BIOAVAILABILITY OF LYSINE IN GRAIN SORGHUMS FOR GROWING CHICKENS

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BIOAVAILABILITY OF LYSINE IN GRAIN

SORGHUMS FOR GROWING CHICKENS

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CHAPTER I

INTRODUCTION

The use of grain sorghums in animal feeding, especially for swine and poultry, has increased during the past few years. Corn and grain sorghum are two of the principal grains used in the formulation of poultry rations. The ready adaptability of grain sorghums to varied growing condition, especially semi-arid climate conditions, has stimulated the usage of this grain for animal feeding in many parts of the world.

The major problem in using grain sorghums in place of corn in animal feeding is determining, from a nutritional standpoint, how much grain sorghum can be used in the ration. The widespread production and utilization of grain sorghums and the use of computer formulation has made it necessary to obtain more data relative to nutrient availability from digestive and metabolic standpoints. It is recognized that the nutrient content of a feedstuff as determined by chemical analysis does not necessarily establish the amount of each nutrient which can actually be utilized when that feedstuff is fed. Therefore, biological tests must be conducted to determine the availability of those nutrients which are present. For this reason, experimental studies in recent years have been conducted for the purpose of determining nutrient availability in grain sorghums when they are used in rations for poultry, swine, and beef cattle.

CHAPTER II

REVIEW OF LITERATURE

Nutritional Comparison Between Yellow Corn and Grain Sorghums

General

The favorable price of sorghum grains in comparison with other cereal grains has made them the prime energy source and principal competitor of corn in feeding poultry and swine. Sorghum grains have in many cases largely replaced corn in poultry and swine feeding. Therefore, many studies have been conducted to compare the feeding value of grain sorghums to that of yellow corn.

Generally corn has a higher feeding value than grain sorghums when the two are compared on a pound for pound basis. In an 84-day feeding trial with laying hens, a diet composed of 100 percent corn resulted in significantly higher egg production than did a diet composed of 100 percent grain sorghum (Peischel *et al*., 1976). The same result was obtained by Petersen (1969) when a comparison was made between corn and sorghum grain in which corn or sorghum grain was fed at a level of 50 percent in test diets.

Ackerson *et al*. (1978) reported a significantly higher starch content in corn grain than in four varieties of grain sorghums. He concluded that the lower feeding value of sorghum grain as compared with corn might be related to a lower starch content, and the chemical or structural composition of the protein matrix which surrounds the starch granules.

Studies have been involved in feeding isonitrogenous rations which contained corn or grain sorghum to chicks. Sanford et al. (1968) found that chicks fed yellow corn grew significantly faster than did those fed sorghum grain when the protein levels in the rations were equal. This result was supported in a study by Deaton et al. (1967) in a 336-day feeding trial starting with day-old egg strain chickens. These chickens were fed 14 percent protein diets which contained either corn or sorghum grain. However, other workers, Thayer et al. (1957) and Hammond (1942), found that yellow corn and improved varieties of grain sorghum are the same from the standpoint of feed efficiency and growth promoting value for growing chickens and laying hens.

In another study conducted by Syed Abu Bakar et al. (1975), sorghum grain replaced part or all of the yellow corn in a series of broiler diets in two trials, each lasting 8 weeks. The control ration contained 60 percent of corn and the grain sorghum rations were formulated to contain 15, 30, 45, and 60 percent of grain sorghum replacing corn on a pound per pound basis. There was no significant difference in feed intake or feed efficiency between the treatment and control groups. A xanthophyll deficiency was noted in the broilers fed the high levels of grain sorghum. Significant differences were observed in the amount of yellow pigment in the shanks and skin of the broilers fed yellow corn as compared to those fed yellow endosperm and the regular sorghum grain, respectively (Sanford, 1972). This was due to the lower xanthophyll and vitamin A content in the grain sorghum. The xanthophyll level of grain sorghum is 0.5 mg percent as compared to 10 mg percent for corn (Wall and Rose, 1970).

Energy

The gross energy level in corn and sorghum grain is approximately the same (1,780 versus 1,775 kilocalories per pound). However, the digestible energy in corn is higher than in grain sorghum (1,640 versus, 1,570 kilocalories per pound). This indicates a higher digestibility for energy in corn (92.3 percent) than in grain sorghum (88.4 percent). Wall and Rose (1970) concluded that digestible energy is translated into a higher metabolizable energy value in corn (1,543 kilocalories per pound) than in sorghum grain (1,468 kilocalories per pound).

Endosperm texture and starch type are two of several factors that might have an effect on the energy value of all grains (Sandstedt et al., 1962). The mature seed of sorghum grain consists of the embryo and the endosperm which are both surrounded by a thin layer called pericarp. Endosperm, which constitutes the major fraction of the seed (80 to 84 percent of the whole seed dry matter, Hubbard et al., 1950), consists of cells filled with starch. The starch content of endosperm comprises 83 percent of the endosperm. On the average, the starch granules of grain sorghum are 15 micrometers in diameter (Schoch and Maywald, 1956).

Different types of starches are found in grain sorghums. One type of starch, amylose, is a polymer of glucose units which are bonded by α-1-4 linkages to form a linear structure. Amylose dissolves with difficulty in water. Another type of starch that is more soluble in water or butanol solution is amylopectin. Amylopectin has about 5 percent of α-1-6 bonds in addition to α-1-4 linkages. This gives a branched type structure to amylopectin. Endosperm texture is classified as flour, intermediate, and corneous. These classes are determined by the ratio of floury to corneous endosperm in longitudial sections of the grain kernels (Manson et al., 1971). Starch type is classified as either normal type which has 75 percent amylopectin (branched starch structure), and 25 percent amylose (a linear starch structure) or waxy type which is composed of 100 percent amylopectin. Starch type is determined by an iodine stain test which gives a blue color for amylose and a red color for amylopectin (Whistler and Paschall, 1967).

Cohen and Tanksley (1973) in growth studies with pigs reported higher coefficients of digestibility for dry matter, organic matter, gross energy, and digestible and metabolizable energy in grain sorghums which contained intermediate endosperms and normal starch as compared to other types of endosperm and starch types. Digestible and metabolizable energy values for either normal or waxy starch type grains were essentially the same. However, slight, but insignificant differences were obtained in weight and feed efficiency with grain which contained waxy starch. This is partly due to the greater enzyme susceptibility of waxy starch, and alterations in the structure of the endosperm of the waxy sorghum kernel (Sullins and Roaney, 1974). They found that the endosperm of waxy sorghums had a more uniform distribution of the protien and starch especially in the peripheral area. The greater feeding value of waxy type starch was supported by the work of Lichtensvalner et al. (1978). These plant breeders introduced incremental dosages of waxy genes into sorghum grain, and by so doing increased the in vitro and in vivo digestibility of the grain sorghums.

Dry matter digestibility and its effect on the energy utilization of several grain sorghums were studied in a feeding trial with chicks (Nelson et al., 1975). A significantly high correlation was observed between dry matter digestion, and (1) kilocalories of metabolizable energy per gram (r=0.98), and (2) percentage of gross energy utilized (r=0.99).

The influence of pericarp and endosperm color on digestibility of grain sorghums was studied in the pig (Noland et al., 1977). They found cultivars of sorghum with a yellow seed color were significantly more digestible than were those with brown seed color. They also indicated that sorghum grain with a yellow endosperm had a greater digestibility of energy and protein, and brought about a greater nitrogen retention than did cultivars with white endosperms. However, Thayer et al. (1957), Damron (1968), and Nelson et al. (1975) in studies conducted with chicks showed that neither seed color nor endosperm color influenced the nutritional value of the sorghum grain.

Grain Sorghum Protein

A wide genetic variability was observed in the protein content of grain sorghum varieties (Deosthale et al., 1970). An analysis of 332 varieties of grain sorghums showed that the protein content ranged from 4.7 to 17 percent. The frequent distribution of protein in these varieties indicated that 62 percent of the total samples ranged within a narrow range of 11.0 to 13 percent with an average of 11.73 percent.

All four classes of protein, (1) albumins, soluble in water, (2) globulins, soluble in solutions of salts, (3) prolamines, soluble in solutions of ethyl alchol, and (4) glutelins, soluble in dilute alkali, are found in grain sorghums from varieties with different endosperm and pericarp structure (Neucere and Sumrell, 1979). The percentages of albumin, globulin, prolamine, and glutelin in the protein of grain sorghums ranged from 2 to 8 percent, 2 to 10 percent, 30 to 60 percent, and 20 to 37 percent, respectively (Viropaksha and Sastry, 1968). The amino acid composition in each of these four protein fractions varied. There were some major trends in the amino acid profiles of the fractions which were attributed to differences in the protein content of the whole grain. The proline content of the albumin fraction increased as the protein content of the whole grain increased. Whereas, the lucine content of the albumin fraction was reduced as the protein content of the whole grain increased.

Like zein in corn, prolamine and glutelin are low in nutritional value since they are deficient in the amino acid lysine and some other essential amino acids (Jones and Beckwith, 1970). Whereas the concentration of lysine in albumin and globulin is much higher than in prolamine. Concentrations of lysine, arginine, glycine, cystine and methionine in the globulin fraction are nearly twice that of the endosperm protein (Virupaksaha and Sastry, 1968; Skoch et al., 1970). Methionine, cysteine, isoleucine, and leucine are the amino acids which are most deficient in the albumin and globulin fractions.

The ultrastructure of the endosperm protein of seven hybrids of grain sorghum as determined by an electron microscope showed a well-developed, two-component structure consisting of a protein body enbedded in a matrix protein (Seckinger and Wolf, 1973). The protein body of almost all of the grain sorghums was 2 to 3 micrometers in diameter. Grain sorghums with a high lysine content had smaller protein bodies which verified the negative correlation (r=-.34) between protein content and lysine concentration. Attempts to increase the protein content in grain sorghum varieties which was associated mainly with an increase in prolamine (the protein mostly deficient in lysine) also verifies a negative correlation between protein and lysine concentration in grain sorghums (Virupaksha and Sastry, 1968, Deosthale et al., 1970; and Barnett, 1976).

Total soluble protein which was extracted from sorghum grains by classic Osborne-Mendel solvent systems and techniques (distilled water to extract albumin; 5% sodium chloride solution for globulins; 80% ethanol plus 0.2% sodium acetate solution for prolamines; and 0.2% sodium hydroxide solution for glutelins) ranged between 26.4-40 percent as compared with (opaque-2) corn 81.1 percent. The insolubility of the protein in grain sorghums may be due to a protein-starch matrix configuration (Skoch et al., 1970).

Limitations in the Use of Grain Sorghum in Poultry Rations

The use of grain sorghums in poultry rations has increased during the past few years. The consumption of the whole grain in the field by wild birds is a major problem and has brought about the development of new bird resistance varieties. The bird resistancy of these new varieties is attributed to the grain's tannin content which in fact has caused concern regarding the efficiency with which the grain sorghums can be used in poultry rations (Damron et al., 1968; McMillian et al., 1972). A major part of the tannin content of grain sorghums is found in the pericarp of the grain (Armstrong et al., 1973). Three experiments were conducted to determine the effect of pericarp removal from two bird resistant sorghum grains on chick performance. The removal procedure involved an alkaline treatment of the whole grain followed by hot water washing. Significantly better chick growth and feed conversion were obtained with the pericarp free grain as compared with that obtained with intact bird resistant grain. Bryan (1973) obtained the same result by feeding peeled sorghum grains to chicks.

Growth depression in chicks, pigs, and rats was observed by several workers when bird resistant grain sorghum were fed (Glick and Joslyn, 1969; Rostagno et al., 1973; Shang and Fuller, 1964; Armstrong et al., 1973; Petersen, 1969; and Featherston et al., 1975). A highly significant negative correlation (r=-.93) was shown between rate of growth and tannin content in grain sorghums in a growth study with chickens (Peischel et al., 1976).

The adverse effect on growth in chicks fed grain sorghums which contained high tannin levels (bird resistant) involved a number of factors (Glick and Joslyn, 1969; and Armstrong et al., 1973).

Unpalatability

Growth retardation of chicks is attributed to the appetite-depression effect of the tannin in the ration (Alumot et al., 1964). Depression in appetite is due to the effect of tannin on the palatability of rations for livestock (Chang et al., 1964; Petersen, 1969; and Connor et al., 1969). However, Damron et al. (1968) concluded that the substitution of the bird resistant varieties of grain sorghums for one-half of the yellow corn in diets of chicks did not depress either feed intake or body weight gain.

Toxicity

Toxicity of the tannins found in plants when they are fed to chicks, rats, and other animals have been reported (Vohra *et al*., 1966; and Chang and Fuller, 1964). Out of a number of plant tannins which have been studied, tannic acid is most deleterious (Vohra *et al*., 1966). The addition of 0.5 percent of dietary tannic acid caused a depression in the growth of chicks. A level of 5 percent tannic acid in the ration caused 70 percent mortality between 7 and 11 days in this experiment.

Pyrocatechol and pyrogallol, the metabolites of tannic acid, are more toxic than tannic acid. Whereas galic acid, another metabolite of tannic acid in the body, is less toxic than tannic acid (Rayudu *et al*., 1970).

The metabolites of the tannins in grain sorghums as they are found in the body of the chicken were studied through the use of both paper and thin-layer chromatography (Potter and Fuller, 1968; and Connor *et al*., 1969). They found galic acid, 4-0 methyl galic acid, and pyrogallol in the urine of hens fed tannin. In fact, tannic acid hydrolized to galic acid and a large part of this substance is 0-methylated to produce 4-0 methyl galic acid. Decarboxylation of galic acid accounts for the pyrogallol. The exact mode of formation of pyrocatechol is not clear (Kadirvel *et al*., 1969).

The source of methyl groups for the 0-4 methylation of galic acid are choline and methionine (Kadirvel *et al*., 1969; and Booth *et al*., 1959). Therefore tannin in grain sorghums induced a deficiency of choline and methionine. Tannin toxicity can be completely alleviated by providing high levels of these two nutrients in the diet (Chang and Fuller, 1964). Detoxification of tannic acid in chicks has also been accomplished by the addition of dietary arginine or arnithine (Fuller et al., 1967).

Nitrogen Retention

Very little research data are available on nitrogen retention. Nitrogen retention was found to be reduced by the feeding of grain sorghums with a high tannin content (Vohra et al., 1966; and Nelson et al., 1975).

Leg Abnormality

A leg abnormality which is characterized by an outward bowing of the legs with a swelling at the hock joints, has been reported in chicks fed grain sorghums. This is especially true when the grain sorghums have a high tannin content (Armstrong et al., 1973). The severity of the leg abnormality increased when the amino acids provided by other feedstuffs in the ration were replaced with crystalline forms of these amino acids (Elkin et al., 1978). It was concluded that a change in the gut pH due to the unbuffered addition of the crystalline amino acids probably resutled in an increased activity of the tannin.

The supplementation of the ration with lysine, carnitine or a mineral mix which contained calcium, manganese, and zinc failed to overcome the leg abnormality. This indicates that tannin does not affect bone mineralization since similar bone ash values for chicks fed high or low tannin grain sorghums have been reported (Armstrong et al., 1973).

Biological Value of Grain Sorghum for Chicks

The nutritive value of a protein is determined by comparing its amino acid composition with that of a reference protein such as whole egg protein (Oser, 1951). In a study concerning the nutritional value of low and high protein sorghums, the essential amino acid content of the proteins of the two sorghums were compared with the essential amino acid content of whole egg protein, and with the amino acid requirements of the growing chicks (Vacich et al., 1958). Evaluation by the Essential Amino Acid Index (EAAI) of Oser (1951) indicated a low biological value for the protein from both grain sorghums (low and high protein). The high protein sorghum was shown to have a lower EAAI than the low protein sorghum (62.8 vs. 67.9).

The biological value of the protein of different varieties of grain sorghums which varied in protein content have also been studied using the growth of chicks as the criterion (Waggle et al., 1966; and Vavich, 1959). Sorghum grains with a high protein content have higher percentages of each amino acid than do those with a low protein content. However, at a constant level of protein in the ration, the percentage of the most limiting amino acids, especially lysine, was higher in the low protein than that in high protein sorghum grains (Waggle et al., 1966).

The higher biological value of grain sorghums with a low protein content as compared with grain sorghums with a high protein content at equal levels of protein has been demonstrated by Waggle et al. (1966) in the rat, and by Vavich et al. (1959) in the chick. There was a significant improvement in weight gain and feed efficiency when the grain sorghum with a low protein content was fed. However, Waggle et al. (1966) found the reverse to be true in the chick.

The Effect of Tannin on Biological Value of Grain Sorghum

The efficiency with which grain sorghums are utilized is related, in part, to both peripheral and internal structure of the seed (Sullins and Rooney, 1974) and to the presence of polyphenolic compounds (tannin) (Axtell, 1976). The combination of the tannins presented in sorghum grains with protein reduce the availability of the amino acids in the protein. Polyphenols (tannins) associated with the pericarp of high tannin grain sorghums causes the inhibition of α-amylase and reduces dry matter digestion in the rat (Maxson et al., 1973).

Rinehart et al. (1974) reported a 10 percent lower feeding value for bird resistant grain sorghums as compared to regular sorghum hybrids. The utilization of energy, and dry matter digestion have been shown to be increased as the tannin content in grain sorghums decreased. This resulted in a significant negative correlation (r=-0.63). Increases in dry matter digestion resulted in an increase in amino acid availability (r=0.54) as shown by Nelson et al. (1975).

The Effects of Processing and Detoxification on Biological Value of Grain Sorghum for Chick

Some advantages of the improved new varieties of grain sorghum are: (1) less damage by bird depredation (McMillian et al., 1972); (2) prevention of pre-harvest germination (Harris and Burns, 1970), and (3) less weathering (Harris and Burns, 1973). However, these improved new varieties have a high tannin content and as a result their feeding qualities are reduced (Armstrong et al., 1974; and Featherston and Rogler, 1975). If an economically feasible treatment of harvested grain could reduce the tannin content without loss or damage to grain nutrients, the grain sorghums would be a much more satisfactory crop in many areas (Price et al., 1978). Extraction of tannins from a high-tannin sorghum results in a marked improvement in weight gain, feed efficiency, protein, and energy digestibility in rats and chicks as compared with rats or chicks fed intact unextracted gtain (Featherston and Rogler, 1975; Price et al., 1978; and Armstrong et al., 1974).

The utilization of nitrogen by rats fed the unextracted sorghum grain was approximately 30 percent less than that in rats fed sorghum grain with a low tannin content. The decreased nitrogen absorption which was observed does not appear to be due to the protein per se since the same amount of protein was digested in both grains. It was more likely a difference in the protein pattern as measured by protein fractionation. Extraction of tannin from high tannin grain sorghums results in protein patterns which more closely resembled those observed in low-tannin sorghums (Featherston and Rogler, 1975; and Price et al., 1979).

Among several simple treatments to diminish the tannin content of bird-resistant grain sorghums and to increase their feeding value, only NH_4OH treatment strikingly improves the nutritional quality of the high tannin sorghum, so that for rats and chicks it gives weight gain and feed efficiency equivalent to low tannin sorghum. Other treatments including gaseous NH^3 under pressure and autoclaving, also improve the nutritional value of high tannin content grain, but to a lesser degree

(Price *et al.*, 1978). The metabolizable energy content of grain sorghum for chicks was increased from 2.68 to 3.21 Kcal/gram, and from 3 to 3.45 Kcal/gram by steam processing and pressure cooking, respectively (Weber *et al.*, 1969).

The Effect of Amino Acid Composition and Availability on Biological Value of Grain Sorghum

Factors other than tannin are involved in observed reductions in the biological value of grain sorghums when they are compared to other varieties of sorghums or to corn (Chang and Fuller, 1964). The performance of chicks fed a high dietary level of tannin in the form of grain sorghums was lower than that of chicks fed a corn ration with the addition of tannic acid at the same level as found in high tannin grain sorghums. This result suggests that the performance was not due solely to the presence of tannic acid (Rostagno *et al.*, 1973).

The protein content of grain sorghums varied between different varieties as well as different hybrids, and this brings about a difference in the protein fractions (albumin, globulin, prolamin and glutelin) as found in the seed protein (Virupaksha and Sastry, 1968; Deosthale *et al.*, 1970; and Skoch *et al.*, 1970). The variation in amino acid composition in the four protein fractions results in different feeding values among the grain sorghums (Virupaksha and Sastry, 1968).

The albumin and globulin proteins of grain sorghums have the best nutritional value since they contain sufficient amounts of lysine, arginine, threonine, and methionine which are the most deficient amino acids in grain sorghums. Prolamin is the poorest and glutelin is intermediate in nutritional value (Wall and Rose, 1970).

Prolamin is the principal protein of the grain sorghum. Since a negative correlation exist between the percent lysine in the protein and the percent protein in the seed, any attempts to breed sorghum of high protein content have been unsuccessful in increasing nutritive value. In fact, increases in the protein level in sorghum varieties is correlated with an increase mainly in prolamin which is most deficient in lysine.

There is little agreement among research workers regarding the limiting amino acids in grain sorghums, but most workers identified lysine, and arginine as the most limiting amino acids (Vachich et al., 1959; Deosthale et al., 1970; Shoup et al., 1969; and Waggle et al., 1966). The lysine content of 332 varieties of grain sorghums ranged from 0.90 to 2.67 gram per 100 grams of protein. The frequency distribution of the lysine in these 332 varieties showed that 68 percent of the samples contained 1.49 to 2.44 grams of lysine per 100 grams protein. Deosthale et al. (1970) and Virupaksha and Sastry (1968) reported 0.117% to 0.413% (mean=0.219%) of lysine in samples of a number of varieties of grain sorghums.

There is a significant negative correlation between protein and lysine in the protein. Whereas, a positive significant correlation has been reported between protein and leucine (Deosthale et al., 1970).

The supplementation of rations which contain grain sorghums with lysine results in better feeding value for chicks (Shoup et al., 1969; and Rostagno et al., 1972). The nutritional value of a high lysine variety of sorghum grain resulted in approximately three times greater weight gain and 50 percent less feed consumption in chicks as compared to a control group fed a regular grain sorghum (Featherston et al., 1975).

The nutritional value of the different parts of the grain sorghum seed varies considerably, since there is a variation in the amounts of four protein fractions (albumin, globulin, prolamin, and glutelin) in each part of the seed. This results in different amino acids patterns. The nutritional value of the protein in a bran-germ combination is superior to both the whole grain and the various endosperms (Shoup et al., 1969). This is due to the high percentages of albumin and globulin in the bran and germ.

Effect of Nitrogen Fertilization, Location, Hybridization, and Irrigation on Protein Content and Amino Acid Composition

The protein content of grain sorghums is affected by such factors as nitrogen fertilization, location, hybridization, and irrigation (Burleson et al., 1956). Protein analysis of four hybrid samples in a study by Miller et al., 1964 indicated a somewhat lower protein content in hybrids than in old standard varieties. The yield, however, was significantly higher in hybrid grain sorghums than in standard varieties.

The effect of nitrogen fertilization on yield, protein content, and amino acid composition has been studied by many workers (Burleson et al., 1956; Miller et al., 1964; Shoup et al., 1968; and Waggle et al., 1967). They reported significant increases in both yield and protein content of the grain sorghums when nitrogen fertilizers were used. The level of 17 amino acids increased significantly in the grain as the nitrogen fertilization was increased. However, the rate of increase was not the same among these 17 amino acids. It was indicated that when the concentration of amino acids in the protein was the basis of consideration, lysine, histidine, arginine, threonine, and glycine were reduced as the level of nitrogen fertilization was increased.

A comparison between the protein content of grain sorghums in irrigated plots and those in non-irrigated indicated a higher protein content in the grains in the non-irrigated plots (Shoup et al., 1968; and Miller et al., 1964). Significant differences in protein content ($P<0.01$) were reported among grain sorghums grown at different locations, whereas no significant differences have been shown in amino acid distribution due to location (Deyoe and Shellenberger, 1965).

Development of Lysine Availability Techniques

A number of biological methods for measuring the availability of amino acids in feedstuffs have been used. Procedures have been suggested for obtaining by chemical means values more likely to approximate the level of lysine nutritionally available, but the usefulness of such values must be checked empirically against the direct results of biological tests (Carpenter et al., 1963).

Carpenter (1960) described a chemical procedure in which fluorodinitrobenzen (FDNB) was used for lysine availability. Although this method has been found to be satisfactory for use with animal protein, it is not useful for plant protein materials which contain large maounts of carbohydrates. Also, Carpenter et al. (1963) found higher values for available lysine in chick bioassays than were found by means of a chemical method when proteins of high quality were used.

Certain of the biological methods employed diets based on a single or a mixture of proteins that were deficient, but not devoid, of the amino acid under study (Deshpande et al., 1957; Gupta et al., 1958; Calhoun et al., 1960; and Carpenter et al., 1963). In these procedures the basal diet was assumed to supply all the nutrients and amino acids, except the amino acid of concern, in sufficient amounts to support normal growth in the experimental animals. The main problem in these procedures is the unknown quantity of the amino acid of concern in the basal diet. The two other criticisms of these procedures are:

1. Unless the growth period is very short and the graded levels have a short range, there can be significant differences in the amount of total diet consumed among the experimental groups due to the palatability of the test diet. Consequently a greater "apparent" level of the amino acid under study appears to be present. So, differences in feed intake may distort the assay values (Hill et al., 1965; Carpenter et al., 1963).

2. Most workers appear to have assumed that the test material and the standard behave in an additive manner with the quantity of amino acid which is contributed by the basal diet (Hill et al., 1965).

In recent procedures, workers attempt to use a basal diet in which protein is composed solely of purified amino acids (Smith and Scott, 1965). This eliminates the problem of an unknown amount of the amino acid under study in the basal diet.

Objectives of the Research Herein Reported

Two feeding trials were conducted in this research. Objectives of the first feeding trial are:

1. To measure the bioavailability of lysine in three different varieties of grain sorghums,

2. To study the effect of the milo amino acid pattern on the bioavailability of dietary lysine for chicks, and

3. To determine the effect of the amino acid pattern as required by chicks on the bioavailability of dietary lysine for chicks.

The objective of the second feeding trial is to compare the bioavailability of the energy in yellow corn with that of two different varieties of grain sorghum.

CHAPTER III

EXPERIMENTAL PROCEDURE

Feeding Trial I

General

A total of 400 male meat-strain day-old chicks were purchased from a commercial hatchery. They were wing banded for individual identification and were placed in electrically heated battery brooders with raised wire floors. Fifty chicks were allocated to each battery section for a pretest period which lasted 15 days. They were fed a broiler starter ration during the pretest period. This ration is presented in Table I.

At nine days of age, the symptoms of Chronic Respiratory Disease (CRD) were observed among the chicks and this brought about a delay in initiating Feeding Trial I. The chicks were treated with soluble Terramycin for five days.

When the chicks were 15 days old, a four-day test period was initiated. All chicks were weighed and 180 of them selected and divided into weight classes. Chicks from these weight classes were randomly distributed into 45 battery sections (4 chicks per section). Details of this procedure are given in the section entitled "Experimental Design and Treatments." The 15 treatments which were used will also be described later.

TABLE I

BROILER RATION FED DURING PRETEST PERIOD

Ingredients	Percent
Tallow, feed grade	4.8
Yellow corn, ground	34.7
Milo, ground yellow	8.9
Oats, pulverized (8.9%)	5.9
Soybean meal (44%)	23.4
Alfalfa meal (17%)	5.2
Blood meal (30%)	2.6
Whey, dried (12%)	2.6
Meat and bone scarp (50%)	10.9
dl Methionine	0.2
Trace Mineral[1]	0.05
Salt	0.5
Vitamin mix (breeder hen)[2]	0.25

[1]Provides in the ration: manganese 120 ppm; zinc 80 ppm; iron 60 ppm; copper 10 ppm and iodine 1.0 ppm.

[2]Contains per pound of vitamin mix: vitamin A 1600,000 iu; vitamin D 400,000 iu; vitamin E 1,600 iu; menadione sodium bisulfite complex 800 mg; riboflavin 1400 mg; niacin 7,000 mg; d-pantothenic acid 2,000; choline 110,000; thiamine 200 mg; vitamin B_{12} 2.2 mg; d-biotin 20 mg; and folic acid 140 mg.

Standard management practices were followed in caring for the chicks during the entire experimental feeding period. These practices included brooding (starting with 35^{o}C, then adjusting downward as the heat requirements of the chicks decreased), cleaning the water fountains, adding fresh water every day, and changing the papers under each battery section for better sanitation. Feed and water were given ad libitum. The feed in the feeders was cleared of dirt and foreign materials, and was renewed every day along with the water throughout the course of the experiment. This feeding trial (Trial I) was completed during the spring of 1980 (January 8 to January 27).

Experimental Design and Treatments

The four-day test period started when the chicks were 15 days old. For the test period, all 400 chicks were weighed and were distributed into 30 different weight classes according to their weight. A five gram weight internal was considered to be a class ranging from the heaviest to the lightest chick. The chicks were distributed into the 45 battery sections one chick at a time in rotation until each battery section contained four chicks. This procedure was designed to provide chicks of near uniform weight within each battery section. The 15 treatments which were used were randomly distributed among the 45 battery sections with three replications per treatment. This constituted a completely randomized design. The 15 treatments used in Trial I are listed in Table II.

The first five treatments with 0.00, 0.2, 0.3, 0.4, and 0.5 percent crystalline L-lysine HCl, respectively, were involved in this feeding Trial to obtain a Standard Growth Curve to graded levels of L-lysine HCl.

TABLE II

TREATMENTS IN FEEDING TRIAL I

Treatment No.	Treatment Description
1	Purified ration[1] plus 0.00% crystalline lysine HCl[2]
2	Purified ration plus 0.2% crystalline lysine HCl
3	Purified ration plus 0.3% crystalline lysine HCl
4	Purified ration plus 0.4% crystalline lysine HCl
5	Purified ration plus 0.5% crystalline lysine HCl
6	As 2 plus 2.06% Simulated Milo Amino Acid Mix[3]
7	As 2 plus 4.13% Simulated Milo Amino Acid Mix[4]
8	As 2 plus 6.19% Simulated Milo Amino Acid Mix[5]
9	As 2 plus 6.19% Chicken Amino Acid Requirement Mix[6]
10	As 2 plus level one[7] of Darset
11	As 2 plus level two[8] of Darset
12	As 2 plus level one[7] of Dwarf Redlan
13	As 2 plus level two[8] of Dwarf Redlan
14	As 2 plus level one[7] of 1133
15	As 2 plus level two[8] of 1133

[1]See Table V.

[2]Contains 80% L-lysine.

[3]See Table III. 2.06% Simulated Milo Amino Acids Mix provides 0.1% L-lysine HCl.

[4]4.13% Simulated Milo Amino Acids Mix provides 0.2% L-lysine HCl.

[5]6.19% Simulated Milo Amino Acids Mix provides 0.3% L-lysine HCl.

[6]See Table IV. 6.19% Chicken Amino Acids Requirement Mix provides 0.3% L-lysine HCl.

[7]Level one = 26 percent.

[8]Level two = 52 percent.

Crystalline L-lysine HCl is assumed to be 100 percent available for chicks. This Standard Growth Curve is used as a criterion to estimate the bioavailability of L-lysine in the three varieties of grain sorghums used in Feeding Trial I.

In order to determine if the growth response due to graded levels of lysine in the milo amino acid pattern can be used as a criterion to estimate lysine availability in grain sorghum varieties, and also if the milo amino acids pattern impairs the bioavailability of the lysine, Treatments 6, 7, and 8 with 0.1, 0.2, and 0.3 L-lysine Hcl and other amino acids found in milo amino acids mix were imposed in this feeding trial. The Simulated Milo Amino Acids Mix is presented in Table III.

Treatment 9 with 0.3 percent L-lysine Hcl and other amino acids found in the Chicks Amino Acids Requirement Mix was assigned in Feeding Trial I to determine if the addition of a balanced amino acid pattern effects the chicks utilization of L-lysine. The Chick Amino Acids Requirement Mix is presented in Table IV.

The three different varieties of grain sorghum Darset, Dwarf Redlan, and 1133, which were tested for the bioavailability of their lysine content were added to the purified ration at two levels (26 and 52 percent). This was done at the expense of corn starch to formulate the ration fed in Treatments 10 through 15.

It should be pointed out that to avoid any loss of weight due to a lysine deficiency in Treatment 6 through 15, 0.2 percent crystalline L-lysine HCl was added to the rations. However, to estimate the bioavailability of lysine in the three different varieties of grain sorghum, the gain due to this amount of supplemental lysine (0.2%) was calculated by regression equations obtained from data on Treatments 1

TABLE III

SIMULATED MILO AMINO ACIDS MIX

Milo amino acids	Percent of amino acids in milo	Grains to stimulate 26% milo	Percent of amino acids in simulated milo
Arginine[1] HCl, H_2O	.39	.122	7.297
Cysteine	.13	.034	2.033
Glycine	.35	.091	5.443
Histidine[2] HCl, H_2O	.26	.091	5.443
Isoleucine	.49	.127	7.596
Leucine	1.77	.460	27.512
Lysine[3] HCl	.25	.081	4.844
Methionine	.14	.036	2.153
Phenylalanine	.62	.161	9.629
Serine	.51	.133	7.955
Threonine	.37	.096	5.742
Tryptophane	.05	.013	.778
Tyrosine	.26	.068	4.067
Valine	.61	.159	9.510
	6.434	1.672	100

[1]Contains 82 percent L-arginine.

[2]Contains 85 percent L-histidine.

[3]Contains 80 percent L-lysine.

TABLE IV

AMINO ACIDS REQUIREMENT IN CHICKS[1]

Amino acid	Percent in chick amino acid requirement mix
Lysine	0.300
Arginine	0.350
Histidine	0.137
Tyrosine	0.137
Trptophane	0.0457
Phenylalanine	0.152
Methionine	0.106
Cysteine	0.106
Threonine	0.198
Leucine	0.300
dl-methionine	0.183
Valine	0.210
Glysine	0.183
Proline	0.122
Glutamic Acid	4.249
Serine	-
Alanine	-
Asparagine	-
Total	6.19

[1]Sasse and Baker, 1973.

through 5,and 6 through 8. These estimated gains were subtracted from the observed weight gains and were used as the actual weight gain due to grain sorghum consumed in Treatments 10 through 15.

Rations

A purified ration (Sasse and Baker, 1973) which met all physiological requirements for chicks, but void of the amino acid lysine (Table V) was used in this feeding trial. The test nutrients were added to this purified ration at the expense of its corn starch content. To obtain the greatest possible degree of uniformity between and among experimental rations, a basal ration was formulated (Table VI). Since the maximum level of the test grain sorghums (three varieties) was 52 percent, the basal ration was composed of all nutrients in the purified ration with a 52 unit reduction in its corn starch content. The three different varieties of grain sorghums and the crystalline amino acids were added to the basal ration and corn starch was added to increase the percentage up to 100 percent. The experimental rations (treatments) are presented in Table VII.

Measurements Made

Measurements were taken at 8:30 a.m. every day during the four-day test period. These measurements included body weight and feed consumption. The body weight of each chick was measured and recorded individually in grams. Feed consumption was recorded on the basis of the total amount of feed in grams consumed by the chicks present in each battery section (replicate) each day.

TABLE V

PURIFIED RATION[1]

Ingredients	Percent
Corn starch	60.69
Corn oil	10.00
Solka floc	3.00
Glista salt	5.37
NaHCo[3]	1.00
Vitamins	0.40
Choline chloride	0.20
dl-α-tocopherol acetate	+
Ethxnyguin (125 mg/kg diet)	+
Amino Acids Mix:	
L-Lysine HCl	0.00
L-Arginine HCl	1.15
L-Histidine HCl·H_2O	0.45
L-Tyrosine	0.45
L-Trptophane	0.15
L-Phenylalanine	0.50
DL-Methionine	0.35
L-Cysteine	0.35
L-Thronine	0.65
L-Leucine	1.00
L-Isoleucine	0.60
L-Valine	0.69
L-Glycine	0.60
L-Proline	0.40
L-Glutamine[2]	12
L-Serine	-
Total	100

[1]Sasse and Baker (1973).

[2]Mono sodium glutamate was added instead of glutamine.

TABLE VI

COMPOSITION OF BASAL RATION

Ingredients	Percent
Corn starch	17.896
Corn oil	20.83
Solka floc	6.250
Glista salts	11.187
NaHCo3	2.083
Turkey vitamin mix[1]	0.833
Choline Chloride	0.416
dl-α-tocopherol acetate	+
Ethoxyguin (125 mg/kg diet)	+
Amino Acid Mix:	
L-Lysine HCl	0.00
L-Arginine HCl	2.3958
L-Histidine HCl·H_2O	0.938
L-Tyrosine	0.938
L-Trptophane	0.313
L-Phenylalanine	1.042
dl-Methionine	0.730
L-Cysteine	0.730
L-Thronine	1.350
L-Leucine	2.080
L-Isoleucine	1.250
L-Valine	1.440
L-Glycine	1.250
L-Proline	0.830
L-Glutamine[2]	25.00
L-Serine	-
Total	100.00

[1]See Table I

[2]Mono sodium glutamate was added instead of glutamine.

TABLE VII

EXPERIMENTAL RATION

Rations (Treatment)	Percent basal ration	Percent crystalline lysine HCl	Percent chick amino acid requirement mix	Percent simulated milo amino acid Mix	Percent grain sorghum	Percent Corn starch
1	48	0.00	0.00	0.00	0.00	52
2	48	0.2	0.00	0.00	0.00	51.8
3	48	0.3	0.00	0.00	0.00	51.7
4	48	0.4	0.00	0.00	0.00	51.6
5	48	0.5	0.00	0.00	0.00	51.5
6	48	0.2	0.00	2.06[1]	0.00	49.74
7	48	0.2	0.00	4.12[2]	0.00	47.68
8	48	0.2	0.00	6.18[3]	0.00	45.62
9	48	0.2	6.19[4]	0.00	0.00	45.61
10	48	0.2	0.00	0.00	26 (Darset)	25.8
11	48	0.2	0.00	0.00	52 (Darset)	0.00
12	48	0.2	0.00	0.00	26 (Dwarf Redlan)	25.8
13	48	0.2	0.00	0.00	52 (Dwarf Redlan)	0.00
14	48	0.2	0.00	0.00	26 (1133)	25.8
15	48	0.2	0.00	0.00	52 (1133)	0.00

[1]2.06 percent of Simulated Milo Amino Acid mix provides 0.1 percent crystalline L-lysine HCl.

[2]4.12 percent of Simulated Milo Amino Acid mix provides 0.2 percent crystalline L-lysine HCl.

[3]6.18 percent of Simulated Milo Amino Acid mix provides 0.3 percent crystalline L-lysine HCl.

[4]6.19 percent of Chick Amino Acid Requirement mix provides 0.3 percent crystalline L-lysine HCl.

Protein Analysis of the Grain Sorghum Varieties for Lysine Content

The lysine content of the grain sorghum varieties used in Feeding Trial I was measured by means of an Amino Acid Analyzer in the Department of Biochemistry. In order to prepare the grain sorghum samples for analysis, the following procedure was followed.

Grain sorghum samples were ground well (powdered). One milligram of each sample was hydrolyzed in 200 µl of 6 N HCl under vacuum. The tubes containing the samples were sealed under vacuum and were placed in a 110^{0}C oven for 24 hours. The tubes were opened and dried in a desiccator. The samples were dissolved in 0.15 milliliter of sodium nitrate, 0.2 N (buffer).

The samples were placed in the Amino Acid Analyzer which provided a chromatogram of the amino acid content including lysine. Each amino acid in the samples provided a peak. Each amino acid peak was compared with a standard. The lysine content of the grain sorghum samples was determined to be 0.22, 0.24, and 0.19 percent in Darset, Dwarf Redlan, and 1133, respectively.

Analysis of the Grain Sorghum Varieties for Tannin Content

The tannin content of the grain sorghum varieties used in Feeding Trial I was done by the Vanillin-HCl Procedure. In order to do this, the following procedure was followed.

A standard solution was prepared by dissolving 100 milliliters of catachin in 50 milliliters of methanol and this standard was used as a criterion to measure the tannin content in the grain sorghum samples.

A standard curve of tannin was obtained by placing different concentrations of the standard solution plus 5 milliliters of vanillin-HCl in a spectrophotometere and then plotting the readings on a graph. One gram of each grain sorghum sample was mixed with 50 milliliters of methanol and shaken for 24 hours. One milliliter of this solution was placed in a spectrophotometere and the results were compared with the standard curve. This procedure determined the tannin content to be 0.171, 0.00, and 0.165 percent in Darset, Swarf Redlan, and 1133, respectively.

Feeding Trial II

Ninety day-old meat-strain chicks were obtained from a commercial hatchery. The chicks were distributed into boxes according to their weight. In order to have chicks with approximately the same body weight in each group, the chicks were distributed into nine battery sections in three electric battery brooder one chick at a time in rotation until each section contained ten chicks. Three battery sections within each of the three battery were used. The chicks were wing banded for better identification and the initial weight of each chick was recorded in grams. The three treatments which were used (Table VIII) in this feeding trial (Trial II) were assigned at random to each of three battery sections within each electric brooder. Thus the experiment design was a randomized block with three replicates per treatment.

In order to make a valid comparison among the two varieties of grain sorghum (Frontier and Redlan) and yellow corn, three isonitrogenous and isocalorieous rations were formulated using the above grains.

In order to obtain the greatest possible degree of uniformity between and among the experimental rations, a basal ration was first mixed in a small electric mixer (50 kg capacity). This mixer was cleaned before and after each experimental ration was mixed. The ingredients in the basal ration are listed in Table IX.

TABLE VIII

EXPERIMENTAL DESIGN OF FEEDING TRIAL II

Treatment Number	Grain fed
1	Grain sorghum (Redlan)
2	Grain sorghum (Frontier)
3	Yellow corn

The protein content of the yellow corn, Frontier, and Redlan sorghum grains were determined to be 9.8, 12.1, and 12.13 percent, respectively, by means of the Kjeldahl Procedure (N x 6.25). Since the protein contents of the two varieties of sorghum grains used in this feeding trial were approximately equal, an equal amount of each of these two varieties were mixed with the basal ration to obtain the experimental rations fed in Treatments 1 and 2. However, the yellow corn which contained less protein (8.9) was included in a greater amount than were the two varieties of sorghum grains. This was done to provide

TABLE IX

BASAL RATION

Ingredients	Percent
Tallow, feed grade	5.500
Soybean meal (44%)	64.200
Alfalfa meal (17%)	5.510
Dried whey (12%)	5.510
Live yeast culture (14%)	5.510
Meat and bone meal (50%)	9.200
dl Methionine	0.184
Phosphorus supplement (Ca27-P18)	1.840
Calcium carbonate	0.920
Trace mineral mix[1]	0.180
Salt	0.920
Turkey vit mix[2]	0.550
Calculated analysis	
Protein %	35.2
Kcal/454g	1037.72

[1]Provides in the ration: manganese, 120 ppm; zinc, 80 ppm; iron, 60 ppm; copper, 10 ppm, and iodine 1.0 ppm.

[2]Contains per 454g of vitamin mix: vitamin A, 1,600,000 I.U.; vitamin D, 600,000 I.U.; vitamin E, 5400 I.U.; menadione sodium bisulfite complex, 400 mg; riboflavin, 100 mg; niacin, 3000 mg; d-pantothenic acid, 3200; choline, 80,000 mg; thiamine, 400 mg; pyrioxine, 400 mg; vitamin B12, 1.6 mg; d-biotin, 20 mg; folic acid, 200 mg.

from the corn protein an amount equal to that provided by the grain sorghums. In addition, tallow (animal fat) was added to the two sorghum grain ration in Treatments 1 and 2 to eliminate any differences in metabolizable energy between the sorghum grains and yellow corn. The experimental rations are presented in Table X.

Standard management practices were followed in caring for the chicks during the entire experimental feeding period. These practices included brooding (starting with 35^{o}C then adjust downward as the heat requirements of the broilers decreased), cleaning the water fountains and adding fresh water every day, changing the papers under each battery section, and keeping the feeders half full of feed. The feed in the feeders was cleared of foreign material and dirt, and was renewed every day. Feed and water were given ad libitum. Each battery section was equipped with a suitable size water fountain, and two feeders.

Measurements Made

The second feeding trial (Trial II) was held during the spring of 1980 (May 2 through May 23). Measurements were taken at the end of first, second, and third weeks. The measurements included body weight and feed consumption. The body weight of each broiler was measured and recorded individually in grams. Feed consumption was recorded on the basis of the total amount of feed in grams consumed by the broilers present in each battery section during each one week period. No mortality was observed during Feeding Trial II.

TABLE X

EXPERIMENTAL RATIONS OF BROILERS FED IN TRIAL II

Ingredients	No. 1 (with Redlan) %	No. 2 (with Frontier) %	No. 3 (with Corn) %
Tallow, feed grade	7.37	7.37	3
Soybean meal (44%)	35	35	35
Grain sorghum (Redlan)	31.2	--	--
Grain sorghum (Frontier)	--	31.2	--
Yellow corn (ground)	--	--	41.55
Alfalfa meal (44%)	3	3	3
Dried whey (12%)	3	3	3
Live yeast culture (14%)	3	3	3
Meat and bone meal (50%)	5	5	5
dl Methionine	0.1	0.1	0.1
Phosphorus supplement	1	1	1
Calcium carbonate	0.5	0.5	0.5
Trace mineral mix[1]	0.1	0.1	0.1
Salt	0.5	0.5	0.5
Turkey vitamin mix[2]	0.3	0.3	0.3
Polyethylene	9.98	9.98	4
Total	100	100	100
Nutrient analysis			
Protein (%)[3]	22.9	22.9	22.86
Kcal/454g[4]	1179	1179	1201

[1]See footnote of Table IX.

[2]See footnote of Table IX.

[3]Kjeldahl analysis.

[4]Calculated.

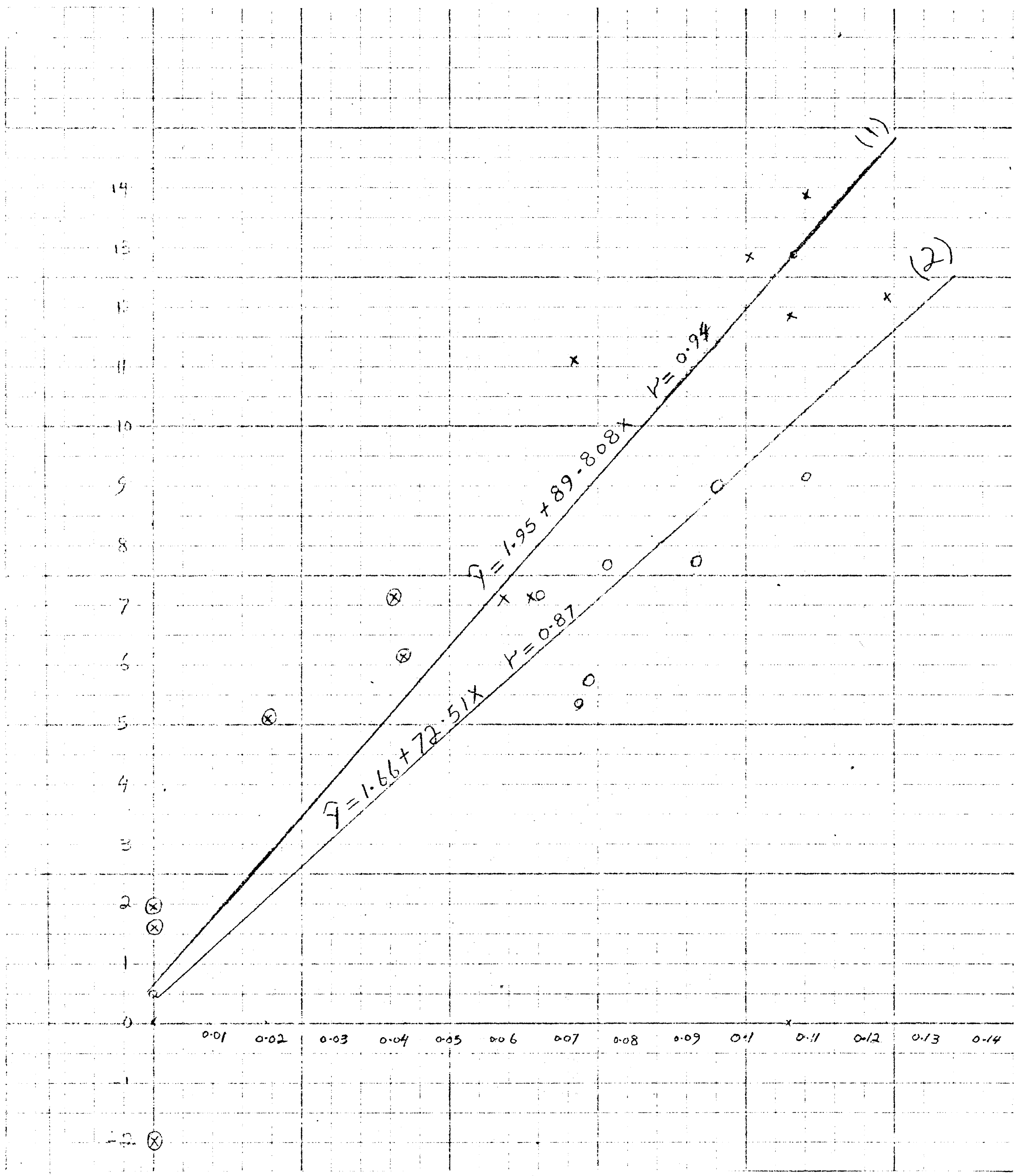
CHAPTER IV

RESULTS AND DISCUSSION

Feeding Trial I

Statistical Analysis

Body weight and feed consumption data were used to calculate average daily gain and feed consumed per replicate for the four-day test period. Regression equations were calculated (Snedecor and Cochran, 1975) from the average daily gain as the dependent variable (y) and grams of crystalline lysine Hcl consumed per each replicate as the independent variable (x) in Treatments 1 through 5 and 6 through 8. These equations were calculated to be $\hat{y} = 1.95 + 89.808x$ and $\hat{y} = 1.66 + 72.510x$, respectively. These equations were named Lysine Standard Regression Equation (LSRE), and Simulated Milo Amino Acid Standard Regression Equation (SMAASRE), respectively. Plotting the average daily gain (grams) per each replicate against the grams of crystalline lysine HCl consumed in Treatments 1 through 5, and 6 through 8 provided a Lysine Standard Growth Curve and a Simulated Milo Amino Acid Standard Growth Curve, respectively. These two curves are presented in Figure I. The confidence intervals were calculated (Steel and Torrie, 1960) for the slopes of the Lysine Standard Growth Curve and the Simulated Milo Amino Acid Standard Growth Curve to determine if the slopes of these



[1]Lysine Standard Growth Curve.

[2]Milo Amino Acid Standard Growth Curve.

Figure 1. Lysine Standard and Simulated Milo Amino Acid Standard Growth Curves

two curves are different or are the same. Overlapping of the calculated confidence intervals for these two curves (89.8 ± 21.15 vs. 72.51 ± 38.9) indicated no significant difference between the two slopes.

The bioavailability estimate of L-lysine in the three varieties of grain sorghums used in Feeding Trial I was made by means of the Slope Ratio Technique. In using this technique, two regression equations on gain due to the grain sorghum consumed were calculated for each variety of grain sorghum. In calculating the first regression equation, the Lysine Standard Regression Equation was used to adjust the average observed gain in each replicate due to the 0.2 percent of supplemental crystalline L-lysine HCl which was already added. However, in calculating the second regression equation, the Simulated Milo Amino Acid Regression Equation was used for this adjustment. These adjustments resulted in the calculation of average daily gain due to the grams of each grain sorghum variety consumed in Feeding Trial I. These adjustment calculations are presented in Table XI. Treatment 2 which contained 0.2 percent crystalline L-lysine HCl (common in all grain sorghum treatments), and zero percent grain sorghum was used along with each of the two other treatments considered for each grain sorghum variety (Treatments 10 through 15) in calculating the two regression equations for each variety of grain sorghum used. The regression equations calculated to be $\hat{y}_{2,10,11} = 0.62 + 0.151x$ and $\hat{y}_{2,10,11} = 1.54 + 0.170x$ for Darset, $\hat{y}_{2,12,13} = 0.44 + 0.200x$ and $\hat{y}_{2,12,13} = 1.13 + 0.219x$ for Dwarf Redlan, and $\hat{y}_{2,14,15} = 1.41 + 0.267x$ and $\hat{y}_{2,14,15} = 2.32 + 0.286x$ for 1133. Confidence intervals and correlation coefficients (r) were calculated (Steel and Torrie, 1960) for the above equations. These data are summarized in Table XII. The confidence intervals for the slopes of the regression equations calculated for

TABLE XI

ADJUSTMENT ON GAIN FOR 0.2 PERCENT SUPPLEMENTAL L-LYSINE HCl

					Adjustment using lysin standard regression equation		Adjustment using milo amino acid standard regression equation	
Treatment	Replicate	Observed gain (g)	L-Lysine HCl consumed (g)	Milo consumed (g)	Gain due L-lysine HCl (g)	Gain due milo (g)	Gain due L-lysine HCl (g)	Gain due milo (g)
2	1	5.13	0.019125	0	3.67	1.46	3.05	2.08
	2	7.19	0.04250	0	5.77	1.42	4.74	2.45
	3	6.13	0.04275	0	5.79	0.34	4.76	1.37
10	1	6.13	0.04500	5.85	5.99	0.14	4.92	1.21
	2	9.75	0.04800	6.22	6.26	3.49	5.14	4.61
	3	6.25	0.04750	5.82	6.22	0.03	5.10	1.14
11	1	11.69	0.05100	13.19	6.33	5.16	5.36	6.33
	2	4.44	0.03660	9.52	5.24	-0.8	4.31	0.13
	3	9.13	0.05187	13.48	6.61	2.52	5.42	3.71
12	1	5.5	0.04037	5.25	5.58	- .08	4.59	0.91
	2	4.56	0.03812	4.96	5.37	0.81	4.42	0.41
	3	6.25	0.0433	5.64	5.84	0.41	4.8	1.45
13	1	8.19	0.04187	10.89	5.71	2.48	4.70	3.49
	2	10.62	0.049	12.74	6.35	4.27	5.21	5.41
	3	8.44	0.0408	10.63	5.61	2.83	4.62	3.82
14	1	3.50	0.0376	4.89	5.33	-1.83	4.39	-0.89
	2	13.19	0.0456	5.93	6.05	7.14	4.97	8.22
	3	11.42	0.0466	6.06	6.14	5.28	5.04	6.38
15	1	15.12	0.0466	12.12	6.14	8.98	5.03	10.08
	2	10.69	0.0461	11.99	6.09	4.60	5.00	5.69
	3	6.38	0.0511	13.29	6.54	-0.16	5.37	1.02

TABLE XII

REGRESSION EQUATIONS, CONFIDENCE INTERVALS, AND CORRELATION COEFFICIENTS FOR EACH GRAIN SORGHUM VARIETY USED IN TRIAL I

Variety	Regression equation using Lysine Standard Equation for adjustment of the gain	Regression equation using Simulated Milo Amino Acid Equation for adjustment of the gain	Confidence internal for slope	Correlation coefficients r
Darset	$\hat{y}_{2,10,11}$[1] = 0.62 + 0.151x	———	0.151 ± 0.37	0.42
	———	$\hat{y}_{2,10,11}$[1] = 1.54 + 0.170x	0.170 ± 0.37	0.46
Dwarf Redlan	$\hat{y}_{2,12,13}$[1] = 0.44 + 0.200x	———	0.200 ± 0.40	0.71
		$\hat{y}_{2,12,13}$[1] = 1.13 + 0.219x	0.219 ± 0.39	0.66
1133	$\hat{y}_{2,14,15}$[1] = 1.41 + 0.267x	———	0.267 ± 0.36	0.40
		$\hat{y}_{2,14,15}$[1] = 2.32 + 0.286x	0.286 ± 0.36	0.42

[1]Treatment number used for calculation.

each grain sorghum variety were broad and indicated considerable variation among the replicates.

The bioavailability of L-lysine in the grain sorghum varieties used which is expressed in grams of lysine per gram of grain sorghum variety fed, were estimated by dividing the slope of the first and the second regression equations calculated for each variety of grain sorghum by the Lysine Standard Regression Equation and the Simulated Milo Amino Acid Standard Regression Equation, respectively. Since the Lysine Standard Regression Equation and the Simulated Milo Amino Acid Standard Regression Equation were calculated on the basis of lysine HCl (synthesized form of lysine containing 80 percent pure lysine), the estimated values for bioavailability of lysine were corrected through the use of this factor (0.80). The bioavailability of L-lysine was found to be 0.0014 and 0.0018 gram/gram for Dorset, 0.0018 and 0.0024 gram/gram for Dwarf Redlan, and 0.0024 and 0.0031 gram/gram for 1133. The percent bioavailability of L-lysine in the grain sorghum varieties was calculated by dividing the grams of available L-lysine per gram of each grain sorghum by its lysine content. The percent available L-lysine was estimated to be 63 and 82 in Dorset, 74 and 99 in Dwarf Redlan, and 123 and 159 in 1133. It should be pointed out that under estimating the lysine in the grain sorghum samples, as measured by the Amino Acid Analyzer, can result in overestimating bioavailability.

In order to study other objectives (effect of the milo amino acid pattern and the chick amino acid pattern on the utilization of L-lysine), average daily gain, feed consumption, and feed efficiency were calculated for all treatments. These data are presented in Table XIII.

TABLE XIII

AVERAGE DAILY GAIN, FEED CONSUMPTION AND FEED EFFICIENCY FOR TRIAL I[1]

	Treatment				
	1	2	3	4	5
Gain (g)	0.50 ± 1.13	6.15 ± 3.0	8.43 ± 1.3	12.94 ± 0.6	13.13 ± 0.8
Feed (g)	13.875 ± 1.12	17.396 ± 3.92	21.54 ± 1.16	27.125 ± .31	28.292 ± 1.82
Feed/Gain	27.7 ± 4.84	2.77 ± .5	2.63 ± .25	2.10 ± .1	2.15 ± .07
Lys. Hcl (mg)	0.0 ± 0	34.8 ± 7.8	66.2 ± 3.49	108 ± 1.25	141.5 ± 9.07
Lys/Gain Hcl		5.66	7.8529	8.35	10.78
	6	7	8	9	
Gain (g)	6.77 ± 0.6	8.34 ± 0.7	7.29 ± 1.9	9.38 ± 1.6	
Feed (g)	23.96 ± 1.14	23.7 ± 0.8	18.094 ± 3.66	20.479 ± 2.04	
Feed/Gain	3.6 ± .4	2.86 ± .23	2.54 ± .12	2.23 ± .16	
Lys. Hcl (mg)	47.9 ± 2.27	47.5 ± 0.16	36.2 ± 7.3	40.9 ± 4.04	
Milo Simulated Lys. Hcl (g)	23.9 ± 1.14	47.5 ± 0.16	54.3 ± 10.97		
Chick Simulated Lys. Hcl (g)	--	--	--	61.4 ± 6.1	

TABLE XIII (Continued)

	Treatment					
	10	11	12	13	14	15
Gain (g)	7.375 ± 1.19	9.896 ± 2.12	5.4375 ± .49	9.083 ± .77	9.368 ± 2.98	10.72 ± 2.53
Feed (g)	22.937 ± .50	23.206 ± 2.45	20.3115 ± .76	21.958 ± 1.28	21.64 ± 1.42	23.97 ± .79
Feed/Gain	3.23 ± .39	3.05 ± .58	3.77 ± .21	2.42 ± .08	3.05 ± 1.17	2.57 ± .74
Lys. Hcl (g)	45.9 ± .93	46.5 ± 4.95	40.6 ± 1.5	43.9 ± 2.6	43.3 ± 2.9	46.2 ± 1.59
Milo (g)	5.964 ± .13	12.06 ± 1.27	5.28 ± .197	11.42 ± 0.07	5.63 ± .37	12.47 ± .4

[1]Means ± SEM within a classification not sharing a common superscript letter differ P<.05, n=3 except for Treatments 8 and 7 which had n=2.

To determine if the chick amino acid requirement pattern has an effect on the utilization of L-lysine in chicks, average daily gain in Treatment 9 with 0.5 percent lysine HCl (0.3 percent is provided by Chick Amino Acid Requirement mix) was compared with that in Treatment 5 which had the same percent lysine HCl (0.5%). Least Significance Different (LSD) was calculated (Steel and Torrie, 1960) on average daily gain using pooled variance obtained from all treatments in Trial I in order to obtain more accuracy. This comparison showed no statistically significance difference between gain in Treatments 5 and 9.

Since Treatments 6, 7, and 8 had the same percent crystalline L-lysine HCl as did Treatments 3, 4, and 5, but the lysine was provided by the Milo Amino Acid Mix in Treatments 6, 7 and 8, comparisons were made between average daily gain in Treatments 5 and 8, 4 and 7, and 3 and 6 by the LSD procedure. It was concluded that the gains in Treatments 7 and 8 are statistically different from those in Treatments 4 and 5, respectively. However, a difference in average daily gain in Treatments 3 and 6 was not statistically significant.

Discussion

Through the use of the slopes of the two regression equations, namely Lysine Standard and Simulated Milo Amino Acid Standard, as the criteria in estimating the bioavailability of lysine, two different values were obtained for each variety of grain sorghums. When the Lysine Standard was used, a lower value for bioavailable L-lysine was obtained than when the Simulated Milo Amino Acid Standard was used. Although both of these two regression equations were used in estimating lysine bioavailability in this study, the Simulated Milo Amino Acid Standard Regression Equation seems most logical. This is true because this regression equation is calculated from data obtained from those treatments (Treatments 1, 2, 6, 7, and 8) in which crystalline lysine HCl was fed as it is found in the milo amino acid pattern. Therefore, the chicks in these treatments consumed lysine in the same amino acid pattern as it is in the grain sorghum varieties which were used in Trial I. The great variation in gain due to 1133 in Treatments 14 and 15 resulted in a high value for the slope of the regression equation which was calculated for this variety. This resulted in the abnormal values for lysine bioavailability as determined for this variety.

The poor correlation between bioavailable lysine and percent tannin ($y = 1 + 1.6x$, $r = .27$) in these grain sorghum varieties is in agreement with the result obtained in a study by Fuller et al., 1966. They found a dietary level of tannin between 0.64 and 0.83 percent was required to cause a depression in chick performance. Since the tannin concentration in the rations fed in Treatments 10 through 15 were below this deleterious level, no depression was observed in chick performance.

The average daily gain of the chicks in Treatments 7 and 8 (milo amino acid treatments) was statistically lower ($P \leq .05$) from that in Treatments 4 and 5 (standard treatments). In fact all milo amino acid treatments (6, 7, and 8) resulted in less gain in chicks than was obtained in the corresponding standard treatments (3, 4, 5) with the same percent of crystalline lysine Hcl. This result is supported by different slopes as calculated by the Lysine Standard Regression Equation and the Simulated Milo Amino Acid Standard Regression Equation (Figure I). Although, the difference between the slopes of these equations was not statistically detected (because of a broad confidence intervals for the slopes), the data obtained tend to indicate that the milo amino acid pattern reduces the lysine bioavailability estimates in these grain sorghums as measured by diminished chick growth performance. In fact, the milo amino acid pattern appears to cause a classical amino acid imbalance when added to the purified ration. This is supported by reduced chick gain and feed intakes in Treatments 6, 7, and 8 in comparison to those in Treatments 3, 4, and 5, respectively (Table XIII). It should be pointed out that the data presented do not suggest that the L-lysine HCl is metabolized less efficiently in the imbalanced rations. The decreased chick growth in Treatments 6, 7, and 8 is accompanied by a concomitant reduction in feed intake which would explain the reduced performance.

No significant difference ($P<.05$) between gains in Treatment 9 (0.5 percent lysine Hcl provided from the chick Amino Acid Requirement mix) and corresponding standard lysine treatment (Treatment 5) demonstrate the lack of an amino acid imbalanced on chick growth and feed intake when balanced amino acids are added to the purified ration.

It can be concluded that among the three varieties of grain sorghum used in Trial I, 1133 and Darset had the highest and lowest bioavailability of their L-lysine content, respectively. Dwarf Redlan was intermediate in this respect. In general, not only the percent bioavailability of L-lysine, but percent L-lysine content which is positively correlated with protein content (Virupaksha and Sastry, 1968), must be considered in selection grain sorghum varieties for poultry feeding. This is true because these two factors determined total bioavailability of L-lysine in grain sorghum. On the other hand in providing equal amount of protein from high versus low protein varieties of grain sorghum, the ration containing the high protein variety should be supplemented with lysine at a higher level than the low protein variety since lysine is the most deficient amino acid especially in the high protein varieties.

Feeding Trial II

Calculation of Data

From the body weight and feed consumption data which were recorded every week, the average daily gain and average daily feed consumed for each chick were calculated for each week (Period 1, 2, and 3). These data (gain per broiler per day and feed per broiler per day) were used to calculate the means for each period. These means were analyzed by period and by the overall average for all three periods. These data are presented in Table XIV.

Statistical Analysis of Data

The data on body weight gain and feed consumed as presented in Table XIV were analyzed statistically using the analysis of variance (A.O.V.) procedure as described by Snedecor and Cochran (1967). Based upon this analysis of variance, F-values were used to compare differences among the three treatments in weight gain and feed consumed per chick per day. The analysis of variance of the data is illustrated in Table XV and XVI. The F-values for weight gain and feed consumed per chick per day in Periods 1, 2, 3, and overall were not statistically significant.

TABLE XIV

MEAN GAIN AND FEED CONSUMED PER CHICK PER DAY FOR PERIOD 1, 2, 3, AND OVERALL IN TRIAL II

		1st Period		2nd Period		3rd Period		Overall	
Treatment no.	Ration type	Gain per chick per day (g)	Feed consumed per day (g)	Gain per chick per day (g)	Feed consumed per day (g)	Gain per chick per day (g)	Feed consumed per day (g)	Gain per chick per day (g)	Feed consumed per day (g)
1	Grain sorghum (Redlan)	16.30	22.70	24.87	40	35	70.20	25.39	44.30
2	Grain sorghum (Frontier)	17.09	22.60	21.50	37.6	38	71.19	25.53	43.79
3	Corn	16.31	21.90	26.50	42	36	65.00	26.27	42.96

TABLE XV

ANALYSIS OF VARIANCE FOR BODY WEIGHT IN TRIAL II

		Mean Squares			
Source	df	Period 1	Period 2	Period 3	Overall
Total	89	5.500	52.440	44.00	103.55
Block	2	3.75	313.870	73.80	215.82
Ration	2	6.060	196.278	76.88	187.29
Block*Ration	4	32.037	653.960	77.77	2439.00
Chick (Block*Ration)	81	4.220	12.730	41.23	348.10

TABLE XVI

ANALYSIS OF VARIANCE FOR FEED CONSUMED IN TRIAL II

		Mean Squares			
Source	df	Period 1	Period 2	Period 3	Overall
Total	8	1.550	82.142	18.86	
Block	2	0.0022	48.02	2.53	140.40
Ration	2	0.587	21.76	33.30	22.23
Block*Ration	4	2.815	129.39	19.79	865.98

Discussion

As was previously described, in order to make a valid comparison in the bioavailability of energy in yellow corn and the two varieties of grain sorghums, the rations used in Trial II were formulated to be the same in dietary protein and energy content. In addition, an attempt was made to formulate these rations with dietary protein levels higher and dietary energy levels lower than the current nutrient standard in use under commercial production conditions. The commercial standard (Scott, 1979) recommends 94.43 grams of protein per pound of feed and 0.076 grams of protein per kilocalorie of energy content. However, the rations used in Trial II have been formulated to have 103.96 grams of protein per pound of feed and 0.088 grams of protein per kilocalorie of energy content. In other words, the amount of the protein per kilocalorie of energy in these rations was 16 percent higher than the standard. At the same time, the energy content of the ration used was 84 percent of the standard. Therefore this reduction in dietary energy forced the chicks to consume a greater amount of feed in order to meet their energy requirements since chickens continue to consume feed until their energy requirements have been met (Connor et al., 1976). This increase in feed consumption provided a protein intake in excess of the chick's actual needs, and prevented any effect on weight gain which might have been due to a possible protein or amino acid deficiency. Therefore, any differences in weight gain among the treatment group if observed would be due to a response to differences in the energy availability in the rations used.

Energy distribution in each ration is presented in Table XVII.

TABLE XVII

ENERGY AND PROTEIN IN RATIONS FED IN TRIAL II

	Ration 1		Ration 2		Ration 3	
Ingredient	Energy Kcal/lb	Protein %	Energy Kcal/lb	Protein %	Energy Kcal/lb	Protein %
All ingredients other than those listed below	460	19.17	460	19.17	460	19.17
Grain sorghum (Redlan)	461	3.77				
Grain sorghum (Frontier)			461	3.77		
Yellow corn					633	3.69
Tallow	258		258		105	
Total	1179	22.9	1179	22.9	1201	22.86

All ingredients other than tallow, grain sorghum varieties, and yellow corn provide 460 kilocalories of energy per pound of ration. Therefore, all rations are equal in this respect. Since the grain sorghums are lower in energy than yellow corn, different levels of tallow (7.37, and 3 percent) as an intensive energy source were added to the rations which contained the sorghum varieties (Redlan and Frontier) and yellow corn, respectively, to equate dietary energy content. However, the different levels of tallow might be considered as a source of variation in the weight gain of the chicks. This is not a valid conclusion however, since Balloun et al. (1956) found that the maximum growth response in male chicks due to tallow was obtained when the tallow was added at a dietary level of one percent. Beyond this supplemental level there was no additional growth response from the tallow per se. Since dietary energy is equal in all rations and feed consumed by the chicks on the three treatments was equal (Table XVI), the energy intake was equal in all treatments. Therefore, the equal growth response on the chicks in Trial II indicates the same bioavailability of energy in the two different varieties of grain sorghums (Redlan and Frontier) and in yellow corn.

CHAPTER V

SUMMARY AND CONCLUSIONS

Two feeding trials which lasted 4 and 21 days, respectively, were conducted during the spring of 1980. In the first feeding trial which was initiated with one hundred and eighty chicks when they were 15-days old, the bioavailability of L-lysine in Darset, Dwarf Redlan, and 1133 was measured as well as the effect of the milo and chick amino acid requirement patterns on L-lysine utilization. A purified ration which met all physiological requirements of chicks, but which was void of L-lysine was used in Trial I. A Slope Ratio Technique was used in this trial. To do so, two regression equations were calculated on data obtained on Treatments 1 through 5 with graded levels of L-lysine Hcl (0.0, 0.2, 0.3, 0.4, and 0.5 percent), and Treatments 6 through 8 with graded levels of L-lysine Hcl (0.1, 0.2, and 0.3 percent) provided from the milo amino acid pattern. Slopes of the regression equations obtained from the grain sorghum variety treatments (10 through 15) were divided by the slope of each of the above equations (lysine Standard and Milo Amino Acid Standard) to calculate the lysine bioavailability in the grain sorghum varieties.

Gain in Treatments 6 through 8 and Treatment 9 were compared with the standard lysine treatments which contained equal percentages of lysine HCl to determine the effect of the milo amino acid and chicks amino acid requirement patterns on the utilization of lysine.

It was concluded that the 1133 and Darset have the highest and the lowest lysine bioavailability, respectively. However, Dwarf Redlan is intermediate from this standpoint. It also appeared that chicks fed lysine HCl from the milo amino acid pattern gained significantly less that those fed on equal fed on equal percentage of lysine Hcl. Since feed intake in the treatments with the milo amino acid pattern were also less than that in the standard treatments, it was concluded that the milo amino acid pattern may not have a deleterious effect on lysine utilization, but causes an amino acid imbalance and reduces feed intake. Therefore in ration formulation using grain sorghums, a supplemental source of a few amino acids especially L-lysine should be considered to prevent an amino acid imbalance. The equal weight gain in treatment 9 when lysine is provided in a balance amino acid pattern when compated with the weight gain in the standard treatment (Treatment 5) indicates the importance of amino acid balance in the ration on lysine utilization and chick performance.

In the second feeding trial, the energy availability for chicks in two varieties of grain sorghums (Redlan and Frontier) was compared to that in yellow corn using isonitrogenous and isocaloric rations. The protein and energy content of these rations were formulated to be higher and lower than recommended levels, respectively, in order to detect any differences in the bioavailability of the energy content provided by these grains.

In the second feeding trial, it was indicated that the energy provided from grainsorghums in the ration has the same availability as it is in yellow corn.

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