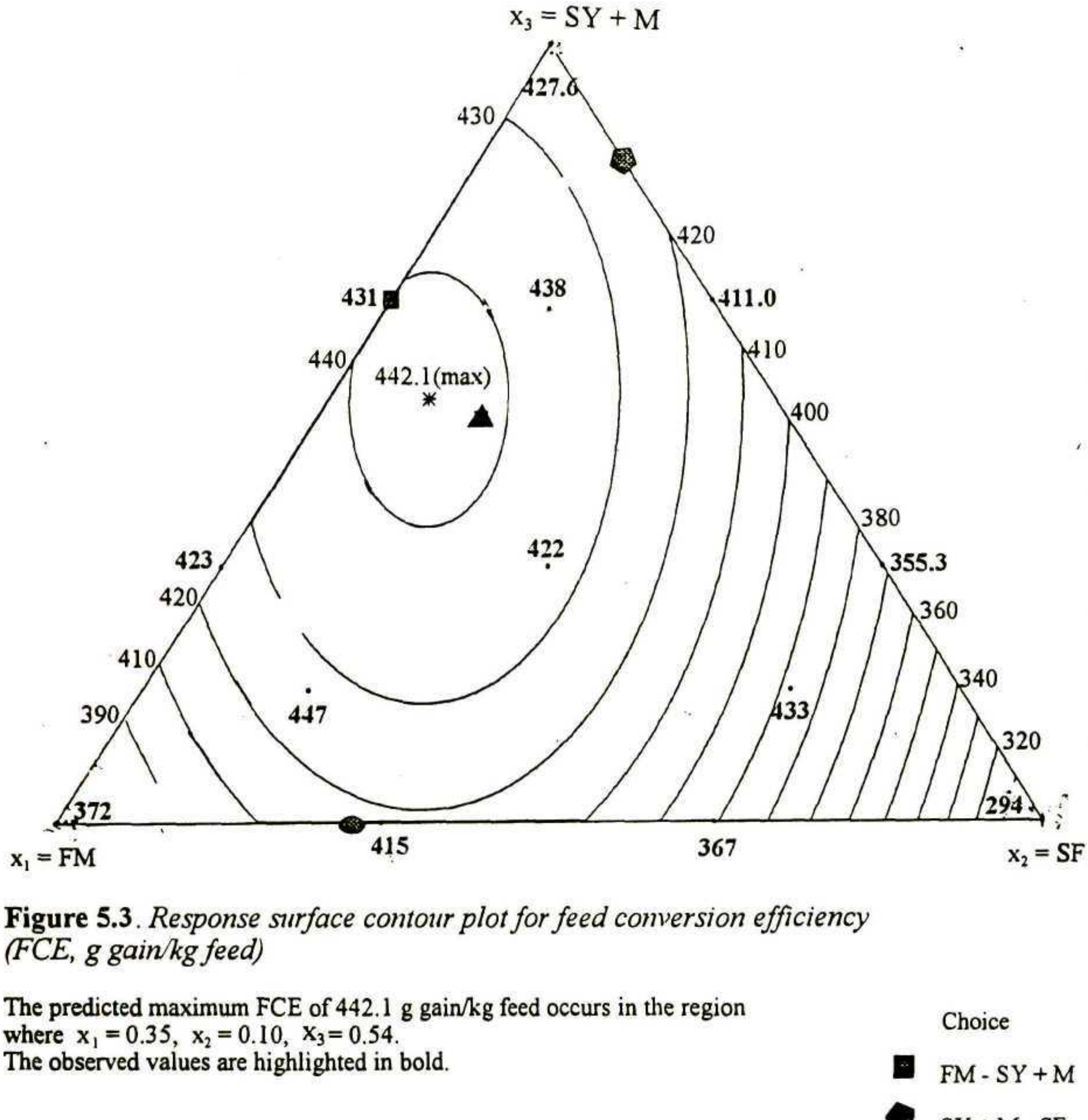


Figure 5.3. *Response surface contour plot for feed conversion efficiency (FCE, g gain/kg feed)*

The predicted maximum FCE of 442.1 g gain/kg feed occurs in the region where $x_1 = 0.35$, $x_2 = 0.10$, $x_3 = 0.54$.
The observed values are highlighted in bold.

Choice

■ FM - SY + M

⬟ SY + M - SF

● FM - SF

▲ FM - SF - SY + M

Table 5.4 *Basal feeds chosen by the birds as a proportion of the total food consumed.*

Treatment	Basal Feed	Mean Proportion	SD	P-Value	
14	FM	0.3310	0.0415	0.0039	*
14	SY+M	0.6690	0.0415	0.0039	*
15	SF	0.1455	0.0507	0.0008	*
15	SY+M	0.8545	0.0507	0.0008	*
16	FM	0.7040	0.0435	0.0026	*
16	SF	0.2960	0.0435	0.0026	*
17	FM	0.3103	0.0408	0.0350	n.s.
17	SF	0.1714	0.0444	0.0068	*
17	SY+M	0.5183	0.0679	0.0120	*

Hypothesis for T 14-T16

Mean = 0.500

Hypothesis for T17

Mean = 0.333

n.s not significantly different from 0.500 (T14-T16) or 0.333 (T17)

otherwise significantly different from 0.500 (T14-T16) or 0.333 (T17) at P<5%(*)

Table 5.5 *Comparison of the predicted and observed performance of broiler chickens.*

	Basal feed proportion		
	FM	SF	SY+M
Predicted proportions to maximise :			
Weight gain	0.15	0.00	0.85
FCE	0.35	0.11	0.54
Choice made to maximise :			
Weight gain	0.33	0.00	0.67
FCE	0.31	0.19	0.51

5.4 DISCUSSION

The results showed the inadequacy for growth of the unsupplemented soybean oilcake meal basal feed (SY) and sunflower oilcake meal basal feed (SF) when offered as a sole protein source in broiler chickens. The poor performance was related to the amino acid composition of the feeds on offer as a proportion of the requirements (Appendix 5.2). The poor performance of broilers fed the unsupplemented soybean oilcake meal basal feed (SY) indicated that this basal feed was severely deficient in methionine. The quadratic positive response (in terms of growth) to methionine supplementation of SY was primarily a stimulatory effect on voluntary feed intake. This resulted not only in the subsequent substantial improvements in body weight gain and FCE, but also yielded superior growth performance of broilers. The beneficial effect is in agreement with previous reports (Askelson and Balloun, 1964; van Weerden, Schute and Sprietsma, 1976; Ross, Damron and Harms, 1972; Ross and Harms, 1970).

In a series of trials, Soares *et al.* (1974) working with broilers till 21 days of age, found that supplementing a corn-soybean meal feed containing 21% protein and 0.66% total sulphur amino acids with 0.15% DL-methionine improved weight gains and FCE significantly ($p<0.05$). When the same feed was supplemented with 0.30% DL-methionine further improvements in the biological performance was not obtained. Waldroup *et al* (1976) observed that at a lower crude protein level, it is beneficial to offer feeds formulated to minimize excesses of each essential amino acid whilst maintaining a correct balance between each essential amino acid. It is therefore likely that by supplementing the SY feeds with methionine a comparatively well balanced amino acid profile with minimal deficiencies or excesses was obtained and therefore adding this to a fairly well balanced mixture (i.e. FM) or to SF elicited an improved biological response (weight gain and FCE).

From examining the results, it is evident that broilers clearly sensed that the SY+M was closer to their requirement than SY alone, thereby choosing significantly larger amounts than was done in the previous experiment.

Wethli, *et al* (1975) suggested that the amino acids in oil-seed cakes occur in such disproportion to the requirements of the chick that the utilization of the first limiting amino acid is severely impaired. On the contrary, several authors have suggested that amino acid excesses have no effect on the utilization of the first limiting amino acid (Harper *et al.*, 1970; Boorman and Ellis, 1996). In explaining the reasons for poor performance on high intakes of poor-quality proteins, Boorman and Ellis (1996) argued that apart from a small decrease 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 amino acid from tissue protein. In the present experiment, it could be speculated that a deficiency of lysine in SF and methionine in the unsupplemented SY feed may have been worsened by a general amino acid imbalance that resulted in a decrease in food intake and an unfavourable biological performance on these feeds and for feed mixtures containing 67% of SF. These findings agree with reports that dietary deficiencies of indispensable amino acids or an amino acid imbalance affects animal performance (Harper, *et al.*, 1970; Wethli, *et al.*, 1975;

Mc Donald *et al.*, 1991; Gous, 1992; NRC, 1994; Forbes, 1995; Boorman and Ellis, 1996). That the depression in growth rate associated with the ingestion of an imbalanced feed is mainly a result of a depression in feed intake is confirmed by Leung *et al* (1968).

Working with rats, they observed that when feed intake was increased appreciably by force feeding, the animals grow normally, and concluded that the basic effect of an imbalance appeared to be on feed intake regulation.

Growth improved following the use of FM-SF-SY+M in equal proportions than if either had been fed singly. Unlike SF, as the proportion of FM and SY+M in feeding mixtures increased from 16.7% to 66.7% of the feeding treatment, biological performance of birds tended to improve. These observations may be due to an overall increase in the blend of feed chosen which was nutritionally adequate in methionine. Additionally, SY+M and the FM component may contribute amino acids lacking in the SF basal feeds thus possibly improving the balance of amino acids or other nutrients and enhanced the acceptability of the feeds on offer. An increase in feed intake, due to enhanced acceptability of the feeds on offer allowed the required amino acid(s) to be available to the cell at the time protein synthesis is taking place.

Emmans (personal communication 1996) suggested that broiler chickens when given appropriate training are able to select from various protein sources offered and compose an adequate diet according to their needs over a period of time if the combinations on offer is non-limiting. The results of this experiment show that, given appropriate prior exposure the young rapidly growing broiler chicken is able to select a blend of feeds differing in amino acid content to support maximum biological performance. Imbalances seemed to be minimized in the choice feeding treatments, consequently these feeds yielded comparably good growth rates. When given a choice between two or three feeds broilers select a higher proportion of the feed closer to adequacy (i.e. FM or SY+M) and between 20-30 % of the relatively less adequate feed (SF). This in line with the observations of Forbes and Shariatmadari (1994) who observed that broilers offered a choice between very low protein (VLP) and average protein (AP) feeds and AP and high protein (HP), selected their intake from the feed closer to adequacy (AP) and consumed less of the relatively less adequate feed.

The experimental results support the hypothesis that birds are able to regulate their intakes of two or more feeds which are individually inadequate, as far as the properties of the feeds allow, to meet their requirements for amino acids and probably certain nutrients (Cowan and Michie, 1978; Mastika and Cumming 1987; Emmans, 1991; Belyavin, 1993; Forbes and Shariatmadari, 1994; Mc Donald *et al.*, 1991).

Even though broilers on the three-choice feeding treatments showed a marked preference for the non-limiting SY+M feed, they continued to eat some FM and sample the relatively less adequate SF feed as predicted by the model. There were variations in proportions of the blend of feed within treatments and between treatments that yielded similar optimal growth. Forbes and Shariatmadari (1994) indicated that in spite of earlier training, variations in dietary selection between individuals do occur because chickens continue to sample the trough with the less adequate feed thus still maintaining their foraging behaviour.

5.5 CONCLUSIONS.

The improvement in growth with the inclusion of SY-supplemented basal feeds in feed mixtures and in the choice fed treatments shows that is beneficial to supplement practical feeds consisting of soybean with DL-Methionine. The improvement in performance may have resulted from an overall increase in the feed blend consumed that was adequate in methionine. Choice fed broilers were able to compose their own diet not only according to their requirement but also the learned associations between the nutritional characteristics (sensory properties and metabolic effects) of each feed.

The biological performance resulting from the feeding of different combinations of the three-component mixtures of protein sources was used to determine the combination of ingredients that maximised biological performance (maximum weight gain and FCE). The combinations of two- or three-component mixtures that maximised performance was then compared with the choices made by chickens when offered a choice of the two or the three components individually, and it was found that in all cases the choices made coincided with those combinations that maximised performance. It is clear from this experiment that broiler chickens will attempt to maximise performance by choosing the best possible combination of protein sources, when given the opportunity to do so.

The results also show that the use of response surface techniques can be successfully applied to determine the proportions of two or more ingredients that can be blended to yield a greater biological performance.

CHAPTER 6

GENERAL DISCUSSION

The exponentially growing human population in Africa is increasing the animal protein food needs and increasing stress on the resource base required to produce such livestock products. This situation is not likely to stabilize for the next decade. Of the different livestock species, poultry (broiler chickens and cull layers) account for 30-50% of the total animal protein supply. It can be argued that the rapid development of the poultry industry will reduce the market incentive to expand other forms of livestock production in future. This trend is creating problems as regards the supply and efficient utilization of protein feed sources. As the protein resource base becomes more limiting, there needs to be an evaluation of how these protein feedstuffs can be used to complement each other in order to maximize biological performance of broiler chickens. In order to achieve the estimated poultry production targets, the major emphasis is, and will continue to be, on improving nutrition with the existing protein feed resources.

Increases in poultry production to meet the rapidly growing demand should be addressed through a balanced feed supply, with a balanced amino acid(s) composition and by correction of any critical deficiencies with low cost supplements. These two considerations represent the most important strategy and are the principal constraints among the nongenetic factors affecting productivity. It was previously accepted that broiler diets must contain protein sources, which must mostly be supplied from animal sources such as fishmeal, in order to obtain a proper balance of amino acids. With a better knowledge of the amino acid needs of poultry, it has become clearer that, in order to maximise the performance of broiler chickens, a mixture of protein feed sources is required. It has also been noted that the lysine and methionine contents of broiler starter feeds are very important to maximise the positive and minimize the negative impacts of an inadequate amino acid composition of protein feed mixtures. With this in mind a series of experiments was conducted to determine response surfaces to mixtures of protein sources in biologically important variables such as weight gain and FCE, the purpose being to compare the combination of feeds that maximise performance with the combination chosen by birds given *ad libitum* access to the three components simultaneously.

The first experiment was designed to test the hypothesis that broiler chickens, when provided with three separate protein feedstuffs on a free-choice basis, select a combination of three feeds that will meet their requirements for maximum growth and production and hence will maximize their biological efficiency.

Four protein sources, differing in amino acid composition, were used either alone or in two- or three- component mixtures *viz*: fishmeal, sunflower oilcake meal, soybean oilcake meal and maize gluten meal. This trial was from 18 to 28 days of age. Maximum growth was obtained on the fishmeal basal feed, but this was not significantly different (P<0.05) than that on the combination of feeds chosen although it was significantly different (P<0.05) than that when the other protein sources were fed alone. Broilers did maximize their FCE on one of the combinations (T4, SY-SY-MG), and did improve their performance (weight gain and FCE) on each of the protein sources other than fishmeal, when given a choice. Broilers on the FM-SF-SY and SF-SY-MG choices met at least 50% of their requirement for each amino acid. Birds chose a higher proportion of the feed closer to adequacy whilst avoiding the MG feed, which was inadequate, to give the highest possible response. Even though MG was selected against, the birds continued to sample this feed. In all cases broilers selected against maize gluten, this being the most badly balanced amino acid containing protein source offered to chickens in the trial.

In the three subsequent experiments broilers between one and three weeks of age were used. In these experiments, one-, two- and three component mixtures of the same protein sources as those used in the first experiment were used together with treatments in which birds were offered choices between the different protein sources. The purpose of the mixture treatments was to determine, firstly, the response surface based on criteria such as weight gain and FCE, and the purpose of the choice treatments was to determine, whether broilers, given access to the components separately, would attempt to maximise either weight gain or FCE. This technique thus provides evidence of the importance of amino acid balance in the feed offered to broilers (by means of the mixture experiment) and of the ability or otherwise, of broilers to make a choice from two or three feeds such that weight gain or FCE is maximised (by means of the choice feeding experiments). Once a mixture experiment has been conducted it is possible to predict with reasonable accuracy the proportions of each feed that will give rise to a particular performance.

The three basal feeds used in Experiment 2, were fishmeal (FM), sunflower oilcake meal (SF) or maize gluten meal (MG). Maximal growth and FCE were obtained on the T5 (0.67-0.33, FM-SF) mixture. The lowest response in all biological variables was obtained on T2 (MG alone). Maximum growth rate among the 13 mixtures was obtained on almost equal amounts of FM and SF (0.52 and 0.48) and with no maize gluten protein. Broilers on the choice feeding treatments, in which FM and SF were offered, chose almost the same proportions of these protein sources as that required.

Based on the evidence that MG was avoided almost entirely because of its poor amino acid balance (Exp 2) soybean was substituted for maize gluten meal in the third experiment. The three protein sources used in this experiment were fishmeal, sunflower oilcake meal and soybean oilcake meal. Results for the mixture experiment show that maximal growth and FCE was obtained on FM:SF:SY (0.67:0.17:0.17) feed mixture.

Broilers on the choice feeding treatments in which FM-SF-SY were offered, chose almost the same proportions of these three as that required to maximise growth rate (0.60-0.28-0.12) and FCE (0.70-0.30-0.00). These experiments provides strong practical evidence for the ability of broilers to choose from two or three feeds differing in amino acid balance and compose their own diet according to their own requirements to maximise performance. It was however surprising that broilers chose very little of soybean basal feed to maximise weight gain.

Experiment 4 was conducted to determine whether the birds would alter the choice made in Experiment 3 when SY was supplemented with methionine. The three protein sources used were fishmeal, sunflower oilcake meal and soybean oilcake meal supplemented with DL-methionine. Chickens fed feed mixtures maximised their weight gain on FM:SF:SY+M (0.33:0.33:0.33) feed mixture. SY fed as a sole source of protein significantly ($P<0.05$) depressed growth rate and FCE unlike SY+M which enhanced performance.

The choice made to maximize weight gain (0.33-0.00-0.67) and FCE (0.31-0.19-0.51) when birds were offered a choice of FM-SF-SY+M were similar to the predicted proportions to maximize weight gain (0.15,0.00,0.85) and FCE (0.35,0.11,0.54). When offered a choice between two or three feeds broilers select a feed closer to adequacy (i.e FM or SY+M) and relatively less of the inadequate feed (SF).

The response surface contour plots for weight gain and FCE showed that these parameters could be predicted with a reasonable degree of accuracy. There was a clear response to supplementary methionine (Compare Fig 4.1 and 5.1, 4.2 and 5.2). The most obvious interpretation of these figures is that for the FM, SF and SY choice on offer to broilers (Exp.3) to maximize biological efficiency much more of the adequate diet (FM) or near adequate (SF) will have to be chosen. Furthermore, very little of SY is required in the feed blend that maximizes performance. When SY is supplemented with DL-Methionine, there is an increase in the amount of this SY+M feed required in the feed blend that maximizes performance. The results of the two experiments showed that methionine addition caused a marked improvement in growth and FCE of broilers.

The results from these series of mixture experiments show that there is a relationship between a particular combination of protein sources and performance , and that this relationship can be better formalised by means of a quadratic equation. The measured response was noted to depend on the type and proportion of the protein basal feeds present in the mixture or its amino acid composition. The performance of birds given a mixture of feeds is most often greater than would be predicted when the components are fed singly (i.e. they exhibit some synergistic effect). As the proportions of well balanced proteins (i.e.FM or SY+M) in the mixtures increased from 16.7% to 66.7% of the total food on offer, the biological performance of the chickens improved. This appeared to be caused primarily by the higher intakes of balanced proportions of amino acids. These components contributed amino acid(s) lacking in the other basal feeds, thus improving the balance of amino acid(s) or other nutrients. Additionally, they provided a more balanced ingested amino acid(s). An increase in the daily intake of the critical nutrients (i.e. lysine and methionine) in the feed blend chosen increased protein synthesis or weight gain of broilers.

A comparison of the mixture results with that obtained when a choice of diets was offered showed that in all cases broilers did make appropriate choices to coincide with those combinations that maximised performance.

The findings from the series of experiments confirm the current knowledge of the effects of amino acid deficiencies and antagonisms on animal performance. Chicks fed diets that were deficient in any one amino acid had comparatively lower biological performance (Harper, *et al.*, 1970; Wethli, *et al.*, 1975; Boorman, 1979; Gous, 1992; NRC, 1994; Forbes, 1995; Mc Donald *et al.*, 1991), due to their inability to make use of all the other essential and non-essential amino acids for protein synthesis. This results in unutilized amino acids. A notable feature of such a deficiency was a rapid reduction in feed intake and weight gain. A moderate deficiency (e.g. SF) led to an increase in feed intake confirming reports by Gous (1992), McDonald *et al.*, (1991) and Forbes, (1995). It is only when the deficiency is severe that food intake declines as observed with the MG and SY basal feeds. An adequate amino acid intake is a prerequisite for protein synthesis to occur. A decline in intake and invariably a deficiency of an amino acid, results in an impairment of body weight gain. This is due to a lowered efficiency of utilization of dietary protein.

Experimental results show that birds chose a higher proportion of the feed closer to adequacy whilst avoiding the MG and SY feeds which were inadequate to give the highest possible response. The results of these studies, using ingredients similar to those used in commercial broiler production, suggest that when broilers are given a choice of two- or three protein feed sources differing in amino acid(s) following prior exposure to these feeds they are capable of composing an adequate diet that maximises performance. This proves that birds are able to regulate their intakes of two or more feeds which are individually inadequate, as far as the properties of the feeds allow, to meet their requirements for amino acids (Forbes and Shariatmadari, 1994; Mc Donald *et al.*, 1991).

Broilers were clearly able to differentiate between different feeds on offer on the basis of their amino acid(s) content, matching their choice with their requirement. This research highlights the importance of accounting for the available amino acid content of feed ingredients when formulating broiler diets with using protein sources differing widely in their amino acid content. Such a practice will ensure that a balanced amino acid composition is present in the feed blend on offer to maximize performance.

This technique could be used to evaluate unusual protein sources against the more commonly-used ingredients such as fishmeal or soybean plus methionine. The advantages would be that the broilers would give an idea of whether the ingredient is acceptable, and in what proportions, relative to the other ingredients, such an ingredient could be substituted for the other two.

The series of experiments reported in this thesis are unique in that three-component mixture experiments have not been reported in the literature to date, and that these mixture experiments, in conjunction with treatments in which broilers are offered choices between two or three of the components used in the mixtures, provide very strong corroborative evidence of the ability of broiler chickens to make a choice between protein sources that will maximise their performance.

ABSTRACT

The series of experiments reported in this thesis were designed to test the hypothesis that broiler chickens, when provided with three separate protein feedstuffs on a free-choice basis, select a combination of these feeds that will meet their requirements for maximum growth. To test this hypothesis, the first experiment involved choice feeding treatments only, but the subsequent experiments were designed as a severe test of the hypothesis. These were made up of two parts: the first consisted of 13 mixtures of three components in each experiment which were designed to map the response in weight gain and FCE to different combinations of the ingredients; the second part consisted of various two- and three component choices. By comparing the choices made with the performance of the birds on the various mixtures it was possible to determine whether those broilers offered a choice were attempting to maximise performance.

Four protein sources viz: fishmeal (FM), soybean oilcake meal (SY), sunflower oilcake meal Fishmeal (FM) and maize gluten meal (MG) differing in amino acid composition were offered in the first trial, using broilers from 18 to 25 days. The basal feeds contained 120g crude protein kg $^{-1}$ and 12.5 MJ AME kg^{-1}. Only one protein source was used in each feed. Maximum growth was obtained on the fishmeal basal feed, but this was not significantly different ($P<0.05$) from that on any of the combinations of feeds chosen although it was significantly different ($P<0.05$) from that when the other protein sources were fed alone.

In the three subsequent experiments broilers between one and three weeks of age were used. In these experiments, one-, two- and three component mixtures of the same ingredients as those offered in first experiment were used together with treatments in which birds were offered choices between the different protein sources. The three basal feeds used in the second experiment were FM, SF and MG. Maximum growth rate was obtained on almost equal amounts of FM and SF (0.52 and 0.48), which was close to that chosen by the broilers offered a choice of protein sources.

In the third experiment the three protein sources used were FM, SF and SY. The appropriate

choice made to maximise weight gain (0.60, 0.28, 0.12) and FCE (0.70, 0.30, 0.00) when birds were offered a choice of FM-SF-SY was similar to the combinations that maximised weight gain (0.57, 0.28, 0.15) and FCE (0.66, 0.34, 0.00), when different mixtures of the protein sources were supplied to the birds.

In Experiment 4, three protein sources *viz*: FM, SF, and SY supplemented with DL-methionine were used. The choice made to maximize weight gain (0.33, 0.00, 0.67) and FCE (0.31, 0.19, 0.51) when birds were offered a choice of FM, SF and SY+M were again close to the proportions that maximized weight gain (0.15, 0.00, 0.85) and FCE (0.35, 0.11, 0.54).

The combination producing the maximum response in the treatments in which SY + M was fed differed markedly from that when SY was unsupplemented with methionine. Similarly, when given the choice, broilers consumed greater proportions of the SY + M than of SY unsupplemented.

When offered a choice between two or three feeds differing in amino acid balance broilers select feeds that are more similar to the required amino acid balance (i.e FM or SY+M), and avoid feeds that are relatively badly balanced, such as SY and MG. In these experiments SF, SY or MG fed as the sole source of protein depressed growth rate and FCE, unlike FM and SY supplemented with methionine which enhanced performance. In all cases broilers selected against maize gluten, this being the most badly balanced amino acid containing mixture offered to the broilers. Evidence from these trials supports the hypothesis that broiler chickens will select effectively from a combination of three feeds to maximise performance.

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Appendix 2.1 *The essential amino acid requirements (g kg^{-1} diet) of broiler chickens between 18 to 25 days of age according to various authors.*

Amino acid	Gous* (1996)	NRC (1994)	Woodham and *Deans* (1975)
Arginine	12.0	11.1	7.6
Histidine	4.6	3.2	3.4
Isoleucine	8.4	7.3	4.8
Lysine	11.0	10.0	8.7
Leucine	12.5	10.9	10.0
Methionine	4.8	3.8	-
Phenylalanine	6.5	6.5	-
Proline	5.5	5.5	-
Threonine	7.2	7.4	5.1
Tryptophan	2.0	1.8	1.4
Valine	9.0	8.2	7.0
TSAA	8.0	7.2	5.8

*EFG Broiler simulation model
TSAA, Total Sulphur Amino Acids.

Appendix 2.2 *The blend of amino acids present in feeding regimes as a proportion of the requirements (AA_R, %) of broiler chickens between 18-25 days of age.*

Amino Acid (AA)	AA_R Gous (1996)*	Feeding treatment 1	2	3	4	5	6	7	8
Arginine	1.20	0.67	0.64	**0.58**	0.65	**0.63**	0.71	0.67	0.29
Histidine	0.42	0.80	0.78	0.84	0.64	1.01	0.62	0.73	0.47
Isoleucine	0.72	0.71	0.69	0.70	0.71	0.70	0.69	0.76	0.59
Lysine	1.20	**0.54**	**0.46**	0.62	**0.36**	0.78	**0.23**	0.59	**0.14**
Leucine	1.26	0.64	0.67	0.81	0.68	0.70	0.54	0.70	1.35
Methionine	0.40	0.63	0.71	0.71	0.47	0.92	0.53	**0.31**	0.72
Threonine	0.76	0.58	0.55	0.62	0.51	0.68	0.46	0.59	0.47
Tryptophan	0.19	0.83	0.73	0.75	0.85	0.71	0.80	1.04	0.37
Valine	0.83	0.68	0.70	0.67	0.64	0.74	0.69	0.60	0.55
TSAA	0.74	0.55	0.60	0.60	0.50	0.67	0.53	0.41	0.67

Limiting amino acids in feed are highlighted in bold.
*Gous(1996), EFG broiler simulation model.

Appendix 3.1 *Mean food intake, body weight gain and food conversion efficiency (FCE) of broiler chickens for the two-week experimental period.*

Treatment	Food Intake (g/bird d)		Weight gain (g/bird d)		FCE. g gain/kg food	
	Male	Female	Male	Female	Male	Female
1	33.30	35.05	10.65	11.88	319.8	329.0
2	18.63	14.97	2.84	1.82	149.5	120.2
3	29.60	29.87	10.97	10.05	370.8	336.6
4	40.35	32.41	11.15	12.00	275.0	369.7
5	50.30	43.07	22.14	19.64	439.9	454.8
6	23.85	21.43	5.56	5.32	231.7	248.0
7	29.60	26.13	9.03	8.30	305.1	314.5
8	40.68	43.35	16.94	17.57	416.2	408.9
9	24.44	30.49	6.56	7.91	268.1	275.5
10	41.26	37.88	15.33	12.99	372.7	352.70
11	41.19	40.09	12.80	11.40	309.2	285.5
12	27.66	31.58	7.17	7.79	260.3	252.6
13	35.67	34.53	12.26	11.34	342.1	327.0
14	52.80	45.99	18.35	18.46	348.2	400.3
15	34.20	34.57	9.10	8.57	266.3	251.1
16	45.03	42.99	16.79	11.97	367.0	270.3
17	54.61	48.54	18.09	18.96	330.5	388.9
SD[1]	1.7638		4.075		32.6	

SD[1] Standard deviation of the mean.

Appendix 3.2 *The blend of amino acids present in feeding regimes as a proportion of the requirements (AA_R, %).*

	AA_R																	
Amino Acid (AA)	Gous 1996	Feeding treatment 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Arginine	1.20	**0.58**	0.77	0.71	0.64	0.62	0.75	0.71	0.67	0.36	0.69	0.63	0.73	0.70	0.67	0.72	0.64	0.69
Histidine	0.42	0.69	0.67	0.75	0.69	0.71	0.70	0.68	0.73	0.30	0.71	0.70	0.69	0.73	0.73	0.74	0.69	0.73
Isoleucine	0.72	0.78	0.65	0.74	0.73	0.76	0.68	0.69	0.75	0.26	0.72	0.75	0.68	0.73	0.75	0.72	0.74	0.74
Lysine	1.20	0.66	0.77	**0.34**	0.61	**0.62**	0.71	**0.43**	**0.48**	0.66	**0.21**	**0.57**	0.67	**0.45**	**0.59**	**0.57**	0.64	0.61
Leucine	1.26	0.73	0.72	0.57	0.73	0.67	0.73	0.62	0.62	0.73	0.29	0.67	0.70	0.62	0.70	0.71	0.68	0.71
Methionine	0.40	0.89	0.68	0.59	0.82	0.79	0.65	0.75	0.69	0.61	0.72	0.81	0.70	0.66	0.69	0.60	0.83	0.71
Phe	0.72	0.70	0.75	0.81	0.72	0.74	0.77	0.73	0.78	0.25	0.75	0.73	0.75	0.78	0.78	0.80	0.71	0.77
Threonine	0.76	0.68	0.55	0.61	0.64	0.66	0.57	0.59	0.64	0.18	0.61	0.65	0.58	0.61	0.64	0.60	0.64	0.63
Tryptophan	0.19	0.75	0.70	1.06	0.73	0.85	0.82	0.72	0.95	0.23	0.84	0.79	0.77	0.95	0.96	1.00	0.74	0.91
Valine 0.83	0.76	0.68	0.68	0.74	0.73	0.68	0.71	0.70	0.23	0.71	0.74	0.69	0.69	0.70	0.68	0.74	0.71	
TSAA	0.74	0.62	0.65	**0.56**	0.63	**0.60**	0.62	0.64	**0.58**	0.22	0.61	**0.61**	0.63	0.58	**0.58**	0.57	**0.63**	**0.60**

*EFG broiler simulation model TSAA, Total sulphur amino acid; Phe, Phenylalanine.

The limiting amino acids are highlighted in bold.

Appendix 3.3 *Analysis of variance when fitting a quadratic model to the growth response data obtained when broilers were offered mixtures of three protein sources.*

Source	DF	SS	MS	P	
Sex	1	2.211	2.211	0.468	n.s.
Regression	5	1079.274	215.885	0.000	*
Linear	2	763.020	105.645	0.000	*
Quadratic	3	316.257	105.419	0.000	*
Residual	45	185.595	4.124	-	
Error					
Lack-of-Fit	19	88.991	4.684	0.287	n.s..
Pure error	26	96.603	3.716		
Total	51	1267.080			

n.s. not significantly different at P<0.05
otherwise significantly different at P<0.05(*)

Appendix 3.4 *Analysis of variance when fitting a quadratic model to the feed intake response data obtained when broilers were offered mixtures of three protein sources.*

Source	DF	SS	MS	P	
Sex	1	19.01	19.01	0.347	n.s.
Regression	5	3280.60	656.12	0.000	*
Linear	2	2330.86	433.91	0.000	*
Quadratic	3	949.74	316.58	0.000	*
Residual	45	945.30	21.01	-	
Error					
Lack-of-Fit	19	266.67	14.01	0.917	n.s.
Pure error	26	679.03	26.12		
Total	51	4244.90			

n.s. not significantly different at P<0.05
otherwise significantly different at P<0.05(*)

Appendix 3.5 *Analysis of variance when fitting a quadratic model to the FCE response data obtained when broilers were offered mixtures of three protein sources.*

Source	DF	SS	MS	P	
Sex	1	219	219.50	0.771	n.s.
Regression	5	261009	52201.70	0.000	*
Linear	2	205375	49467.80	0.000	*
Quadratic	3	55633	18544.40	0.000	*
Residual	45	115676	2570.60	-	
Error					
Lack-of-Fit	19	53286	2804.50	0.350	n.s.
Pure error	26	62390	2399.60		
Total	51	376904			

n.s. not significantly different at $P<0.05$
otherwise significantly different at $P<0.05$(*)

Appendix 3.6 *Regression coefficients obtained by fitting a quadratic model to the biological responses.*

Term	Regression coefficients for:		
	Food intake	Weight gain	FCE
x_1(FM)	34.688	11.353	305.847
x_2(MG)	16.488	2.793	156.596
x_3(SF)	28.766	9.901	344.209
x_1*x_2	32.132	11.056	315.278
x_1*x_3	57.206	34.825	387.216
x_2*x_3	12.837	-1.004	-4.693

x_1 proportion of FM present in the mixture
x_2 proportion of MG present in the mixture
x_3 proportion of SF present in the mixture

Appendix 4.1. *Nutrient composition (g kg⁻¹) of the protein feed sources used in the experiment.*

Amino acid	Protein source FM	SF	SY
Arginine	38.1	28.5	3.1
Histidine	15.9	8.7	1.2
Isoleucine	30.6	14.3	2.0
Lysine	50.7	12.4	2.7
Leucine	49.8	22.2	3.4
Methionine	19.5	8.4	0.6
Phenylalanine	27.5	16.6	2.2
Proline	31.0	17.6	4.9
Threonine	28.2	12.9	1.7
Tryptophan	7.80	4.10	0.7
Valine	34.6	17.4	2.1
TSAA	25.0	14.8	1.3
Proximate analysis of protein sources:			
Crude protein	656.0	380.4	435.3
Calcium	22.8	3.8	2.8
Phosphorus	17.5	9.4	6.1
Fat	146.9	21.3	21.5
Ash	123.4	67.6	75.4
Moisture	84.5	86.5	107.5
MJ AME kg^{-1}	13.3	6.4	8.3

FM, fishmeal; SF, sunflower oilcake meal; SY, soybean oilcake meal.
TSAA, Total Sulphur Amino Acids.

Appendix 4.2 *Mean food intake, body weight gain and food conversion efficiency (FCE) of broiler chickens for the two-week experimental period.*

Treatment	Food Intake (g bird d^{-1}) Male	Female	Weight gain (g bird d^{-1}) Male	Female	FCE. g gain/kg food Male	Female
1	45.33	45.38	14.94	15.82	329.8	348.4
2	51.33	51.93	6.52	8.04	127.3	154.7
3	44.34	42.25	10.78	10.69	244.9	254.6
4	49.20	47.47	16.71	16.85	340.4	356.5
5	46.74	44.65	15.86	16.50	339.3	369.6
6	49.11	47.96	13.41	13.97	273.3	291.3
7	42.33	38.79	15.74	15.74	375.7	417.3
8	46.33	50.92	13.21	16.24	287.4	317.3
9	46.82	46.83	14.72	14.99	314.5	319.9
10	47.40	46.75	19.17	16.71	404.7	357.5
11	40.62	45.29	19.59	18.73	480.6	413.6
12	47.23	47.67	14.02	13.96	296.7	293.1
13	47.26	48.13	15.62	12.92	330.7	268.6
14	47.53	46.67	21.18	19.96	445.8	427.6
15	34.86	38.06	13.48	13.22	385.9	352.2
16	43.58	42.10	19.19	16.81	439.4	399.3
17	47.36	46.14	19.26	19.08	406.9	413.7
SD[1]	1.88		1.07		28.46	

SD[1] standard deviation of the mean.

Appendix 4.3 The blend of amino acids present in feeding regimes as a proportion of the requirements (AA_R, %).

Amino Acid (AA)	AA_R Gous 1996	Feeding treatment 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Arginine	1.20	**0.58**	0.66	0.82	0.61	0.66	0.71	0.63	0.74	0.34	0.69	0.63	0.67	0.76	**0.60**	0.73	0.63	0.63
Histidine	0.42	0.69	0.57	0.88	0.65	0.75	0.67	0.61	0.82	0.27	0.71	0.70	0.64	0.80	0.66	0.71	0.73	0.68
Isoleucine	0.72	0.78	0.55	0.86	0.70	0.80	0.65	0.62	0.83	0.23	0.73	0.75	0.64	0.79	0.71	0.69	0.79	0.72
Lysine	1.20	0.77	**0.29**	0.71	0.61	0.75	**0.43**	**0.45**	0.73	**0.21**	0.59	0.68	**0.44**	0.65	0.62	**0.48**	0.75	0.62
Leucine	1.26	0.72	0.49	0.85	0.64	0.76	0.61	0.57	0.81	0.16	0.69	0.71	0.59	0.77	0.65	0.65	0.75	0.67
Methionine	0.40	0.89	0.58	**0.49**	0.79	0.76	0.55	0.68	0.62	0.44	0.65	0.77	0.62	0.57	0.80	0.54	0.81	0.76
Phenylalanine	0.72	0.70	0.64	0.95	0.68	0.78	0.74	0.66	0.86	0.27	0.76	0.73	0.70	0.85	0.68	0.78	0.75	0.71
Threonine	0.76	0.68	0.47	0.71	0.61	0.69	0.55	0.54	0.70	0.16	0.62	0.65	0.54	0.67	0.62	0.58	0.69	0.62
Trypthophan	0.19	0.75	0.60	1.23	0.70	0.91	0.81	0.65	1.07	0.20	0.86	0.81	0.73	1.04	0.70	0.89	0.85	0.76
Valine	0.83	0.76	0.58	0.79	0.70	0.77	0.65	0.64	0.78	0.19	0.71	0.74	0.64	0.75	0.71	0.68	0.77	0.71
TSAA	0.74	0.62	0.55	0.54	**0.60**	**0.59**	0.55	0.57	**0.57**	0.18	**0.57**	**0.60**	0.56	**0.56**	**0.60**	0.55	**0.60**	**0.59**

*EFG broiler simulation model; The limiting amino acids are highlighted in bold.

Appendix 4.4 *Analysis of variance when fitting a quadratic model to the weight gain data obtained when broilers were offered mixtures of protein sources.*

Source	DF	SS	MS	P	
Regression	5	422.25	84.45	0.000	*
Linear	2	181.26	85.16	0.000	*
Quadratic	3	240.99	80.33	0.000	*
Residual	45	114.99	2.56	-	
Error					
Lack-of-Fit	19	48.56	2.55	0.491	n.s.
Pure error	26	66.43	2.56		
Total	51	537.29			

n.s. not significantly different at P<0.05
otherwise significantly different at P<0.05(*)

Appendix 4.5 *Analysis of variance when fitting a full cubic model to the food intake data obtained when broilers were offered mixtures of protein sources.*

Source	DF	SS	MS	P	
Regression	9	319.96	35.55	0.000	*
Linear	2	54.95	72.12	0.000	*
Quadratic	3	106.05	41.33	0.005	*
Special cubic	1	158.70	0.26	0.86	n.s.
Full cubic	3	158.70	52.70	0.0001	*
Residual	41	343.00	8.37	-	
Error					
Lack-of-Fit	15	154.77	10.32	0.21	n.s.
Pure error	26	188.23	7.24		
Total	51	662.95			

n.s. not significantly different at P<0.05
otherwise significantly different at P<0.05 (*)

Appendix 4.6 *Analysis of variance when fitting a quadratic model to the FCE data obtained when broilers were offered mixtures of protein sources.*

Source	DF	SS	MS	P	
Regression	5	226851	45370.20	0.000	*
Linear	2	105673	45064.30	0.000	*
Quadratic	3	121178	40392.80	0.000	*
Residual	45	109003	2422.30	-	
Error					
Lack-of-Fit	19	60543	3186.50	0.101	n.s.
Pure error	26	48460	1863.80		
Total	51	335876			

n.s. not significantly different at $P<0.05$
otherwise significantly different at $P<0.05$ (*)

Appendix 4.7 *Regression coefficients obtained by fitting the responses with a quadratic[1] or a full cubic model[2] to the biological responses.*

Term	Regression coefficients for: Feed intake[1]	Feed intake[2]	Weight gain[1]	FCE[1]
x_1 (FM)	45.60	45.45	15.47	339.89
x_2 (SF)	50.13	51.44	7.31	150.86
x_3 (SY)	44.30	43.24	10.45	238.19
x_1*x_2	-16.72	-18.44	22.91	601.37
x_1*x_3	9.45	10.45	11.91	201.50
x_2*x_3	3.85	3.18	22.23	433.17
x_1*x_2 (x_1-x_2)	-	42.72	-	-
x_1*x_3 (x_1-x_3)	-	-37.26	-	-
x_2*x_3 (x_2-x_3)	-	3.68	-	-
x_1*x_2*x_3	-	7.46	-	-

x_1, FM basal feed as proportion of total
x_2, SF basal feed as proportion of total
x_3, SY basal feed as proportion of total

Appendix 5.1 *Mean food intake, body weight gain and food conversion efficiency (FCE) of broiler chickens for the two-week experimental period.*

Treatment	Food Intake ($g\ bird\ d^{-1}$)		weight gain ($g\ bird\ d^{-1}$)		FCE. g gain/kg food	
	Male	Female	Male	Female	Male	Female
1	46.43	41.58	20.30	12.74	436.3	307.9
2	24.60	26.00	7.08	7.82	287.6	300.5
3	50.50	43.24	21.55	18.46	428.7	426.4
4	48.12	43.67	20.60	17.54	427.8	402.1
5	40.99	46.28	17.16	19.81	418.2	427.8
6	31.08	37.65	11.05	13.14	355.5	355.0
7	37.03	42.54	14.31	14.50	386.2	347.4
8	45.78	45.22	19.80	19.48	432.1	430.1
9	45.17	43.13	18.65	17.64	413.0	409.0
10	46.24	49.04	19.81	20.25	428.6	414.4
11	44.21	43.77	20.18	19.15	456.4	437.0
12	28.28	34.90	11.79	15.80	413.3	452.1
13	42.45	40.98	18.66	17.93	439.3	437.4
14	52.58	50.00	22.97	20.37	425.3	419.5
15	40.20	42.53	18.57	19.62	460.8	460.7
16	47.39	41.43	17.46	16.71	380.0	404.2
17	51.30	48.10	22.12	20.88	434.0	434.3
18	28.50	29.67	8.52	9.59	298.3	323.2
SD[1]	3.6788		1.4719		22.951	

SD[1] - standard deviation of the mean.

Appendix 5.2 *The blend of amino acids present in feeding regimes as a proportion of the requirements (AA_R, %).*

Amino Acid (AA)	AA_R Gous (1996)*	1 2	3	4	5	6	7	8	9	Feeding treatment 10	11	12	13	14	15	16	17	
Arginine	1.20	**0.58**	0.77	0.71	0.64	0.62	0.75	0.71	0.67	0.36	0.69	0.63	0.73	0.70	0.67	0.72	0.64	0.69
Histidine	0.42	0.69	0.67	0.75	0.69	0.71	0.70	0.68	0.73	0.30	0.71	0.70	0.69	0.73	0.73	0.74	0.69	0.73
Isoleucine	0.72	0.78	0.65	0.74	0.73	0.76	0.68	0.69	0.75	0.26	0.72	0.75	0.68	0.73	0.75	0.72	0.74	0.74
Lysine	1.20	0.77	**0.34**	0.61	0.62	0.71	**0.43**	**0.48**	0.66	0.21	**0.57**	0.67	**0.45**	0.59	0.66	**0.57**	0.64	0.61
Leucine	1.26	0.72	0.57	0.73	0.67	0.73	0.62	0.62	0.73	0.29	0.67	0.70	0.62	0.70	0.73	0.71	0.68	0.71
Methionine	0.40	0.89	0.68	0.59	0.82	0.79	0.65	0.75	0.69	0.61	0.72	0.81	0.70	0.66	0.69	0.60	0.83	0.71
Phenylalanine.	0.72	0.70	0.75	0.81	0.72	0.74	0.77	0.73	0.78	0.25	0.75	0.73	0.75	0.78	0.78	0.80	0.71	0.77
Threonine	0.76	0.68	0.55	0.61	0.64	0.66	0.57	0.59	0.64	**0.18**	0.61	0.65	0.58	0.61	0.64	0.60	0.64	0.63
Tryptophan	0.19	0.75	0.70	1.06	0.73	0.85	0.82	0.72	0.95	0.23	0.84	0.79	0.77	0.95	0.96	1.00	0.74	0.91
Valine	0.83	0.76	0.68	0.68	0.74	0.73	0.68	0.71	0.70	0.23	0.71	0.74	0.69	0.69	0.70	0.68	0.74	0.71
TSAA	0.74	0.62	0.65	**0.56**	**0.63**	**0.60**	0.62	0.64	**0.58**	0.22	0.61	**0.61**	0.63	**0.58**	**0.58**	**0.57**	**0.63**	**0.60**

*EFG broiler simulation model, The limiting amino acids are highlighted in bold.

Appendix 5.3 *Analysis of variance when fitting a quadratic model to the weight gain response data obtained when broilers were offered mixtures of three protein sources.*

Source	DF	SS	MS	P	
Regression	5	665.73	133.15	0.000	*
Linear	2	517.53	240.01	0.000	*
Quadratic	3	148.20	49.40	0.000	*
Residual	45	229.87	5.11	-	
Error					
Lack-of-Fit	19	131.62	6.93	0.075	n.s.
Pure error	26	98.24	3.78		
Total	51	899.03			

n.s. not significantly different at P<0.05
otherwise significantly different at P<0.05(*)

Appendix 5.4 *Analysis of variance when fitting a quadratic model to the feed intake response data obtained when broilers were offered mixtures of three protein sources.*

Source	DF	SS	MS	P	
Regression	5	1915.01	383.00	0.000	*
Linear	2	1539.65	785.09	0.000	*
Quadratic	3	375.36	125.12	0.004	*
Residual	45	1088.95	24.20	-	
Error					
Lack-of-Fit	19	492.96	25.95	0.373	n.s.
Pure error	26	595.99	22.92		
Total	51	3007.89			

n.s. not significantly different at P<0.05
otherwise significantly different at P<0.05(*)

Appendix 5.5 *Analysis of variance when fitting a quadratic model to the feed conversion efficiency response (FCE) data obtained when broilers were offered mixtures of three protein sources.*

Source	DF	SS	MS	P	
Regression	5	76001	15200.10	0.000	*
Linear	2	45827	20642.10	0.000	*
Quadratic	3	30174	10058.00	0.000	*
Residual	45	52581	1168.50	-	
Error					
Lack-of-Fit	19	33875	1783.00	0.016 n.s	
Pure error	26	18706	719.50		
Total	51	130959			

n.s. not significantly different at P<0.01
otherwise significantly different at P<0.01(*)

Appendix 5.6 *Estimated regression coefficients obtained by fitting the responses with a quadratic model.*

Term	Regression coefficients for:		
	Feed intake	Weight gain	FCE
x_1(FM)	44.0 (2.26)*	16.8 (1.04)*	375.3 (15.7)**
x_2(SF)	24.1 (2.26)*	6.7 (1.04)*	294.7 (15.7)**
x_3(SY+M)	47.1 (2.26)*	19.9 (1.04)*	421.2 (15.7)**
x_1*x_2	37.0 (9.99)*	24.1 (4.58)*	314.2 (69.4)**
x_1*x_3	-5.2 (9.99) n.s.	4.4 (4.58)n.s.	55.0 (69.4)**
x_2*x_3	12.5 (9.99) n.s.	8.5 (4.58)n.s.	150.4 (69.4)**

Standard deviation of the mean in parenthesis
n.s. not significantly different at P<0.05 for Feed intake and mass gain otherwise significantly different at P<0.05(*)

n.s. not significantly different at P<0.01 for FCE
otherwise significantly different at P<0.01(**)