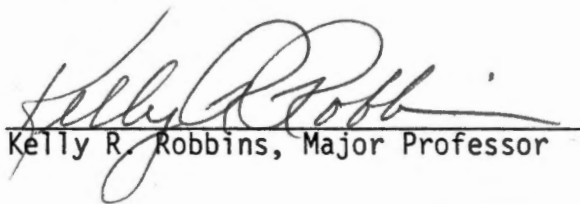
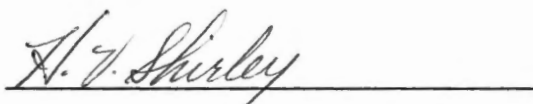


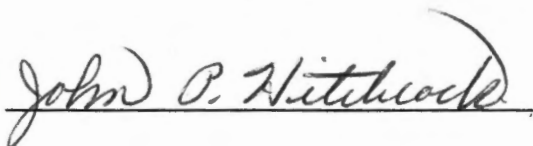
To the Graduate Council:

I am submitting herewith a thesis written by Nancy S. Mitchell entitled "Sulfur Amino Acid Utilization By Growing Broilers Fed Practical and Semi-Purified Diets at Two Energy Levels." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.


Kelly R. Robbins, Major Professor

We have read this thesis
and recommend its acceptance:





Accepted for the Council:


The Graduate School

SULFUR AMINO ACID UTILIZATION BY GROWING BROILERS
FED PRACTICAL AND SEMI-PURIFIED DIETS AT
TWO ENERGY LEVELS

A Thesis
Presented for the
Master of Science
Degree
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Nancy S. Mitchell

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ABSTRACT

A series of six experiments was conducted utilizing either corn-soybean meal or semi-purified basal diets. All experiments utilized a total of 1807 Hubbard x White Mountain strain chicks with ages ranging from 1 to 49 days posthatching. The objective of these experiments was to determine total sulfur amino acid (methionine + cystine) requirements for growing broilers fed practical and semi-purified diets at two energy levels.

Experiment 1 employed a corn-soybean basal diet at two energy levels to establish the sulfur amino acid requirement for maximal feed efficiency of chicks during the period 8 to 22 days posthatching. Five graded levels of DL-methionine (0 to .24% in the presence of .80% and .79% methionine + cystine, for low and high energy basal diets, respectively) were employed in a 2 x 5 factorial treatment arrangement. There was a marked growth response due to increased energy level. There was no significant gain response due to supplemental DL-methionine. Gain/feed ratio was maximized at .98% methionine + cystine for the low energy diet (2970 kcal ME/kg) and at .91% methionine + cystine for the high energy diet (3232 kcal ME/kg).

Experiment 2 was conducted to determine the sulfur amino acid requirement for maximal feed efficiency of chicks during the period 21 to 34 days posthatching. DL-methionine additions were identical to those used in Experiment 1. As with the younger chicks

there was a marked growth response to increased energy level and no significant gain response to supplemental DL-methionine. Gain/feed ratio was maximized at .89% methionine + cystine for the low energy diet (3045 kcal ME/kg) and at .95% methionine + cystine for the high energy diet (3307 kcal ME/kg).

Experiment 3 was conducted to confirm results of Experiment 1 and 2 that utilized battery-reared chicks. This trial was conducted in a grower house and in a manner more closely resembling those currently employed in the broiler industry. The basal diets were the same as employed for Experiment 2; however, methionine additions were decreased to .05, .10, .15, and .20%. There was a significant methionine effect for both the low and high energy diets. As DL-methionine was incrementally added from 0 to .20% (.91% methionine + cystine) there was a significant improvement in gain/feed ratio for birds on the high energy diet. However, unlike Experiments 1 and 2 there was no marked growth response due to increased energy levels. To further substantiate the results with practical corn soybean diets a semi-purified basal diet was employed for Experiments 4, 5, and 6.

In Experiment 4, five graded levels of DL-methionine (0 to .60% in the presence of .59% methionine + cystine) were employed in a 2 x 5 factorial treatment arrangement. As with previous experiments, there was a marked growth response in young broiler chicks due to increased energy levels. Gain/feed ratio was maximized at .89% methionine + cystine for the low energy diet

(2750 kcal ME/kg) and at 1.04% methionine + cystine for the high energy diet (3200 kcal ME/kg). Based on gain data, the total sulfur amino acid requirement was estimated to be $.78 \pm .227\%$ for the low energy basal diet and $.81 \pm .095\%$ for the high energy diet. Both of these estimates are lower than the recommended .93% methionine + cystine requirement listed by the National Research Council (N.R.C.) for broiler chicks 0 to 3 weeks of age.

Experiment 5 was conducted to determine if the same effects of energy level and methionine supplementation would occur in the older chicks (4 to 6 weeks of age). Similar diets and methionine additions were used for the older birds. A marked growth response occurred due to increased energy levels. There was also a significant gain response due to supplemental DL-methionine for both energy levels. Gain/feed ratio was significant over all levels of supplemental DL-methionine for the high energy diet and was maximized at .90% methionine + cystine for the low energy diet. Based on gain data, the total sulfur amino acid requirement was estimated to be $.96 \pm .085\%$ for the low energy diet and $.75 \pm .130\%$ for the high energy diet. These estimates are both higher than the recommended .72% methionine + cystine requirement by the N.R.C. for broiler chicks 3 to 6 weeks of age.

In Experiment 6 choline was incrementally added, from 600 to 1800 mg/kg diet, to a semi-purified basal diet containing .803% methionine + cystine, 22.1% crude protein and 3200 kcal ME/kg. Results indicated no significant differences for gain, feed consumption, or gain/feed due to choline supplementation.

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CHAPTER I

INTRODUCTION

Indispensable amino acids are of major importance to the monogastric nutritionist. To maintain proper levels of these amino acids in the diet, it has been suggested that rations be balanced for specific amino acids rather than by the popular method of using crude protein. In practical corn-soybean meal poultry diets, the sulfur amino acids (methionine + cystine) are usually first limiting. Poultry utilize relatively large amounts of sulfur amino acids during both the growing and reproductive portions of their life. Therefore, studies on their requirements must be conducted at several ages. In proportion to body weight, feed consumption increases with increasing size. As weight increases, more of the feed is used for maintenance and a smaller portion is used for protein synthesis. Since amino acids contribute to this protein, it has been suggested that their levels may also decrease in the growing animal. However, there is still some debate in the case of sulfur-bearing amino acids.

Another very important factor in the determination of methionine + cystine requirements is the evaluation of feedstuffs. Results from many related experiments vary greatly due in part to the popular practice of using table values to estimate the levels of methionine, cystine, and choline in the basal diets. Table values are unable to allow for variation in different lots

of the same ingredient. These discrepancies can result in the need of additions of up to one pound per ton of supplemental sulfur containing amino acids.

Therefore, studies were conducted with practical corn-soybean meal diets as well as semi-purified diets to better determine the methionine + cystine requirement of broilers 0 to 3 weeks of age and 4 to 6 weeks of age. To improve the validity of requirement estimates and to further characterize the experimental diets, methionine + cystine bioavailability was determined in the corn and soybean meal used.

Methionine and choline are two related nutrients required for optimum performance of poultry. Methionine fulfills three major metabolic roles. It is an indispensable amino acid, a precursor of the amino acid cystine, and a precursor of choline. It is through choline that methionine provides one carbon unit for purine and pyrimidine synthesis, for example. However, the birds ability to synthesize choline is low. Thus, it is necessary to supplement most poultry diets with both methionine and choline. But, the quantitative aspects of this have been debated since it was first reported in 1943. Therefore, an additional study was conducted to determine if the methionine requirement was affected by supplemental choline in a corn-soybean basal diet.

CHAPTER II

LITERATURE REVIEW

I. FORMS OF SUPPLEMENTAL METHIONINE

Because most poultry feed in the United States today is composed primarily of corn and soybean meal, the latter of which is naturally lacking in sulfur amino acids (methionine + cystine), various compounds have been used by the feed industry to fulfill the chicken's sulfur amino acid requirement. Much work has been done recently on the comparison of the efficacy of two available forms of synthetic methionine, OH-methionine and DL-methionine, for the growing chick when fed both in practical and purified diets. On the basis of the ratio of the molecular weights of OH-methionine and methionine as adjusted by purity, it is often assumed that commercial OH-methionine has a value of 82.8% that of DL-methionine on a weight basis. Typical relative values determined with corn-soybean meal diets tend to support this conclusion (Harms et al., 1976 a,b; 1977).

Christensen and Anderson (1980) conducted work with practical and amino acid diets containing varying levels of methionine that revealed a pronounced effect due to methionine additions. OH-methionine was found to be slightly less efficacious than methionine when the diet was deficient in methionine. However, when methionine levels were closer to the requirement, efficacy was similar for both compounds.

Results with semi-purified diets have not been as consistent as those with practical or crystalline amino acid diets. Some investigators have found the same level of efficacy for OH-methionine and DL-methionine as measured by feed efficiency and rate of growth (Creger et al., 1968; Romoser et al., 1976), whereas other researchers have found OH-methionine to be less efficacious than DL-methionine (Halloran and Bishop, 1966; Tipton et al., 1966).

Other studies have evaluated the comparative utilization of the D- and L-isomers of OH-methionine (Katz and Baker, 1975). It appears that man utilizes D-methionine poorly relative to L-methionine under conditions of either intact protein or crystalline amino acid consumption (Baker and Boebel, 1980). Similarly, work with chicks has shown faster growth results when L-methionine is fed rather than D-methionine, particularly when the methionine compounds were added to a crystalline amino acid diet distinctly deficient in sulfur amino acids (Katz and Baker, 1975). In other work by Baker and Boebel (1980) it was reported that differences among the isomers of methionine and OH-methionine are small when intact protein diets are fed, but large when protein-free, crystalline amino acid diets are fed.

In one study, Baker and Boebel (1980) fed chicks a methionine deficient (.10% DL-methionine) cystine adequate crystalline amino acid diet. Chicks fed supplemental methionine gained faster and more efficiently than those fed isomolar quantities of OH-methionine, regardless of the isomeric form

administered. L-methionine was clearly more efficacious than D-methionine, but L-OH-methionine was clearly inferior to D-OH-methionine. While DL-methionine tended to be more efficacious than D-methionine, DL-OH-methionine appeared equal in efficacy when compared with D-OH-methionine. A toxicity study that accompanied this work clearly showed that the D- and L-isomers of OH-methionine are not equivalent biologically. An isomolar quantity (2.0%) of D-OH-methionine severely depressed growth and resulted in hemolytic anemia and iron deposition in the spleen. However, the same quantity of L-OH-methionine was essentially safe. DL-OH-methionine produced responses intermediate between D- and L-OH-methionine.

More recently, work has centered around the use of the liquid free acid form of OH-methionine as an alternative to the conventional dry supplements. It is felt that this liquid intermediate will allow for a more even distribution in the diet, easier handling and a lower manufacturing cost. Results showed that the liquid methionine product OH-methionine appeared equal to a commercial DL-methionine product in efficacy for broilers grown to market weights. Performance of chicks fed diets fortified with OH-methionine was equal to that of chicks fed diets fortified with L-methionine, DL-methionine or the calcium salt of methionine hydroxy analogue (Waldroup et al., 1981).

However, work conducted by Van Weerden et al. (1982) seemed to disagree with these results. Performance of chicks fed the

OH-methionine supplemented diets was distinctly inferior to those fed equimolar quantities of DL-methionine. The DL-OH-methionine was only 76% as effective as DL-methionine on an equimolar basis, which is 66% by weight of the commercial product used. The conflicting results between purified and practical diets seem to indicate that the basal diet content may influence the efficiency of the supplemental methionine products. However, it seems more important to note the differences in methionine level and total sulfur amino acid content when purified and practical diets are fed. In practical diets, 80 to 90% of the sulfur amino acid requirement is provided by the protein in the basal portion. This leaves only a small amount that may be added by a methionine source. In contrast, when purified diets are used a greater amount of sulfur amino acids comes from the methionine test source. This allows for greater and more easily detectable differences between treatments.

Given that the degree of methionine deficiency in the basal diet is the primary factor influencing the apparent efficacy of OH-methionine, the dietary methionine:cystine ratio may also affect the response of supplemental OH-methionine. It was found that OH-methionine activity in crystalline amino acid diets was poorest when added to a basal diet adequate in cystine but devoid of methionine, better when the basal diet was adequate in methionine but devoid of cystine, and best when the basal diet

was deficient in total sulfur amino acids but contained an equal weight contribution of both methionine and cystine (Scott et al., 1966).

These results were confirmed with the additional observation by Sasse and Baker (1974) that chicks fed OH-methionine as the sole source of dietary sulfur amino acids in a chemically-defined diet performed as poorly as those fed a combination of OH-methionine and cystine. It is important to note the portion of the total sulfur amino acid that can be provided by cystine. This varies from as little as 40% (or less) at deficient sulfur amino acid levels to at least 50% at the sulfur amino acid requirement (Sasse and Baker, 1974). Therefore, it seems that the differences in efficacy between DL-methionine and DL-OH-methionine that are so easily detectable in crystalline amino acid diets also occur in the practical type diets. The problem seems to be with the lack of sensitivity inherent in diets that are only marginally deficient in methionine (Boebel and Baker, 1982).

II. CYSTEINE

As stated previously, methionine has several important functions in the body, one of which is a precursor of cysteine. The pathway for this function is also the metabolic scheme for methionine catabolism. Since both methionine and cysteine are physiologically essential for normal maintenance and growth of the animal, their combined dietary total and individual levels

within that total have been an area of experimentation. Therefore, when cysteine is deficient and methionine is in excess, methionine is probably converted to cysteine. This catabolism achieves two functions: (1) it removes excess methionine which is extremely toxic (Sauberlich, 1961), and (2) it overcomes the deficiency of cysteine. The reverse of the methionine to cysteine pathway does not occur. Cysteine has been shown to be capable of replacing part but not all of the dietary need for methionine. Much work has been done to determine the maximum amount of the animal's total sulfur amino acid needs that can be supplied by cysteine. These estimates have varied depending on species, age, physiological function, and method of measurement. However, in general, dietary cysteine can contribute approximately 50% of the total sulfur amino acid needs for the young animal and about 80 to 90% for the mature nonproducing animal (Graber et al., 1971).

Poultry utilize relatively large amounts of sulfur amino acids during the growing and reproductive portions of their life. Therefore, studies on their requirements must be conducted at several stages of their life cycle. In proportion to body weight, feed consumption increases with increasing size. Further, as weight increases more of the feed is used for maintenance and a smaller portion is used for protein synthesis. Since amino acids contribute to this protein it has been suggested that their levels may also decrease in the growing animal. However, there is still some debate in the case of the sulfur-bearing amino acids (Chung et al., 1973).

Previous work has suggested a direct relationship between the amino acid composition of keratoid tissue and the amino acid needs for maintenance of the older bird. Feathers represent 4 to 7% of the chicken's weight and are a keratoid tissue high in protein which utilizes a large portion of circulating sulfur amino acids during synthesis. Also, feathers contain 10 to 15 times more cysteine than methionine. Therefore, the increasing need for cysteine conflicts with the concept of a decreasing amino acid need with age (Block and Weiss, 1956).

Since cysteine is the major sulfur-bearing amino acid in feathers, the sparing ability of cysteine for methionine would be expected to increase with increasing age. Studies have shown an increase in the cysteine requirement coupled with a decrease in the methionine requirement in addition to a replacement value of 55% cysteine of the total sulfur amino acid requirement in maturing birds (Block and Weiss, 1956).

Females from feather-sexed broiler strains have been shown to have a more extensive feather development than males through the starting phase of production. Studies indicate that feather growth decreases markedly (0-3 weeks posthatching) with the female Leghorn at a smaller weight and younger age than for the male. Therefore, continuing feather growth by the male through the latter portion of production would lead to a converse situation for cysteine (Kuenzel et al., 1979).

Combinations of methionine and cysteine have also been investigated to see what effects these combinations may have on the total sulfur amino acid requirement. When considering inter-relationships between cysteine and methionine it is important to review absorption of these amino acids. One site exists in the chick's mucosal epithelial membrane which provides for the absorption of both D- and L-methionine. L-cysteine inhibits the uptake of both isomers by competing with them for the common site (Lerner and Taylor, 1967).

Generally, amino acid requirements are expressed as a percentage, either of the diet or per unit of dietary energy. Because of the conversion of methionine to cysteine (or cystine) and the difference in molecular weight between methionine and cysteine, it would be more accurate to state requirements in a manner that equalizes numbers of molecules. The same number of molecules is supplied by 1.25 g of methionine or 1.00 g of cysteine. Therefore, it appears more efficient to express the sulfur amino acid requirement on a mole per unit of feed basis (Wheeler and Latshaw, 1981).

III. METHIONINE--CHOLINE RELATIONSHIP

Methionine and choline are two related nutrients required for optimum performance of poultry. As stated previously, methionine is a precursor of choline. It is through choline that methionine provides one carbon units for purine and pyrimidine

synthesis. However, the birds ability to synthesize choline is low. Thus, it is necessary to supplement most poultry diets with both methionine and choline. But the quantitative aspects of this have been debated since it was first reported in 1943. Therefore, it appears that there is considerable confusion concerning the choline-methionine interrelationship in avian nutrition.

The dietary choline requirement of chickens is age dependent. Young chicks have a readily demonstrated choline requirement, but after 8 weeks of age, a dietary choline need is difficult to show (Nesheim et al., 1971). However, laying hens fed corn-soy-meat meal diets appear to require supplemental choline to maintain maximum egg weight and production. The total choline requirement appears to be greater than 1000 ppm but no more than 1500 ppm. Adequate methionine is needed to provide the carbon skeleton for the sulfur amino acids in the egg proteins (Tsiagbe et al., 1982).

The methylation of aminoethanol to methylaminoethanol seems to be the rate-limiting step in choline biosynthesis. High levels of dietary methionine or other methyl donors therefore cannot completely spare the chick's requirement for dietary choline which is in contrast to what occurs with growing mammals (Nesheim and Johnson, 1950). When Molitoris and Baker (1976) fed a choline-devoid crystalline amino acid diet containing a balanced mixture of excess dietary amino acids (except for methionine) the young chick's requirement for dietary choline was increased. All the amino acids, except methionine, which was a dietary variable,

were at minimal levels necessary to support maximum growth. These results indicate that excess dietary protein, but not methionine increases in the young chick's choline requirement and that dietary alterations do not alter the concentration of body choline (Molitoris and Baker, 1976). This agrees in part with earlier reports that show dietary protein and methionine have a marked effect on the choline required for maximum growth and for the prevention of perosis in growing chicks (Ketola and Nesheim, 1974).

Perosis is a malformation of the hock joint. The signs usually found are swelling and flattening of the hock joint, sometimes followed by slipping of the Achilles tendon from its condyles. The tibia and the tarsometatarsus may exhibit bending near the joint and lateral rotation. One or both legs may be affected. One way to prevent perosis is to feed a diet adequate in all indispensable nutrients, especially manganese, choline, niacin, biotin, and folic acid. After deformities have occurred they cannot be corrected by feeding an adequate diet.

The disagreement between the two reports can be based on the relative level of methionine used to make the comparisons as well as the criteria used for quantification. Ketola and Nesheim (1974) compared a diet deficient in methionine with one containing excess methionine and used perosis score as their measurement criterion. Whereas Molitoris and Baker (1976) compared excess levels of methionine with an adequate level of methionine and used weight gain as their measurement criterion.

To further complicate interpreting data, many researchers estimate from feed composition tables the methionine, cystine, and choline levels used in the basal diets. There is a large amount of variability when this procedure is used although it is an accepted practice in industry. Also, variability may occur within cultivars of the same ingredient. Some soybean cultivars may have as much as 20% more methionine than others (Rubin et al., 1979). In addition, relationships to other environmental factors besides methionine and choline content must first be known before the diet can be correctly evaluated. Some factors that may increase the chick's methionine requirement under practical conditions include dietary cystine, protein, energy, and copper levels, as well as environmental temperature, coccidiostats and disease (Baker et al., 1982).

Recent studies utilizing both a purified diet and a corn-soybean meal diet have indicated that when young chicks are fed a diet deficient in choline, methionine spares choline; and, when fed a diet deficient in methionine, choline spares methionine. However, when each nutrient is at its physiological requirement, an excess of either nutrient has minimal capacity to reduce the dietary need for the other (Baker et al., 1982). Therefore, in contrast to the situation that occurs in mammalian species, no amount of excess methionine can eliminate the dietary need of chicks for choline. And since supplemental methionine and choline typically account for 1.5 to 3% of total feed costs for broiler

production, with the price of methionine per unit weight about 2-1/2 times that of choline, it would not be feasible to attempt to meet the choline requirement by feeding excess levels of methionine.

IV. EFFECT OF DRUG ADDITIVES ON METHIONINE REQUIREMENT

Much interest has been exhibited in the effects caused by feeding antibiotics and other drug additives on the dietary requirement of broiler type chicks. Anticoccidial activity has been claimed for many polyether antibiotics including monensin (Haney et al., 1970) and lasalocid (Mitrovic and Schildknecht, 1974). Both antibiotics are ionophores capable of transporting metal ions through biological membranes, but they differ in their ion selectivity. Monensin optimally transports monovalent ions like Na^+ and K^+ but is ineffective in transporting divalent ions (Pressman, 1968). Lasalocid, in contrast, is capable of transporting divalent metal ions like Mg^{++} and Ca^{++} in addition to monovalent ions (Pressman, 1973).

It has been suggested that environmental and/or nutritional stress conditions may result in feather pecking over the back in monensin-fed birds (Kiker and Sherwood, 1974). Also noted were problems of poor feathering, growth depression, and flightiness, but these reports are now well documented. Since feathers are high in cystine, some concern has been expressed that monensin, and possibly other anticoccidials, might affect the chick's need

for sulfur amino acids. It was found that monensin levels higher than 75 ppm depressed chick growth (Damron et al., 1977). Increasing the level from 99 to 121 ppm of monensin resulted in a further significant reduction of body weights. However, no significant interaction of coccidiostat with methionine was found indicating that lasalocid and monensin did not influence the methionine requirement. In studies conducted on the methionine response of chicks fed either corn-pea-soybean meal or corn-soybean meal diets in the presence and absence of either monensin (121 mg/kg) or lasalocid (125 mg/kg) results indicated that lasalocid exerts an "antibiotic-like" methionine-sparing effect when a diet distinctly deficient in sulfur amino acids is fed (Patel et al., 1979). They further suggested that growth may plateau at a lower level of supplemental methionine when 121 mg/kg is fed. Also, they observed that the growth depression associated with the use of monensin was not overcome or counteracted by the addition of levels of methionine higher than the N.R.C. (1977) requirement. The growth depressing effect of monensin was associated with continued intake of the drug as evidenced by the observation that as soon as the drug was withdrawn from the diet, growth rate increased immediately and feed efficiency improved during the drug withdrawal period (Patel et al., 1979).

Lasalocid, also a polyether antibiotic approved for use in commercial broiler production has been found to have a high degree of efficacy against various strains of Eimeria. It was

noted that medicated birds were heavier, had an improved feed efficiency, exhibited a lesser degree of pathology, and had a lower mortality when compared to infected non-medicated controls. In general, it would appear that .0075% is the optimum level for lasalocid in feed. This projected use level (.0075%) was effective, palatable, and safe for chicks (Mitrovic and Schildknecht, 1974). At the recommended level, the drug proved very effective in reducing morbidity and preventing mortality due to coccidiosis (Taylor et al., 1974). Lasalocid was found to provide better or equal performance than eight other coccidiostats (Mitrovic et al., 1975). Lasalocid exerted a methionine sparing effect when added to diets that were deficient in sulfur amino acids based on increased growth and feed efficiency and increased feather weight. The cause of this growth response to lasalocid in diets severely deficient in sulfur amino acids is unknown. Lasalocid was also found to have growth depressing effects when used at the upper (125 ppm) level of the range approved for use in poultry feed (Taylor et al., 1974). In later work it was found that feeding levels of lasalocid greater than 75 ppm numerically depressed body weights (Damron et al., 1977).

Feed efficiency was greatly affected by supplementation of methionine but not by the supplementation of different coccidiostats. Feed efficiency data showed that the growth depression associated with the use of monensin was not due to impairment in digestion or absorption of nutrients but probably

was due to a depression in feed consumption. It was suggested that lasalocid used in a broiler diet decreased methionine requirement for growth, feed efficiency, and for feather growth (Patel et al., 1980). However, it may also be noted that the marked sulfur amino acid X lasalocid interaction is not of practical importance since at the levels of sulfur amino acid normally encountered in practical-type diets no interaction was apparent (Willis and Baker, 1980).

V. EVALUATION OF FEEDSTUFFS

Increased emphasis has been placed in recent years on the amino acid requirements and recommendations for poultry. Methionine and total sulfur amino acid requirements (methionine + cystine) have been of particular interest since they generally influenced least-cost feed formulas (corn-soy-based diets). Also it is important to note that amino acid requirements are not absolute values. They depend on a variety of factors. The poultry industry today utilizes more high energy diets which frequently are relatively low in crude protein. Also other factors besides the methionine and choline content of the diet must be evaluated before the amino acid requirements can be determined. These factors include dietary cystine and copper content, environmental temperature, coccidiostats, and the general health of the chicks (Baker et al., 1982).

Another very important factor is the evaluation of feedstuffs. Results from any related experiments vary greatly due in part to

the popular practice of using table values to estimate the levels of methionine, cystine, and choline in the basal diets. However, table values are unable to allow for variation in different lots of the same ingredient. These discrepancies can result in the need of additions of up to 1 pound per ton of supplemental sulfur containing amino acids.

Kornegay et al. (1975) determined the protein and amino acid content of several varieties of commercially grown opaque-2 corn obtained from several locations in Illinois in 1972 and in Virginia in 1973 and 1974. In the Illinois study crude protein content of opaque-2 corn ranged from 7.5 to 10.5% and averaged 8.9% when values were corrected to 90% dry matter. Only lysine content was determined for the samples due to discrepancies in the duplication of individual samples.

In the Virginia study in 1973 the protein content of the opaque-2 corn ranged from 7.3 to 9.6% and averaged 8.4% when values were corrected to 90% dry matter. In 1974 the protein content of the opaque-2 corn ranged from 6.8 to 10.6% and averaged 8.6% when values were corrected to 90% dry matter. In both 1973 and 1974 the cystine and methionine content averaged .32 and .16%, respectively for the opaque-2 varieties.

In the normal corn samples, protein content ranged from 7.5 to 9.7% in 1973 and averaged 8.7%. The samples contained a cystine and methionine content of .32 and .20%, respectively. Whereas in 1974 the protein content ranged from 7.0 to 10.3% and

averaged 8.5% with a cystine and methionine content of .29 and .18%, respectively. These results clearly indicate variations in feedstuffs not only from varieties of corn utilized but also from different sections of the country.

Another area of concern in formulating rations is the availability of nutrients within each feedstuff that is utilized. Netke and Scott (1974) reported the availability of total sulfur amino acids (TSAA) and available methionine in soybean meal and corn. Available cystine was determined by difference. Due to large variations between laboratories and also between analytical procedures, methionine and cystine availability estimates were reported without regard to the amount found to be present by analytical procedures. It was calculated from regression coefficients that each gram of soybean meal consumed exhibited an activity equivalent to 12 mg of available sulfur amino acids of 1.2% of soybean meal. In the second assay the basal diet was supplemented with L-cystine at the requirement (.35%). It was calculated from regression coefficients that each mg of crystalline methionine consumed resulted in a weight gain of .282 grams and each gram of soybean meal consumed resulted in a weight gain of 1.613 grams. By calculation each gram of soybean meal has the growth promoting activity of 5.72 mg of methionine (.572% of soybean meal). By difference soybean meal contains .628% cystine. When these values were expressed as a percent of soybean protein the values were 1.15% and 1.26% for methionine

and cystine, respectively. These values agree with analytical values (1.1 and 1.2%) reported by Block and Weiss (1956). This could indicate that sulfur amino acids present in good grade soybean meal are highly available to the chick for growth purposes.

For the corn assays conducted by Netke and Scott (1974) it was reported that each gram of corn consumed resulted in a gain of .592 grams, with the methionine + cystine activity in 1 gram of corn being 4.26 mg (.426% of corn). It was calculated that each gram of corn consumed had the activity of 1.19 mg of methionine (.119% of corn). Since the total sulfur amino acid activity in corn was found to be .426%, then by difference the cystine content of corn would be .307%. In the samples of corn used the protein of corn contained 1.3% available methionine and 3.4% available cystine. Subsequent work carried out by Sasse and Baker (1972) would indicate that the availability of total sulfur amino acid in corn approaches 100%.

CHAPTER III

MATERIALS AND METHODS

I. EXPERIMENTAL ANIMALS AND TREATMENTS

A series of six experiments was conducted using either corn-soybean meal or semi-purified basal diets. Broiler chicks Hubbard x White Mountain strain, were used in all experiments and work was conducted at The University of Tennessee, Knoxville poultry farm. All chicks, except for Experiment 3 which will be discussed later, were housed in heated thermostatically-controlled wire-floored starter batteries, and a 24-hour constant light schedule was maintained. All chicks were feather-sexed upon hatching and fed a corn-soybean meal starter diet during the first seven days posthatching. After an overnight fast, the chicks were inspected, weighed, wing banded, and randomly allotted to treatments by weight (Sasse and Baker, 1974). Experimental groups were selected to have the same initial mean weight and a similar weight distribution. Feed and water were provided ad libitum. Mortality was recorded daily and feed consumption data were adjusted when deaths occurred.

The first experiment was conducted utilizing 180 8-day-old chicks with an average initial weight of 148 grams. The assay period was 8 to 22 days posthatching. Male and female chicks were allotted to experimental groups. The composition of the corn-soybean

meal basal diet is presented in Table 1. Basal diet 1 was calculated to contain 2970 kcal ME/kg and .80% methionine + cystine; whereas diet 2 contained 3232 kcal ME/kg and .79% methionine + cystine. Both basal diets were calculated to contain 22.0% crude protein. Ten experimental diets consisting of a factorial arrangement of 2 energy levels x 5 levels of supplemental methionine were used. Each treatment was randomly assigned to triplicate groups of seven chicks each. In diets 1-5 methionine was incrementally added, thus increasing methionine + cystine from .80% to 1.04%. Diets 6-10 contained the same methionine levels but employed the high energy basal diet (diet 2).

Experiment 2 was conducted utilizing 180, 21-day-old male and female chicks with an average initial weight of 438 grams. The assay period was 21 to 34 days posthatching. The composition of the corn-soybean basal diet is presented in Table 2. Basal diet 1 was calculated to contain 3045 kcal ME/kg and .71% methionine + cystine; whereas diet 2 contained 3307 kcal ME/kg and .71% methionine + cystine. Both basal diets were calculated to contain 19.0% crude protein. Ten experimental diets consisting of a factorial arrangement of 2 energy levels x 5 levels of supplemental methionine were used. Treatments were randomly assigned to triplicate groups of six chicks each with an average initial weight of 438 grams. In diets 1-5 methionine was incrementally added, thus increasing methionine + cystine from .71% to .95%. The same methionine levels were used in diets 6-10 but the energy level was increased.

TABLE 1. Composition of the Corn-Soybean Meal Basal Diets
(Experiment 1)

Ingredient	%	
	Basal 1	Basal 2
Corn (7.8% CP) ^a	60.63	54.56
Soybean meal (47.1% CP) ^a	36.42	37.49
Corn oil	0.	5.00
Limestone	0.50	0.50
Dicalcium phosphate	1.20	1.20
Salt	0.50	0.50
Vitamin premix ^b	0.60	0.60
Coban ^c	0.10	0.10
BMD-25 ^d	0.05	0.05
Nutrient levels		
Metabolizable energy kcal/kg ^e	2970	3232
Crude protein, % ^f	22.0	22.0
Methionine, % ^f	0.391	0.344
Methionine + cystine, % ^f	0.798	0.791

^aDetermined by laboratory analysis.

^bVitamin premix provided per kilogram of diet: vitamin A, 4175 IU; vitamin D₃, 750 ICU; riboflavin, 4.74 mg; niacin, 41.7 mg; D-calcium pantothenate, 7.3 mg; vitamin B₁₂, 11.0 µg; choline chloride, 468.75 g.

^cMonensin premix (45 g/lb), Elanco Products Co., Indianapolis, IN.

^dBacitracin methylene disalicylate premix (25 g lb), A. L. Laboratories, Inc., Englewood Cliffs, NJ.

^eCalculated from published values (NRC, 1977).

^fCalculated from chemical analysis of corn and soybean meal employed.

TABLE 2. Composition of the Corn-Soybean Meal Basal Diets
(Experiments 2-3)

Ingredient	%	
	Basal 1	Basal 2
Corn (7.8% CP) ^a	68.29	62.21
Soybean meal (47.1% CP) ^a	28.86	29.94
Corn oil	0.	5.00
Limestone	0.50	0.50
Dicalcium phosphate	1.20	1.20
Salt	0.40	0.40
Vitamin premix ^b	0.60	0.60
Coban ^c	0.10	0.10
BMD-25 ^d	0.05	0.05
Nutrient levels		
Metabolizable energy, kcal/kg ^e	3045	3307
Crude protein, % ^f	19.0	19.0
Methionine, % ^f	0.351	0.347
Methionine + cystine, % ^f	0.712	0.705

^aDetermined by laboratory analysis.

^bVitamin premix provided per kilogram of diet: vitamin A, 4175 IU; vitamin D₃, 750 ICU; riboflavin, 4.74 mg; niacin, 41.7 mg; D-calcium pantothenate, 7.3 mg; vitamin B₁₂, 11.0 µg; choline choride, 468.75 g.

^cMonensin premix (45 g/lb), Elanco Products Co., Indianapolis, IN.

^dBacitracin methylene disalicylate premix (25 g/lb), A. L. Laboratories, Inc., Englewood Cliffs, NJ.

^eCalculated from published values (NRC, 1977).

^fCalculated from chemical analysis of corn and soybean meal employed.

Experiment 3 was conducted utilizing 1000 day old chicks with an average initial weight of 45 grams and the assay period was 1 to 49 days posthatching. Male and female chicks were utilized. The experiment was conducted in a naturally ventilated grower house that was 9.75 meters x 30.48 meters. The pens which contained 25 male and 25 female chicks each were 3.05 meters x 3.66 meters, thus allowing .21 square meters per bird. All chicks were vaccinated for infectious bronchitis and Newcastle disease at seven days of age. Ten experimental diets consisting of a factorial arrangement of 2 energy levels x 5 levels of supplemental methionine were used. Each treatment was randomly assigned in duplicate to one of 20 pens. The composition of the corn-soybean basal diet is presented in Table 2. The basal diets were the same as employed for Experiment 2, however; methionine additions were decreased. In diets 1-5 methionine was incrementally added, thus increasing methionine + cystine from .71% to .91%. The same methionine levels were used in diets 6-10, however, the energy level was increased.

In Experiment 4, 150 male chicks with an average initial weight of 95 grams were used. The experimental period was 7 to 18 days posthatching. The composition of the semi-purified basal diet is presented in Table 3. Basal diet 1 was calculated to contain 2750 kcal ME/kg and .59% methionine + cystine; whereas basal diet 2 contained 3200 kcal ME/kg and .59% methionine + cystine. Both basal diets were calculated to contain 22.1% crude protein. Ten experimental diets that consisted of a factorial arrangement

TABLE 3. Composition of the Semi-Purified Basal Diets
(Experiment 4)

Ingredient	%	
	Basal 1	Basal 2
Cornstarch	15.40	25.30
Dextrose	25.00	25.00
Isolated soy protein (81.7% CP) ^a	27.00	27.00
Glista salts ^b	5.37	5.37
Purified vitamin mix ^c	0.20	0.20
Choline chloride	0.20	0.20
Cellulose	14.83	10.93
Corn oil	5.00	6.00
Sand	7.00	0.
Nutrient levels		
Metabolizable energy, kcal/kg ^d	2750	3200
Crude protein, % ^e	22.1	22.1
Methionine, % ^e	0.313	0.313
Methionine + cystine, % ^e	0.593	0.593

^aDetermined by laboratory analysis.

^bBaker et al., 1982.

^cVitamin premix provided per kilogram diet: thiamin·HCl, 100 mg; riboflavin, 16 mg; niacin, 100 mg; D-calcium pantothenate, 20 mg; pyridoxine·HCl, 6 mg; biotin, 0.6 mg; cyanocobalamin, 20 µg; folic acid, 4 mg; menadione, 5 mg; vitamin A, 10,000 IU; cholecalciferol, 600 ICU; DL-α-tocopheryl acetate, 20 mg.

^dCalculated from published values (NRC, 1977).

^eCalculated from chemical analysis of the isolated soy protein employed.

of 2 energy levels x 5 levels of supplemental methionine were used. Treatments were randomly assigned to triplicate groups of six chicks each. In diets 1-5 methionine was incrementally added, thus increasing methionine + cystine from .59% to 1.19%. Diets 6-10 contained the same methionine levels but employed the high energy basal diet (diet 2).

Experiment 5 utilized 150 male chicks with an average initial weight of 829 grams. Chicks were raised in a grower house up to 28 days posthatching and were fed a corn-soybean meal pre-test diet. The assay period was 28 to 42 days posthatching. The composition of the semi-purified basal diet is presented in Table 4. Basal diet 1 was calculated to contain 2750 kcal ME/kg and .45% methionine + cystine; whereas basal diet 2 contained 3200 kcal ME/kg and .45 methionine + cystine. Both basal diets were calculated to contain 16.7% crude protein. Ten experimental diets that consisted of a factorial arrangement of 2 energy levels x 5 levels of supplemental methionine were used. Treatments were randomly assigned to triplicate groups of five chicks each. In diets 1-5 methionine was incrementally added, thus increasing methionine + cystine from .45% to 1.05%. The same methionine levels were used in diets 6-10, however, the energy level was increased.

In Experiment 6, 147 male chicks averaging 158 grams initially were used. The assay period was 8 to 19 days posthatching. The composition of the semi-purified basal diet is presented in Table 5. The basal diet was calculated to contain 3200 kcal ME/kg,

TABLE 4. Composition of the Semi-Purified Basal Diets
(Experiment 5)

Ingredient	%	
	Basal 1	Basal 2
Cornstarch	21.65	31.56
Dextrose	25.00	25.00
Isolated soy protein (81.7% CP) ^a	20.45	20.45
Glista salts ^b	5.37	5.37
Purified vitamin mix ^c	0.20	0.20
Choline chloride	0.20	0.20
Cellulose	15.13	2.53
Rice hulls	7.00	8.69
Corn oil	5.00	6.00
Nutrient levels		
Metabolizable energy, kcal/kg ^d	2750	3200
Crude protein, % ^e	16.7	16.7
Methionine, % ^e	0.237	0.237
Methionine + cystine, % ^e	0.449	0.449

^aDetermined by laboratory analysis.

^bBaker et al., 1982.

^cVitamin premix provided per kilogram diet: thiamin·HCl, 100 mg; riboflavin, 16 mg; niacin, 100 mg; D-calcium pantothenate, 20 mg; pyridoxine·HCl, 6 mg; biotin, 0.6 mg; cyanocobalamin, 20 µg; folic acid, 4 mg; menadione, 5 mg; vitamin A, 10,000 IU; cholecalciferol, 600 ICU; DL- α-tocopheryl acetate, 20 mg.

^dCalculated from published values (NRC, 1977).

^eCalculated from chemical analysis of the isolated soy protein employed.

TABLE 5. Composition of the Semi-Purified Basal Diet
(Experiment 6)

Ingredient	% of diet
Cornstarch	24.86
Dextrose	25.00
Isolated soy protein (81.7% CP) ^a	27.00
Glista salts ^b	5.37
Purified vitamin mix ^c	0.20
Choline chloride	0.20
Cellulose	10.93
Corn oil	6.00
DL-methionine	0.21
Nutrient levels	
Metabolizable energy, kcal/kg ^d	3200
Crude protein, % ^e	22.1
Methionine, % ^e	0.523
Methionine + cystine, % ^e	0.803

^aDetermined by laboratory analysis.

^bBaker et al., 1982.

^cVitamin premix provided per kilogram diet: thiamin·HCl, 100 mg; riboflavin, 16 mg; niacin, 100 mg; D-calcium pantothenate, 20 mg; pyridoxine·HCl, 6 mg; biotin, 0.6 mg; cyanocobalamin, 20 µg; folic acid, 4 mg; menadione, 5 mg; vitamin A, 10,000 IU; cholecalciferol, 600 ICU; DL-α-tocopheryl acetate, 20 mg.

^dCalculated from published values (NRC, 1977).

^eCalculated from chemical analysis of the isolated soy protein employed.

.803% methionine + cystine and 22.1% crude protein. Seven experimental diets that contained graded levels of choline from 600 to 1800 mg/kg were employed. Each treatment was randomly assigned to triplicate groups of seven chicks each.

II. DATA AND SAMPLE COLLECTION

Chicks utilized in all experiments were weighed initially and at the end of each assay period. Feed consumption was determined and blood samples were taken at the termination of Experiments 4 and 5. Blood samples were taken via heart puncture using a heparinized syringe.

III. PROCESSING AND ANALYSIS OF SAMPLES

Whole blood samples were centrifuged, the plasma was collected and proteins were precipitated by the addition of 25 mg 5'-sulfo-salicylic acid per ml. The protein-free plasma was collected, filtered, and appropriately diluted with lithium citrate buffer (pH 2.2). Amino acid concentrations were determined using an automated amino acid analyzer (Dionex D-400) utilizing a 5 buffer lithium citrate system with norleucine as an internal standard.

IV. STATISTICAL ANALYSIS

Data were analyzed using analysis of variance techniques. Orthogonal and non-orthogonal single-degree-of-freedom comparisons

were made to determine the significance of treatment effects. Amino acid requirements in Experiment 4 were estimated using the nonlinear (4 parameter) point estimation procedure described by Robbins et al. (1979). However, gain data for Experiment 5 could not be adequately fitted to the nonlinear model, thus requirements were estimated using the 3 parameter broken line model (Robbins et al., 1979).

CHAPTER IV

RESULTS AND DISCUSSION

A series of experiments was conducted to better determine the sulfur amino acid requirement of the growing broiler. The first series of Experiments (1-3) utilized corn-soybean meal diets that are similar to those utilized in the poultry industry today and the second series of Experiments (4-6) utilized semi-purified basal diets.

I. EXPERIMENT 1

Data for Experiment 1 are presented in Table 6. Results clearly demonstrated a marked growth response when energy was increased from 2970 to 3232 kcal ME/kg. The energy level generally recommended by the 1977 N.R.C. report is 3200 kcal ME/kg for growing broilers. This growth response occurred without a significant alteration in feed intake, thus resulting in a substantial feed efficiency response. Addition of methionine to the low-energy basal diet did not significantly affect either gain or feed efficiency. Thus, it appears that at this energy level .80% methionine + cystine meets the bird's sulfur amino acid requirement. This is substantially lower than the .93% recommended by the N.R.C. (1977). However, methionine addition to the high-energy basal diet improved gain and gain/feed by 8% and 9%, respectively. These data thus suggest that energy, not sulfur amino acids, is

TABLE 6. Effect of Supplemental Methionine on Performance of 1-to-3 Week Old Broilers Fed Corn-Soybean Meal Diets at Two Energy Levels (Experiment 1)^{a,b}

Added DL-methionine ^e (% of diet)	Dietary energy level (kcal ME/kg) ^c					
	2970			3232 ^d		
	Gain (g)	Feed Consumed (g)	Gain/feed (g/kg)	Gain (g)	Feed Consumed (g)	Gain/feed (g/kg)
0	352	791	445	397	795	499
.06	353	800	442	415	794	523
.12	355	797	445	435	793	549
.18	384	797	482	430	785	548
.24	367	796	461	433	787	550

^aMeans of triplicate groups of six chicks each (3 males + 3 females) fed the experimental diets from day 8 to day 22 post-hatching. Average initial weight was 148 g.

^bPooled SEM for gain, feed consumed, and gain/feed were 15.6, 4.3, and 20.0, respectively.

^cEffect of dietary energy level was significantly ($P < .01$) for gain and gain/feed.

^dThe comparison of 0% vs. .06 - .24% supplemental DL-methionine was significant ($P < .05$) for gain and gain/feed.

^eThe basal diets (Table 1, page 32) contained .798% (low energy) and .791% (high energy) total sulfur amino acids.

the first-limiting factor in corn-soybean meal diets for broiler chicks. When fed diets containing 3232 kcal ME/kg (a level near that recommended by N.R.C.), the sulfur amino acid requirement is apparently near .85%.

II. EXPERIMENT 2

Work was conducted to determine if the same effects of energy level and methionine supplementation would occur in older chicks. Performance data are presented in Table 7. Results indicated a marked growth response when energy was increased from 3045 to 3307 kcal ME/kg. Gain was increased by an average of 10% and gain/feed was increased by an average of 9% in chicks fed the high energy diet. There was no significant gain response to supplemental methionine in birds fed either the low energy diet or the high energy diet, thus for 3-to-5 week old broilers, .71% methionine + cystine appears to be adequate for maximal growth and feed efficiency. Similar to Experiment 1, the energy level in a fat-supplemented corn-soybean meal diet is inadequate to achieve maximum growth.

III. EXPERIMENT 3

Experiments 1 and 2 were both conducted in a laboratory environment. Therefore, Experiment 3 was conducted in a grower house and under conditions more closely resembling those currently utilized in the broiler industry. Performance data are presented

TABLE 7. Effect of Supplemental Methionine on Performance of 3-to-5 Week Old Broilers Fed Corn-Soybean Meal Diets at Two Energy Levels (Experiment 2)^{a,b}

Added DL-methionine ^d (% of diet)	Dietary energy level (kcal ME/kg) ^c					
	3045			3307		
	Gain (g)	Feed Consumed (g)	Gain/feed (g/kg)	Gain (g)	Feed Consumed (g)	Gain/feed (g/kg)
0	653	1025	637	717	1015	706
.06	645	988	653	705	989	713
.12	665	986	674	705	1010	698
.18	653	975	670	680	1024	664
.24	612	1026	596	737	975	756

^aMeans of triplicate groups of six chicks each (3 males + 3 females) fed the experimental diets from day 21 to day 34 post-hatching. Average initial weight was 438 g.

^bPooled SEM for gain, feed consumed, and gain/feed were 14.7, 21.0, and 23.1, respectively.

^cEffect of dietary energy level was significantly ($P < .01$) for gain, and gain/feed.

^dThe basal diets (Table 2, page 33) contained .712% (low energy) and .705% (high energy) total sulfur amino acids.

in Table 8. Results indicated that there was a significant methionine effect. For both basal diets, as methionine was incrementally added from 0-.20% (.71 to .91% methionine + cystine) there was a significant linear decrease in gain and feed consumed. The net effect was a substantial significant improvement in feed efficiency for both basal diets.

Results did not indicate a significant difference in growth response when energy was increased from 3045 to 3307 kcal ME/kg. However, feed intake was significantly lower for the high energy diet. The net effect was a substantially significant improvement in feed efficiency for birds on the high energy diet. It should be noted that results from all three experiments conducted with corn-soybean meal diets clearly indicated a significant improvement in feed efficiency due to increased energy levels. However, the marked growth response that occurred in Experiments 1 and 2 due to increased energy levels was not apparent in Experiment 3. In Experiments 1 and 2, feed consumption was not affected by energy level, and gain was significantly improved. In Experiment 3 gain was not affected, but feed consumption of birds fed the high energy diet was significantly reduced. This may be due to the high environmental temperatures experienced during this trial. Mean daily high temperature in the broiler house was approximately 32°C, with a mean low of approximately 27°C.

TABLE 8. Effect of Supplemental Methionine on Performance of Growing Broilers Fed Corn-Soybean Meal Diets at Two Energy Levels (Experiment 3)^{a,b,c}

Added DL-methionine ^d (% of diet)	Dietary energy level (kcal ME/kg)					
	3045			3307		
	Gain (kg)	Feed Consumed (kg)	Gain/feed (g/kg)	Gain (kg)	Feed Consumed (kg)	Gain/feed (g/kg)
0	1.55	3.51	441	1.60	3.10	516
.05	1.56	3.07	508	1.57	2.94	536
.10	1.54	3.01	512	1.47	2.62	562
.15	1.41	3.14	450	1.51	2.76	548
.20	1.51	2.89	522	1.45	2.64	549

^aData represent means of two groups of 50 chicks each (25 males + 25 females) fed the experimental diets from day 1 to day 49 posthatching. Average initial weight was 48 g.

^bPooled SEM for gain, feed consumed, and gain/feed were .037, .137, and .015, respectively.

^cThe linear effect of methionine was significant ($P < .02$) for gain, feed, and gain/feed. The linear effect of energy was significant ($P < .01$) for feed and gain/feed.

^dThe basal diets (Table 2, page 33) contained .712% (low energy) and .705% (high energy) total sulfur amino acids.

IV. EXPERIMENT 4

To further substantiate results with corn-soybean meal diets, another series of Experiments (4-6) was conducted utilizing a semi-purified basal diet. Performance and plasma methionine + cystine data are presented in Tables 9 and 10, respectively. Results clearly showed a marked growth response in young broiler chicks (1 to 2 weeks of age) when energy was increased from 2750 to 3200 kcal ME/kg. Feed intake was significantly lower for the high energy diet. These results differ from those reported by Robbins (1981). In experiments conducted with Hubbard broiler chicks he reported that feed consumption exhibited marked increases in feed intake as dietary energy level increased. He also reported that the energy requirement is near 2700 kcal ME/kg for Hubbard broiler chicks. This energy level is somewhat lower than those utilized in this work. There was a significant gain response to supplemental methionine in birds fed the low energy diet with the largest significant response obtained with the first addition of methionine (.15%). In birds fed the high energy diet, the largest gain response also occurred with the first addition of supplemental DL-methionine. The feed efficiency responses paralleled the gain responses.

Based on gain data, the total sulfur amino acid requirement was estimated to be $.78 \pm .227\%$ for the low energy basal diet. This estimate is equivalent to .19% supplemental DL-methionine. Based on gain data for the high energy basal diet, the total

TABLE 9. Effect of Supplemental Methionine on Performance of 1-to-2 Week Old Broilers Fed a Semi-Purified Basal Diet at Two Energy Levels (Experiment 4)^{a,b}

Added DL-methionine ^c (% of diet)	Dietary energy level (kcal ME/kg)					
	2750			3200		
	Gain ^{d,e} (g)	Feed Consumed ^f (g)	Gain/feed ^g (g/kg)	Gain ^{d,h} (g)	Feed Consumed ^f (g)	Gain/feed ^g (g/kg)
0	110	314	350	86	214	402
.15	208	455	457	223	411	543
.30	236	406	581	246	386	637
.45	238	457	521	259	391	662
.60	227	464	489	246	391	629

^aMeans of triplicate groups of five male chicks fed the experimental diets from day 7 to day 18 posthatching. Average initial weight was 95 g.

^bPooled SEM for gain, feed consumed, and gain/feed were 11.5, 16.1, and 27.5, respectively.

^cThe basal diets (Table 3, page 35) contained .593% (low energy) and .593% (high energy) total sulfur amino acids.

^dGain: cubic effect of methionine significant ($P < .01$). Linear effect of methionine X linear effect of energy significant ($P < .09$).

^eBased on gain data, the total amino acid requirement was estimated to be $.78\% \pm .227$ (this is equivalent to .19% supplemental DL-methionine).

^fFeed consumed: quadratic effect of methionine X linear effect of energy significant ($P < .03$).

^gGain/feed: linear effect of methionine X linear effect of energy significant ($P < .05$). Linear effect of energy significant ($P < .01$). Quadratic effect of methionine significant ($P < .01$).

^hBased on gain data, the total amino acid requirement was estimated to be $.81\% \pm .095$ (this is equivalent to .22% supplemental DL-methionine).

TABLE 10. Plasma Concentrations of Free Methionine and Cystine in 1-to-2 Week Old Chicks Fed a Semi-Purified Basal Diet at Two Energy Levels (Experiment 4)^{a,b}

Added DL-methionine (% of diet)	Dietary energy level (kcal ME/kg)			
	2750		3200	
	Methionine (nmol/ml)	Cystine (nmol/ml)	Methionine (nmol/ml)	Cystine (nmol/ml)
0	45	213	73	241
.15	83	259	58	221
.30	60	285	60	199
.45	97	280	76	255
.60	99	249	129	201

^aMeans of triplicate groups of five male chicks fed the experimental diets from day 7 to day 18 posthatching.

^bPooled SEM for methionine and cystine were 22.4 and 42.6, respectively.

sulfur amino acid requirement was estimated to be $.81 \pm .095\%$. This estimate is equivalent to .22% supplemental DL-methionine. The level of methionine + cystine in both the low energy and high energy basal diets are lower than those recommended by the N.R.C. They suggest a requirement of .93% methionine + cystine for broiler chicks 0 to 4 weeks of age when diets containing 3200 kcal ME/kg are fed. Minear and Marion (1981) reported on the nutrient requirements of male and female broilers. Their results for the methionine + cystine requirement were .256% per 1000 kcal/kg for male broilers 0 to 4 weeks of age. Therefore, these findings seem to agree with their estimates especially when the high energy basal was employed (.253% for the high energy basal).

Plasma methionine + cystine results for Experiment 4 are presented in Table 10. Merrick (1981) reported supplemental dietary methionine elevated plasma methionine in young chicks when levels three times the requirement or greater were fed. Also young chicks had increased plasma free cystine concentrations when levels three times the requirement were fed. With elevated dietary methionine being consumed, it is reasonable to expect increases in plasma cystine since methionine can be metabolized to cystine. Results from this work showed no significant response in plasma concentrations of free methionine or cystine to incremental additions of DL-methionine from .593% to 1.19% for young chicks (1 to 2 weeks of age). The results agree with Merrick (1981) in that no significant response was observed when dietary methionine additions were not three times the requirement level.

V. EXPERIMENT 5

Experiment 5 was conducted to determine if the same effects of energy level and methionine supplementation would occur in older birds (4 to 6 weeks of age). Data concerning performance and plasma methionine + cystine for Experiment 5 are presented in Tables 11 and 12, respectively. Results clearly demonstrated a marked growth response in the older chicks when energy was increased from 2750 to 3200 kcal ME/kg. There was also a substantially significant improvement in feed efficiency over all levels of supplemental DL-methionine for the high energy diet, and an improvement up to .45% added DL-methionine for the low energy diet. There was a significant gain response to supplemental DL-methionine for both energy levels. Peak gain for the low energy diet occurred in birds fed .60% supplemental DL-methionine. In birds fed the high energy diet, maximum gain occurred with diets containing .30% supplemental DL-methionine. Based on gain data, the total sulfur amino acid requirement was estimated to be $.96 \pm .085\%$ for the low energy basal diet. This estimate is equivalent to .51% supplemental DL-methionine. Based on gain data for the high energy basal diet, the total sulfur amino acid requirement was estimated to be $.75 \pm .130\%$. This estimate is equivalent to .30% supplemental DL-methionine. The level of sulfur amino acids in both the low and high energy basal diets are higher than those recommended by the N.R.C. They suggest a requirement of .72% methionine + cystine

TABLE 11. Effect of Supplemental Methionine on Performance of 4-to-6 Week Old Broilers Fed a Semi-Purified Basal Diet at Two Energy Levels (Experiment 5)^{a,b}

Added DL-methionine ^c (% of diet)	Dietary energy level (kcal ME/kg)					
	2750			3200 ^d		
	Gain ^{d,e} (g)	Feed Consumed ^f (g)	Gain/feed ^g (g/kg)	Gain ^{d,h} (g)	Feed Consumed ^f (g)	Gain/feed ^g (g/kg)
0	419	1206	347	498	1223	407
.15	501	1258	398	541	1298	417
.30	499	1239	403	629	1282	491
.45	583	1266	460	580	1172	495
.60	595	1340	444	593	1186	500

^aMeans of triplicate groups of five male chicks fed the experimental diets from day 28 to day 42 posthatching. Average initial weight was 829 g.

^bPooled SEM for gain, feed consumed, and gain/feed were 19.8, 35.7, and 15.4, respectively.

^cThe basal diets (Table 5, page 38) contained .449% (low energy) and .449% (high energy) total sulfur amino acids.

^dGain: quadratic effect of methionine significant ($P < .03$). Linear effect of energy significant ($P < .01$). Quartic effect of methionine X linear effect of energy significant ($P < .01$).

^eBased on gain data, the total sulfur amino acid requirement was estimated to be $.96 \pm .085\%$.

^fFeed consumed: cubic effect of methionine significant ($P < .05$). Linear effect of methionine X linear effect of energy significant ($P < .01$).

^gGain/feed: Linear effect of methionine significant ($P < .01$). Linear effect of energy significant ($P < .01$). Quartic effect of methionine X linear effect of energy significant ($P < .03$).

^hBased on gain data, the total sulfur amino acid requirement was estimated to be $.75 \pm .130\%$.

TABLE 12. Plasma Concentrations of Free Methionine and Cystine in 4-to-6 Week Old Chicks Fed a Semi-Purified Basal Diet at Two Energy Levels (Experiment 5)^{a,b}.

Added DL-methionine (% of diet)	Dietary energy level (kcal ME/kg)			
	2750		3200	
	Methionine (nmol/ml)	Cystine (nmol/ml)	Methionine (nmol/ml)	Cystine (nmol/ml)
0	7	252	8	277
.15	70	221	35	207
.30	29	229	81	235
.45	129	189	119	192
.60	119	168	80	273

^aMeans of triplicate groups of five male chicks fed the experimental diets from day 28 to day 42 posthatching.

^bPooled SEM for methionine and cystine were 17.3 and 29.3, respectively.

for broiler chicks 3 to 6 weeks of age, when diets containing 3200 kcal ME/kg are fed.

Minear and Marion (1981) reported that the total sulfur amino acid requirement for male broilers 4 to 6 weeks of age was .251% per 1000 kcal/kg. This is equivalent to a recommendation of .69% for the low energy diet and .80% for the high energy diet.

Plasma methionine + cystine results for Experiment 5 are presented in Table 12. Results from this work exhibited no significant response in plasma concentration of free methionine or cystine to incremental additions of DL-methionine from .449 to 1.05% for broiler chicks 4 to 6 weeks of age. This work was conducted to see if there were any noticeable differences due to age. However, similar to the younger birds, this work agreed with Merrick (1981) in that no significant response was observed when dietary methionine additions were not three times the required level.

VI. EXPERIMENT 6

It is well known that a growth response to a deficient amino acid will not be exhibited unless it is the first-limiting amino acid in the diet. It has been suggested by Pesti et al. (1980) that the level of supplemental methionine needed to achieve maximal performance of broiler chickens may be reduced by feeding excess levels of choline chloride. Other recent work, however, has not agreed with this conclusion (Anderson and Dobson, 1982). Therefore, it appeared that there was considerable confusion concerning the choline-methionine interrelationship in avian nutrition.

The trial was conducted to evaluate the potential sparing effect of choline on the methionine requirement. Performance data are presented in Table 13. Choline was incrementally added from 600 to 1800 mg/kg diet to a semi-purified basal diet containing .803% total sulfur amino acids (methionine + cystine), 22.1% crude protein, and 3200 kcal ME/kg. These dietary levels had been tested in Experiment 4.

The results indicated no significant differences for gain, feed consumption, or gain/feed due to choline supplementation. Baker et al. (1982) reported that at deficient levels of choline, methionine spares choline; and at deficient levels of methionine choline spares methionine. However, when each nutrient is at its physiological requirement (i.e., the requirement in the presence of excess levels of the nutrient doing the sparing) an excess of either nutrient has minimal capacity to reduce the dietary need for the other.

TABLE 13. Effect of Supplemental Choline on Performance of 1-to-2 Week Old Broilers Fed Semi-Purified Diets (Experiment 6)^{a,b}

Choline (mg/kg diet) ^c	Gain (g)	Feed Consumed (g)	Gain/feed (g/kg)
600	294	410	717
800	287	402	714
1000	288	413	697
1200	298	402	741
1400	279	413	675
1600	299	413	724
1800	304	420	724

^aMeans of triplicate groups of several male chicks fed the experimental diets from day 8 to day 19 posthatching. Average initial weight was 158 g.

^bPooled SEM for gain, feed consumed, and gain/feed were 11.6, 9.5, and 21.1, respectively.

^cThe basal diet (Table 4, page 37) contained .803% total sulfur amino acids.

CHAPTER V

SUMMARY

Results indicated that energy and not sulfur amino acids was the first-limiting nutrient in corn-soybean meal diets for broiler chicks. In the low energy diets the response to methionine additions was variable. However, for the high energy diet the response to methionine additions was constant.

Minear and Marion (1981) suggest that the methionine + cystine requirement increases proportionately with the dietary energy level. These suggestions seem to hold true when used with a narrow range of energy levels. However, these recommendations did not seem applicable to this work due to the variable response in the low energy diets. It appears from this data that a requirement of .80% methionine + cystine is adequate for the young broiler chick 0 to 4 weeks of age. This recommendation is considerably lower than the .93% methionine + cystine recommended by the N.R.C. when diets containing 3200 kcal ME/kg are fed. However, for the older chicks 4 to 6 weeks of age our results agreed with the N.R.C. recommendation of .72% methionine + cystine. It is important to note that the following recommendations are based on results from corn and soybean samples that were analyzed by ion exchange chromatography. Therefore, these recommendations would not be applicable for all parts of the country unless similar tests were conducted on feed samples.

Also it should be noted that in a corn-soybean basal diet with adequate methionine (.803%) and adequate energy (3200 kcal ME/kg) the choline requirement is met without additional supplementation. This is not to say, however, that the addition of supplemental choline would not aid in the prevention of leg problems.

LITERATURE CITED

LITERATURE CITED

- Anderson, J. O., and D. C. Dobson, 1982. Comparison of DL-methionine and its sodium salt in water solution in broiler starter diets with two choline levels. *Poultry Sci.* 61:2288-2290.
- Baker, D. H., K. M. Halpin, G. L. Czarnecki, and C. M. Parsons, 1982. The choline-methionine interrelationship for growth of the chick. *Poultry Sci.* 62:133-137.
- Baker, D. H., and K. P. Boebel, 1980. Utilization of the D-and L-isomers of methionine and methionine hydroxy analogue as determined by chick bioassay. *J. Nutr.* 110:959-964.
- Block, R. J., and K. W. Weiss, 1956. *Amino Acid Handbook*. Charles C. Thomas, Springfield, Illinois.
- Boebel, K. P., and D. H. Baker, 1982. Efficacy of the calcium salt and free acid forms of methionine hydroxy analogue for chicks. *Poultry Sci.* 61:1167-1175.
- Christensen, A. C., and J. O. Anderson, 1980. Factors affecting efficacy of methionine hydroxy analogue for chicks fed practical diets. *Poultry Sci.* 59:2485-2491.
- Chung, E., P. Griminger, and H. Fisher, 1973. The lysine and sulfur amino acid requirement at two stages of growth in chicks. *J. Nutr.* 103:117-122.
- Creger, C. R., J. R. Couch, and H. L. Ernst, 1968. Methionine hydroxy analogue and L-methionine in broiler diets. *Poultry Sci.* 47:229-232.
- Damron, B. L., R. H. Harms, A. S. Arafa, and D. M. Janky, 1977. The effect of dietary lasalocid or monensin in the presence of roxarsone and graded methionine levels on broiler performance and processing characteristics. *Poultry Sci.* 56:1487-1491.
- Graber, G., H. M. Scott, and D. H. Baker, 1971. Sulfur amino acid nutrition of the growing chick: effect of age on the dietary methionine requirement. *Poultry Sci.* 50:854-858.
- Halloran, H. R., and R. B. Bishop, 1966. A new approach to evaluating the relative efficacy of DL-methionine and methionine hydroxy analogue calcium in practical nutrition. *Poultry and Livestock Comment* 23(2):1-4. E. I. DuPont DeNemours and Company, Inc., Wilmington, D.E.

- Haney, M., M. Hoehn, and J. McGuire, 1970. Novel Antibiotic A-3823. Complex and process for production thereof. U.S. Patent 3,401,568.
- Harms, R. H., A. R. Eldred, and J. A. Blakeslee, 1976a. The relative efficiency of DL-methionine and methionine hydroxy analogue-calcium (MHAC) in the diet of turkey poults. *Poultry Sci.* 55:1594-1595.
- Harms, R. H., A. R. Eldred, and B. L. Damron, 1976b. The relative efficiency of DL-methionine and methionine hydroxy analogue-calcium (MHAC) in broiler diets. *Poultry Sci.* 55:1794-1797.
- Harms, R. H., A. R. Eldred, and J. A. Blakeslee, 1977. The relative efficiency of DL-methionine and methionine hydroxy analogue-calcium (MHAC) in the diet of turkey poults. *Poultry Sci.* 56:186-188.
- Katz, R. S., and D. H. Baker, 1975. Efficacy of D-, L- and DL-methionine for growth of chicks fed crystalline amino acid diets. *Poultry Sci.* 54:1667-1674.
- Ketola, H. G., and M. C. Nesheim, 1974. Influence of dietary protein and methionine levels on the requirement for choline by chickens. *J. Nutr.* 104:1484-1489.
- Kiker, J. T., and D. H. Sherwood, 1974. "Barebacks" in broiler type chickens. *Poultry Sci.* 53:1639.
- Kornegay, E. T., J. D. Hedges, K. E. Webb, Jr., H. R. Thomas, D. H. Baker, G. R. Carlisle, B. G. Harmon, and A. H. Jensen, 1975. Protein and amino acid evaluation of commercially grown opaque-2 corn. *J. Anim. Sci.* 41:1546-1554.
- Kuenzel, W. J., J. H. Walther, and M. M. Schaefer, 1979. Body temperature and its effect on growing broilers. Pages 111-115 in *Proc. Maryland Nutr. Conf., Washington, D. C.*
- Lerner, J., and M. W. Taylor, 1967. A common step in the intestinal absorption mechanisms of D- and L-methionine. *Biochem. Biophys. Acta* 135:990-999.
- Merrick, J. M., 1981. Effects of excess dietary methionine consumption on performance, hematology and plasma free amino acid concentrations in young poultry and swine. M.S. thesis. University of Tennessee, Knoxville, TN.
- Minear, L. R., and J. E. Marion, 1981. Nutrient requirements of male and female broilers. *Zoo Tecnica Int.* Oct. 1981. Pp. 16-19.

- Mitrovic, M., and E. G. Schildknecht, 1974. Anticoccidial activity of lasalocid (X-537 A) in chicks. *Poultry Sci.* 53:1448-1455.
- Mitrovic, M., E. G. Schildknecht, and W. L. Marusich, 1975. Comparative anticoccidial activity and compatability of lasalocid in broiler chickens. *Poultry Sci.* 54:757-761.
- Molitoris, B. A., and D. H. Baker, 1976. Choline utilization in the chick as influenced by levels of dietary protein and methionine. *J. Nutr.* 106:412-418.
- National Research Council, 1977. Nutrient requirements of domestic animals. No. 1. Nutrient requirements of poultry. Nat. Acad. Sci., Washington, D. C.
- Nesheim, R. O., and B. C. Johnson, 1950. Effect of a high level of methionine on the dietary choline requirement of the baby pig. *J. Nutr.* 41:149-152.
- Nesheim, M. C., M. J. Norvell, E. Ceballos, and R. M. Leach Jr., 1971. The effect of choline supplementation of diets for growing pullets and laying hens. *Poultry Sci.* 25:373-375.
- Netke, S. P., and H. M. Scott, 1974. The sulfur amino acid activity in soybean meal and corn measured by chick growth. *Poultry Sci.* 53:592-596.
- Patel, M. B., K. O. Bishawi, and J. McGinnis, 1979. Effect of drug additives and type of diet on methionine requirement of chicks for growth and feed efficiency. *Poultry Sci.* 58:549-554.
- Patel, M. B., K. O. Bishawi, C. W. Nam, and J. McGinnis, 1980. Effect of drug additives and type of diet on methionine requirement for growth, feed efficiency, and feathering of broilers reared in floor pens. *Poultry Sci.* 59:2111-2120.
- Pesti, G. M., A. E. Harper, and M. L. Dunde, 1980. Choline/methionine nutrition of starting broiler chicks. Three models for estimating the choline requirement with economic considerations. *Poultry Sci.* 59:1073-1081.
- Pressman, B. C., 1968. Ionophorous antibiotics as models for biological transport. *Fed. Proc.* 27:1283-1288.
- Pressman, B. C., 1973. Properties of ionophores with broad range cation selectivity. *Fed. Proc.* 32:1698-1703.
- Robbins, K. R., 1981. Effects of sex, breed, dietary energy level, energy source, and calorie:protein ratio on performance and energy utilization by broiler chicks. *Poultry Sci.* 60:2306-2315.

- Robbins, K. R., H. W. Norton, and D. H. Baker, 1979. Estimation of nutrient requirements from growth data. *J. Nutr.* 109:1710-1714.
- Romoser, G. L., P. L. Wright, and R. B. Grainger, 1976. An evaluation of the L-methionine activity of the hydroxy analogue of methionine. *Poultry Sci.* 55:1099-1103.
- Rubin, M., W. J. Kenworthy, and E. Bossard, 1979. Protein and methionine content of soybeans as influenced by cultivar. *Poultry Sci.* 58:1101-1102.
- Sasse, C. E., and D. H. Baker, 1972. Availability of sulfur amino acids in corn proteins for growing chicks. *Poultry Sci.* 51:1858.
- Sasse, C. E., and D. H. Baker, 1974. Sulfur utilization by the chick with emphasis on the effect of inorganic sulfate on the cystine-methionine interrelationship. *J. Nutr.* 104:244-251.
- Sauberlich, H. E., 1961. Studies on the toxicity and antagonism of amino acids for weanling rats. *J. Nutr.* 25:61-72.
- Scott, H. M., M. Kelly, and R. L. Huston, 1966. L-methionine versus methionine hydroxy analogue in basal diets containing either isolated soybean protein or crystalline amino acids. *Poultry Sci.* 45:1123.
- Taylor, W. M., H. G. Eisenbeis, L. J. Hanson, W. L. Marusich, and E. G. Schildknecht, 1974. The safety and efficacy of the anticoccidial drug lasalocid, in chickens under floor pen conditions. *Poultry Sci.* 53:1983.
- Tipton, H. C., B. C. Dilworth, and E. J. Day, 1966. A comparison of D-, L-, DL-methionine and methionine hydroxy analogue calcium in chick diets. *Poultry Sci.* 45:381-386.
- Tsiagbe, V. K., C. W. Kang, and M. L. Sunde, 1982. The effect of choline supplementation in growing pullet and laying hen diets. *Poultry Sci.* 61:2060-2064.
- van Weerden, E. J., H. L. Bertram, and J. B. Schutte, 1982. Comparison of DL-methionine, DL-methionine-Na, DL-methionine hydroxy analogue-Ca, and DL-methionine hydroxy analogue free acid in broilers by using a crystalline amino acid diet. *Poultry Sci.* 61:1125-1130.
- Waldroup, P. W., C. J. Mabray, J. R. Blackman, P. J. Slagter, R. J. Short, and Z. B. Johnson, 1981. Effectiveness of the free acid of methionine hydroxy analogue as a methionine supplement in broiler diets. *Poultry Sci.* 60:438-443.

Wheeler, K. B., and J. D. Latshaw, 1981. Sulfur amino acid requirements and interactions in broilers during two growth periods. Poultry Sci. 60:228-236.

Willis, G. M., and D. H. Baker, 1980. Lasalocid-sulfur amino acid interrelationship in the chick. Poultry Sci. 59:2538-2543.

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