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EVALUATIONS OF PROTEIN QUALITY AND THE RELATIONSHIP OF AMINO ACID BALANCE TO APPETITE

by

Richard Lawrence Dorflinger

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For the Degree of

DOCTOR OF PHILOSOPHY

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THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by Richard Lawrence Dorflinger entitled Evaluations of Protein Quality and the Relationship of Amino Acid Balance to Appetite be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

B. L. Reid
Dissertation Director

4/18/72
Date

After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

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*This approval and acceptance is contingent on the candidate's adequate performance and defense of this dissertation at the final oral examination. The inclusion of this sheet bound into the library copy of the dissertation is evidence of satisfactory performance at the final examination.

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TABLE OF CONTENTS

TABLE OF CONTENTS--Continued

LIST OF TABLES

Table

LIST OF TABLES--Continued

LIST OF TABLES--Continued

ABSTRACT

The effects of dietary level and quality of protein on plasma free amino acid (PAA) levels were studied using three-week-old Hubbard broiler chicks. Initial studies determined guidelines for sampling times. It was observed that no more than 2 hours of fasting were required to produce minimal levels in most PAA in chicks fed ad libitum. Beyond this point some amino acids began to rise in concentration, particularly lysine. Maximal PAA values in force-fed chicks were obtained at approximately 1½ hours of fasting.

Next, the relationship between dietary protein level and PAA levels was studied with chicks fasted 1-3/4 hours prior to force-feeding 5 grams of a soybean meal diet ranging from 3 to 18% protein with and without supplemental methionine. Blood samples taken 1-3/4 hours after feeding showed that all essential PAA, except lysine, threonine and methionine plus cystine, were significantly correlated with dietary protein level in the unsupplemented diet; methionine plus cystine became significantly correlated in chicks fed the methionine supplemented diet.

Force-feeding 5 grams of a 21% protein diet based on soybean meal or cottonseed meal or a 9% protein diet of milo generally allowed for detection of the limiting dietary amino acid by comparing the PAA levels of chicks fed the test protein to levels of chicks fed a reference diet. Fasting for 1-3/4 hours prior to force-feeding did not improve this detection procedure.

Ad libitum fed chicks consuming diets calculated to contain 5.2 grams of protein for each 100 kcal of metabolizable energy showed feed consumptions which varied among diets according to the degree of amino acid deficiency and appeared to explain the variations in PAA levels. When small amounts of protein were ingested, PAA values did not reflect the dietary pattern, while ingestion of large amounts of protein allowed the limiting amino acid to show the greatest percent drop during fasting. When intermediate amounts of protein were consumed, comparison of PAA levels of chicks fed test diets with levels of chicks fed a reference diet was shown to indicate the most limiting amino acid.

A further study was done under conditions of short-term feed consumption. Test diets were fed for only 1-3/4 hours without prior fasting. Blood samples taken at the end of the 1-3/4 hour period showed PAA levels for chicks fed corn, soybean meal, cottonseed meal or milo in relation to PAA levels of chicks fed the reference diet to identify the limiting amino acid in each protein, provided the amino acid intake was not above the requirement.

Amino acid levels in several tissues were measured in Leghorn cockerels fed diets deficient in methionine or with a 5% excess of leucine. Elevated levels of leucine were found in plasma, thigh and breast muscle from chicks fed the high leucine diet and appeared to indicate a toxicity of that amino acid. The methionine deficiency was reflected in plasma and thigh muscle. However, other than this difference which did not appear significant, there was no amino acid pattern that appeared variant enough to explain the decreased appetite resulting from consumption of the methionine deficient diet except that very low levels of histidine were noted in both muscle samples. This result was also noted in chicks receiving the high leucine diet.

CHAPTER 1

REVIEW OF LITERATURE

In the past the evaluation of protein quality has involved laborious, time-consuming methods which generally utilized unnatural conditions. Often the measurement did not include all the factors necessary to determine protein quality. An accurate, rapid method which takes into account all factors influencing protein quality would be most beneficial. Research applications would be numerous. Proteins could be evaluated to more closely determine their overall quality than is presently possible. A rapid method would allow for evaluation prior to performing a desired experiment, and diets could be calculated more effectively to contain the proper amounts of nutrients. Such a method could also be applied commercially. The animal and feed industries are becoming concentrated into larger, but fewer operations. Such an increase in size allows for more exacting specifications in feed formulation than was possible or necessary in smaller units. Improved methods of protein quality evaluation could result in the elimination of excess nutrients being formulated into the diet. This study will be concerned with rapid methods for the evaluation of protein quality and the effect of several parameters on plasma free amino acid levels.

Factors Determining Protein Quality

The quality of a dietary protein is judged in relation to how well it meets the amino acid requirements of a particular animal under given conditions. Two factors are involved: first, the amino acid contents of the protein; second, the availability of each amino acid to the animal. There are many tables showing the average amino acid contents of various feedstuffs. The figures do not reflect the possible changes in each shipment of a feedstuff. If a particular lot of a protein feedstuff is analyzed, it is usually only analyzed for protein and not amino acid content. In monogastric nutrition the amino acid content is of more value than protein level in ration formulation.

The availability of an amino acid, independent of amino acid content, can vary widely among different feedstuffs and as a result of processing methods. Various methods have been devised to measure protein quality, but their usefulness even under laboratory conditions is limited. An accurate, rapid measurement of protein quality is needed to evaluate both amino acid content and amino acid availability.

Classic and Gross Determinations of Protein Quality

Several techniques for protein quality estimation may be regarded as classical methods. Thomas (1909) proposed the "biological value" approach. Osborne, Mendel and Ferry (1919) suggested what has become known as the "protein efficiency ratio." Mitchell and Block (1946) devised a "chemical score" technique.

Many other techniques have been proposed to estimate gross protein quality. Some of these include: urease determination for soybean meal (Bird et al., 1947); an index which takes into account the total gossypol content and nitrogen solubility for cottonseed meal

(Lyman, Chang and Couch, 1953); estimation of epsilon amino groups of lysine in heat treated proteins (Carpenter, 1960); a dye binding capacity for soybean meal (Moran, Jensen and McGinnis, 1963); pepsin-pancreatin digest index (Akeson and Stahman, 1964); and blood urea levels resulting from feeding a test protein (Eggum, 1970).

Determination of Amino Acid Availability

In order to evaluate amino acid availability in a living organism, some type of feeding regime must be employed. Methodology for the determination of amino acid availability generally falls into one of two categories, growth trials or fecal analyses. The growth assay procedure must meed certain criteria. Growth must be a function of the amino acid being tested; the procedure must be able to produce a constant growth response on repeated trials; a linear and parallel response must be obtained upon inclusion of graded amounts of the standard and of the test amino acid into the diet (Gutteridge, Lewis and Morgan, 1961).

Smith (1968) using crystalline amino acid diets determined standard response curves from including graded amounts of several amino acids into a low protein basal diet devoid of that amino acid. Availability of the amino acid upon feeding a test protein was then determined from the regression equation for that amino acid on growth. The validity of the availability figure was confirmed by growth trials in diets of normal protein level. Netke and Scott (1970), utilizing regression analysis and the slope-ratio technique, obtained differences in availability values of almost 10% when using different computation methods.

The use of animal growth involves several disadvantages. These include the time required, cost of crystalline amino acids, and a calculated figure is sometimes employed in the computation of availability. The time requirement is particularly critical for industrial applications. Ivy, Bragg and Stephenson (1971) and De Muelenaere, Chen and Harper (1967) criticized the use of growth trials for determining amino acid availability because differences in availability could be due to the amino acid balance of the protein fed.

Fecal analysis for the estimation of amino acid availability involves one of two basic methods. An inert marker material may be added to the feed. Feed and resulting feces are then analyzed for marker and amino acid content, and the appropriate calculations performed (Crampton, 1956). The other method directly measures the disappearance of amino acids from the gut by the difference between amino acid intake and excretion in the feces, with a correction for metabolic amino acids (Bragg, Ivy and Stephenson, 1969). Soares and Kifer (1971) have estimated amino acid availability by measuring the residual dietary amino acids in the ilia of chicks. Problems with the fecal method include the estimation of amino acids from metabolic sources, effects of feed processing which would render the amino acid undetectable, and the effect of microbial activity (De Muelenaere et al., 1967).

Amount and Effects of Endogenous Nitrogen

Todd and Stilwell (1963) and Kleiner and Orten (1966) estimate the bacterial content of dry feces to be from one-fourth to one-half of the total. Nasset (1968) has stated that digestive secretions and the shedding of mucosal cells cause a significant protein contribution in an animal. Imondi and Bird (1965) found a four- to six-fold increase in nitrogen from the feed to the duodenum in the chicken. Crompton and Nesheim (1969) estimated that endogenous nitrogen contributed approximately 50% of the exogenous nitrogen in the duck. Elwyn (1968) found the total amino acid output of the gut to be greater than the amount of protein ingested in dogs.

It has been shown in rats that at 2 hours after feed had been removed the endogenous nitrogen had extensively diluted the exogenous nitrogen and that intestinal contents resulting from different diets were relatively stable. However, several factors were mentioned which may affect these conclusions. It is not known whether digestion of endogenous and exogenous protein occurs at the same rate. Handling may be a factor which causes increased excretion of endogenous protein. The time of sampling may also be an important factor. It was suggested that at the time of sampling a large enough portion of the exogenous nitrogen may have been digested and absorbed so that endogenous nitrogen may have accounted for the major part of the total gut amino acids. Thus, similarity in amino acid content of the gut would reflect circumstances and not a homeostatic condition (Bergen and Purser, 1968).

Kuiken (1952) fed 2% succinlysulfathiazole to rats and concluded that bacteria had no significant effect on amino acid availability. However, Payne, Combs and Kifer (1968) found that intact chickens on a low protein diet showed higher amino acid availabilities than did cecectomized chickens. Salter and Coates (1971) concluded from their data that the gut microflora had a major influence on the products of protein digestion in the lower gut and excreta. It was postulated that microbial action might produce non-nutritious nitrogenous compounds which would be absorbed and thus lead to erroneously high availability figures. Yoshida et al. (1971) found the feces of conventional rabbits to contain more total amino acids per gram of nitrogen than did feces from germfree rabbits.

Plasma Amino Acid Levels and Protein Quality

Plasma amino acid (PAA) levels, which are relatively easily and quickly measured, are influenced by both dietary amino acid content and availability. Studies employing PAA levels have often been inconclusive, which may be a result of variations in feeding practices, unknown availabilities of amino acids in the reference diets, and unequal absorption rates among amino acids from different feedstuffs (Smith and Scott, 1965). Other variables influencing PAA levels are dietary protein level, dietary protein quality, duration of feeding and the growth rate (McLaughlan and Morrison, 1968).

PAA levels are also affected by fasting. Zimmerman and Scott (1967) found most of the essential amino acids to increase in chick plasma during a 3 to 24 hour fast. The length of fast used by Richardson, Cannon and Webb (1965), 18 hours, may account for their failure to correlate dietary protein quality with PAA levels in the chick. Boomgaardt and McDonald (1969) have reported that fasting PAA levels in the chick are much more variable than in the rat or pig.

The processing of protein feedstuffs affects absorption time. Wheeler and Morgan (1958) noted that rats fed raw pork showed maximal PAA levels 3/4 to 1 hour after eating, while PAA concentrations in rats fed autoclaved pork did not peak until about 5 hours after eating. Peraino and Harper (1963) found that the force-feeding of zein to rats had little effect on PAA concentrations, while force-feeding casein produced an increase in portal blood PAA levels. When hydrolyzates of these proteins were force-fed, much larger increases in PAA levels were demonstrated.

McLaughlan (1963) showed that after a meal of good quality protein PAA levels increased and tended to reflect the amount and amino acid composition of the protein fed. However, it was concluded that since other dietary constituents, such as butter and sugar, could lower PAA levels through alterations in feed passage time, the magnitude of the PAA levels was not a valid criteria for evaluating protein quality.

Numerous workers have demonstrated a general positive relationship between dietary amino acid content and PAA levels in chicks (Richardson, Blaylock and Lyman, 1953; Almquist, 1954; Dean and Scott, 1966; Smith, 1966; Tasaki and Ohno, 1971) and in the dog (Longenecker and Hause, 1959). Zimmerman and Scott (1965) have shown that the first limiting dietary amino acid remained at a constant low level in the plasma until supplied at its required amount at which time it begins to increase in concentration. The rise in PAA concentration coincided with the break in the growth curve. Young et al. (1971) found that plasma tryptophan levels in young men were constant above and below tryptophan intakes of 5 and 3 mg/kg body weight/day and changed linearly with altered tryptophan intakes within this range. From these data it was inferred that the tryptophan requirement was about 3 mg/kg body weight/day for young men. Nitrogen balance data indicated a tryptophan requirement of 2.0 to 2.6 mg/kg body weight, a range thought to be low as tryptophan losses from skin and sweat were not included.

Thus, there appears to be some degree of relationship between dietary amino acid levels and PAA levels, even though blood is only a means of transport between supply and sites of amino acid usage.

Amino Acid Imbalance

Associated with protein and amino acid metabolism is the problem of abnormal dietary amino acid levels. Harper, Benevenga and Wohlhueter (1970) make the following distinctions in terminology. An amino acid imbalance is defined as a dietary deficiency of one amino acid, with no other amino acid present in toxic amounts. The associated adverse effects can be prevented by supplementation of the limiting amino acid. An amino acid antagonism is a competition between amino acids resulting from high level of one amino acid. The adverse effects in this case may be prevented by addition of an amino acid structurally similar to the amino acid in excess. An amino acid toxicity is caused by the ingestion of large amounts of an individual amino acid and may not be reversed with any supplementation. The central result of consuming an imbalanced diet is a reduction in appetite. While much work has been done on this subject, the cause of the hypophagia is unknown.

A comprehensive review on the subject of amino acid imbalance has been compiled by Harper et al. (1970). Harper and Benevenga (1970) have proposed that the response of animals to an amino acid imbalanced diet was either an attempt to maintain normal PAA levels or a response that occured because the capacity of the body's homeostatic mechanisms had been exceeded. Harper and Benevenga (1970) suggest that PAA levels are under the control of homeostatic mechanisms since fasting PAA levels tend to be constant for an individual (Adibi, 1968), and fasting patterns within a species are similar (Munro, 1969). Also, PAA levels tend to return to a normal level following ingestion of a meal. Adibi (1968) noted during prolonged starvation that PAA levels do not fall markedly. However, these observations may indicate a shuttling of nutrients within the body to areas of greatest need under different circumstances rather than a homeostatic regulation of PAA levels per se.

Other studies employing various approaches indicate that amino acids and protein can not be stored to any appreciable extent within the body (Greiger, 1947; Mayer and Vitale, 1958; Maramatsu and Ashida, 1962; Holt, Halac and Kajdi, 1962).

Normally only very small amounts of amino acids are lost in the urine, and this loss is only slightly affected by diet (Harper, 1967). The primary means of regulating PAA levels is through degradation (Harper and Benevenga, 1970). The activity of amino acid degrading enzymes of the liver tend to adapt to protein intake (Harper and Benevenga, 1970), but only a few of these enzymes are affected by high intakes of individual amino acids (Knox and Greengard, 1965). Toshizo and Tahara (1971) concluded from their work that the specific effects of a leucine or valine deficiency depended upon the difficulty in catabolism of amino acids. Thus, when the capacities for protein synthesis and amino acid degradation have been exceeded, remaining amino acids tend to increase in concentration within body fluids and the animal will react by reducing feed intake (Harper and Benevenga, 1970).

The mechanism by which an amino acid imbalanced diet reduces appetite remains unknown. The hypothalamus has been shown to possess centers which affect appetite. Injections of balanced amino acid solutions into the area of the hypothalamus where consumatory behavior is obtained with electrical stimulation was found to inhibit feeding in rats (Panksepp and Booth, 1971). Glycine has been shown to non-specifically inhibit neural firing in cats (Werman, Davidoff and Aprison, 1967; Curtis, Hosli and Johnston, 1968a; Curtis et al., 1968b). However, rats made hyperphagic by lesions in the ventromedial area of the hypothalamus have been shown to still reduce feed intake when fed amino acid imbalanced diets (Leung and Rogers, 1970; Scharrer, Baile and Mayer, 1970). Anand, Banerjee and Chhina (1965) infused a protein hydrolysate and measured the resulting neuronal activity in the lateral and ventromedial areas of the hypothalamus. They concluded that the amino acid content of the blood did not provide a signal to the lateral or ventromedial areas of the hypothalamus for regulation of appetite. Other areas of the brain, however, do appear to be sensitive to PAA levels as demonstrated by Leung and Rogers (1969). These workers were able to prevent the reduction in appetite caused by an amino acid imbalanced diet through the infusion of the limiting amino acid into the carotid artery; infusion into the jugular vein did not increase appetite.

CHAPTER 2

EVALUATIONS OF PROTEIN QUALITY

Experimental Procedures

General Procedures

Three-week-old Hubbard broiler chicks were raised under continuous lighting in electrically heated batteries having raised wire floors. Water and feed were supplied ad libitum except during experimental regimes where stated otherwise. From the time of hatching to the experimental period, chicks were fed a 22% protein broiler starter diet balanced in all nutrients (Table 1, Appendix).

All feed that was force-fed was ground through a 30 mesh screen. Feed was weighed and transferred into the barrel of a plastic 10-ml syringe which had the needle-end drilled out to 3/16 inch and was fitted with a 5 inch rubber tube (3/16 inch inside diameter). Enough water was added into the syringe and mixed with the feed to make 8-10 mls of slurry. The plunger was used to eject the slurry once the tube had been inserted into the esophagus. All feed supplied ad libitum was ground through a 1/16 inch screen if particle size was large enough to allow feed selection.

Blood samples were taken by cardiac puncture using heparinized equipment. Each bird was bled only once. Blood plasma was combined with 20% sulfosalicylic acid in a 2:1 ratio. After mixing, samples were allowed to stand overnight under refrigeration and then were centrifuged before being analyzed on a Beckman amino acid analyzer. Plasma amino acid levels of chicks fed test proteins were compared with levels of chicks fed broiler starter under identical conditions (Table 30). Chicks in Experiment 5 were compared with chicks in Experiment 1.

Experiment 1

Chicks were fed broiler starter diet of the composition listed in Table 1. Blood samples were taken while the chicks were on feed and 0.5, 1, 2, and 3 hours after feed was removed. Blood from 5-10 chicks was pooled for analysis.

Kimber 137 hens were fed a 14.5% protein diet of the amino acid composition listed in Table 5. Hens were fed the diet for 4 days, fasted 12 hours on the fifth day, fed the diet for 1 hour and then bled after 4, 6 and 12 hours of fasting.

Experiment 2

Sixteen-day-old chicks averaging 275 grams in weight were fasted 1-3/4 hours, force-fed 5 grams of a 15% protein soybean meal-glucose monohydrate diet, and then bled at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5.25 and 6 hours of fasting. Blood from 4-11 chicks was pooled per sample.

Experiment 3

Chicks were fasted 1-3/4 hours prior to being force-fed 5 grams of diet ranging from 3-18% protein. Soybean meal was diluted with glucose monohydrate to obtain the desired protein content. One group received soybean meal (2 chicks/protein level), and another group received soybean meal supplemented with DL-methionine (3.75 gm/16 gm N), (4-6 chicks/protein level). Chicks were bled 1-3/4 hours after force-feeding.

Experiment 4

Chicks averaging 450 grams in weight were either nonfasted or fasted for 1-3/4 hours prior to force-feeding 5 grams of either a 21% protein soybean meal-glucose monohydrate diet, a 21% protein cottonseed meal-glucose monohydrate diet, or a 9% protein milo diet. Blood from six chicks per diet was taken and pooled 1-3/4 hours after feeding.

Experiment 5

Chicks were fed, ad libitum, diets of the composition listed in Table 12 for 2 days. Diets were calculated to contain 5.2 grams of protein for each 100 kcal of metabolizable energy, except for the milo diet. Chicks were bled while on feed and after 1 hour of fasting. Blood from 10 chicks was pooled per sample.

Experiment 6

Chicks were fed ground corn, soybean meal, cottonseed meal or ground milo for 1-3/4 hours without prior fasting. Soybean meal and cottonseed meal were diluted with glucose monohydrate to obtain the protein levels indicated in their respective tables. Blood samples were taken at the end of the 1-3/4 hour period from 5-21 chicks per diet and pooled.

Results and Discussion

Experiment 1: Effect of Fasting Time on Plasma Amino Acid Levels in Chicks and Hens Fed *Ad Libitum*

The initial investigations in this series were undertaken to develop guidelines for sampling times which were to be utilized in succeeding determinations of protein quality. In this experiment chicks were fed *ad libitum* and plasma amino acid (PAA) levels determined at various times of fasting. The composition of the broiler starter diet used in these experiments is listed in Table 1. The effectiveness of this diet as a reference or base line for the evaluation of protein quality would be dependent upon how well the broiler starter meets the amino acid requirements of the chick with neither exaggerated excesses nor deficiencies. Methionine and lysine are the amino acids most commonly found to be deficient in natural feedstuffs, and the broiler starter contained these amino acids and glycine in amounts very close to NRC requirements (Table 2).

Ad libitum feeding of the diet resulted in a nonfasting PAA pattern as shown in Table 3. There was a drop in all amino acids within 30 minutes after removal of the feed, and most of the essential amino acids continued to decrease in concentration through 2 hours of fasting. Between 2 and 3 hours plasma lysine increased by 50%, while threonine, valine, methionine, cystine and isoleucine appeared to increase slightly. These data suggest that no more than 2 hours of fasting are required to produce minimal values in most of the essential amino acids in chick blood plasma and that beyond this time there is a substantial rise in plasma lysine and perhaps others.

Comparisons of the differences between nonfasting and 2 hour fasting PAA values with the dietary pattern produced ratios of PAA difference:dietary amino acids (as a % of the protein) which varied from 0.9 for phenylalanine plus tyrosine to 8.1 for threonine (Table 4). Those amino acids which were just adequate by calculation showed ratios ranging between that of glycine, 7.2, and that of the sulfur amino acids, 2.9. Thus, under these conditions, there appears to be no relationship between dietary amino acids and the change in PAA level in relation to fasting. Longenecker and Hause (1959) proposed an evaluation method based on the assumption that PAA were removed at rates proportional to the requirements of the organism. However, the present experiment indicates that such relationship may not exist. Perhaps it would be more valid to relate the PAA level produced from feeding a test diet to the PAA level produced from feeding a reference diet with amino acids present in their required amounts.

Calculations of the percentage decline in PAA after 30 minutes indicated that lysine declined 45% and methionine plus cystine declined 39% (Table 4). These two amino acids were 105% and 99%, respectively, of the NRC requirements in the diet fed. Valine declined 40% and was 125% of the NRC requirement. These data suggest that this method would be inadequate for predicting the limiting amino acids in the chick. All of the amino acids measured were found to decline initially with time suggesting that the broiler starter diet developed was adequate in amino acid content and balance, since it can be presumed that they all increased as a result of <u>ad libitum</u> feeding.

In a second portion of this study the effect of fasting on PAA in the laying hen was studied. A diet adequate in essential amino acid content (Table 5) was fed ad libitum and the fasting PAA values measured. The extended period of fasting caused a continued elevation of PAA levels (Table 6). Lysine accounted for 19% and 38% at 6 and 12 hours, respectively, of the total increase in PAA levels. Other amino acids which increased during the extended fast included glycine, valine, isoleucine, phenylalanine, arginine and methionine. Values for threonine were unavailable.

Summary. The fasting PAA pattern in chicks showed a drop in all amino acids within 30 minutes after removal of the feed. Most essential amino acids continued to decrease in concentration through 2 hours of fasting. Between 2 and 3 hours several amino acids, noticeably lysine, began to increase in concentration. Studies with hens, conducted from 4-12 hours of fasting, confirmed that PAA, namely lysine, do increase in concentration beyond 4 hours of fasting. These data suggest that no more than 2 hours of fasting are required to produce minimal values in most of the essential amino acids in chick blood plasma and that beyond this time there is a substantial rise in plasma amino acids, particularly lysine.

Experiment 2: Effect of Fasting Time on Plasma Amino Acid Levels in Force-Fed Chicks

It was postulated that protein quality might be determined by force-feeding a given amount of protein and then evaluating the resulting PAA patterns. This experiment determined PAA patterns during fasting in chicks force-fed 5 grams of a 15% protein soybean meal-glucose monohydrate diet. Total PAA levels peaked ½ hour after feeding, although lysine peaked at 1½ hours and comparable values for many of the PAA were obtained at 1½ hours (Table 7). Plasma essential amino acid levels then decreased slowly and linearly until about 4 hours of fasting and rose abruptly at 5 and 6 hours. The slower rate of decline of PAA levels than was observed from the data in Table 3 may have resulted from the greater amount of digestion and absorption required as a result of force-feeding.

Summary. Chicks were force-fed a soybean meal diet and the resulting PAA levels measured through 6 hours of fasting. Total PAA levels peaked at ½ hour after feeding, although lysine peaked at 1½ hours and comparable values for many of the amino acids were obtained at 1½ hours. PAA levels rose at 5 and 6 hours of fasting.

Experiment 3: Relationship of Dietary Protein Level to Plasma Free Amino Acid Concentrations

Once conditions for the measurement of PAA levels were established, the relationship of dietary amino acid adequacy to PAA levels was studied. The evidence in the literature tends to support two hypotheses related to the relationship of PAA and dietary protein. The work of Nasset (1968) and others tends to suggest that the endogenous nitrogen and amino acid levels provide a constant medium for absorption and is often used to explain the lack of response in PAA to the feeding of different dietary sources. Other studies suggest that PAA can be correlated to dietary amounts in rats, pigs, humans and chicks under some conditions. The work of Tasaki and Ohno (1971), employing force-fed casein diets, developed four patterns in PAA changes in which some of the amino acids increased with increasing amounts of casein from 3 to 21% while others remained constant, decreased and increased only at higher protein levels.

In this experiment dietary protein levels from 3 to 18%, with soybean meal as the sole protein source, produced significant positive correlations for all essential amino acids in blood plasma except for lysine, threonine and methionine plus cystine (Table 8).

In the second series identical protein levels were fed with a supplement of DL-methionine at the level of 0.65% of the protein to provide an adequate level of the sulfur amino acids (3.75 gm/16 gm N). The sulfur amino acids showed a significant positive correlation (0.89) in the second series. These data support those of Zimmerman and Scott (1965) in that once an amino acid requirement is met, that amino acid increases in the plasma when fed in additional amounts.

The nonsignificant correlations for lysine and threonine suggest a special problem with these two amino acids. The reported variability in the fasting amino acids in the chick (Zimmerman and Scott, 1967; Boomgaardt and McDonald, 1969) suggests an explanation. The intercept values for lysine were 22.7 and 31.5 respectively, in the unsupplemented and supplemented series. The threonine values varied from 33.7 to 75.4 micromoles/100 mls for the two series indicating a disparity in the initial condition of the chicks which did not affect the majority of the essential amino acids, but could have been responsible for the erratic results obtained with lysine and threonine (Table 8).

Summary. When a soybean meal diet was force-fed, all plasma essential amino acids except lysine, threonine and methionine plus cystine were significantly correlated with protein level. When soybean meal was supplemented with DL-methionine, methionine plus cystine also became significantly correlated. Lysine and threonine showed erratic plasma values which appeared to explain their lack of correlation. These data demonstrate that PAA levels can reflect the amount of protein fed once an amino acid has been supplied at a level to meet its requirement in relation to the other amino acids present.

Experiment 4: Effect of Fasting on Plasma Amino Acid Levels in Chicks Force-Fed Various Protein Sources

Since a direct relationship was demonstrated between amino acid level in the diet and that in the plasma, the evaluation of protein quality by the use of PAA was investigated. For this purpose Dean and Scott (1966) proposed computing a ratio between PAA levels resulting from feeding a test protein and levels obtained from feeding a standard diet. This method would require that the standard diet contain no excesses in the essential amino acids to be detected. The broiler starter diet from Experiment 1, just adequate in lysine, glycine and the sulfur amino acids (methionine and cystine), was used as the standard diet and could not be expected to be valid for detection of other amino acids as limiting due to the excesses it contains. Under these conditions protein sources of known amino acid deficiencies were tested. Lysine, glycine, or the sulfur amino acid group was considered as being most deficient in a protein when calculated as most limiting and subsequently produced the lowest PAA ratio when the test diet was fed.

Soybean meal is most deficient in the sulfur amino acids. These amino acids showed the lowest ratio when PAA levels of chicks fed soybean meal were compared with levels of chicks fed broiler starter in both nonfasted and fasted chicks (Table 9). These results were produced in spite of the elevated levels of lysine and threonine in the fasted chicks which would appear to indicate undesirable experimental conditions.

Fasting prior to force-feeding showed little effect on PAA levels in chicks fed cottonseed meal (Table 10). Lysine was calculated to be most limiting in this protein. When plasma values of control chicks for this particular experiment were used to compute PAA ratios, lysine could not be demonstrated as the first limiting amino acid. This appeared to result from the abnormally low lysine values in chicks fed the reference diet. When average PAA values for control chicks (Table 30) were employed, lysine, glycine and the sulfur amino acids showed ratios of 0.81, 0.80 and 0.66 respectively in nonfasted birds and 0.77, 0.88 and 0.81 respectively in fasted birds. By this method, lysine was shown to be first limiting in fasted chicks but not in nonfasted.

Fasting prior to force-feeding showed little effect on PAA levels in chicks fed milo (Table 11). Lysine was calculated to be first limiting with glycine present at 90% of its requirement. When PAA levels of chicks fed milo were compared with levels of control chicks for this experiment, lysine could be demonstrated most limiting in nonfasted but not fasted chicks. An average of reference plasma amino acid levels (Table 30) was employed due to the low lysine values in control birds from this particular experiment. With these values lysine was shown most limiting in both nonfasted and fasted birds.

Summary. Force-feeding a given amount of diet prior to measuring PAA values generally allowed for detection of the limiting dietary amino acid in soybean meal, cottonseed meal and milo by the use of PAA ratios. Fasting 1-3/4 hours prior to force-feeding did not increase the accuracy of the method.

Experiment 5: Effect of Dietary Amino Acid Composition on Plasma Amino Acid Levels in Chicks Fed *Ad Libitum*

It was postulated that allowing *ad libitum* feed consumption might produce similar results as force-feeding. This would require much less effort and larger numbers of birds could be utilized.

Diets were formulated with either dried whole egg, soybean meal, cottonseed meal or milo as the sole protein sources diluted with glucose monohydrate to equalize the calorie-protein ratio, except for the milo diet (Table 12). The protein content of these mixtures was not equalized.

Chicks in this study were fed the respective mixtures for 2 days and blood samples drawn while the birds were on feed and after a 1 hour fast. The amount of feed consumed over the 2 day period varied widely among the groups of chicks and indicated some overconsumption of energy in order to compensate for the amino acid inadequacies of the protein sources fed. The soybean meal diet, with a 15% deficiency in sulfur amino acids, was consumed at a rate of 124 kcal metabolizable energy (M.E.)/chick/day (Table 13). The cottonseed meal diet resulted in a consumption of 160 kcal M.E./chick/day with a calculated deficiency of 29% in lysine. Chicks fed the whole egg diet, 50% deficient in glycine, consumed an average of 182 kcal M.E./chick/day. The lysine content of milo represents 51% of the required amount and at the lower protein level appears to have created an imbalance resulting in decreased appetite, since chicks fed this diet consumed only 37.1 grams protein/chick/day.

In an effort to identify the limiting dietary amino acid from PAA data the following observations were made. Chicks fed milo definitely consumed inadequate amounts of protein as PAA levels were low (Table 13), and no amino acid changed much during a 1 hour fast (Table 14). Thus, the decline in PAA during fasting could not be used to identify the limiting amino acid. Ratios of the PAA levels of chicks fed milo to PAA levels of chicks fed broiler starter indicated lysine and the sulfur amino acids to be limiting (Table 14). From standard table values lysine was calculated to be first limiting while methionine plus cystine was calculated to be adequate. It is not unreasonable that this method failed to detect the limiting amino acid since nutrient intake was so low that PAA levels virtually reflected a fasting condition rather than the diet. Gan and Jeffay (1967) have shown in normally fed rats that liver protein catabolism contributed about 50% of the liver's intracellular amino acid pool, and muscle protein degradation contributed 30% of its intracellular amino acid pool. These percentages increase to as high as 90% and 65% for liver and muscle, respectively, during early stages of fasting (3 days).

The sum of the essential amino acids in the plasma of chicks fed soybean meal equalled that of chicks fed broiler starter (Table 13). However, the levels of lysine and threonine were higher than in chicks fed the broiler starter diet and caused the sum of essential amino acids in the plasma to show a distorted picture. Amino acids declined little during the 1 hour fast for chicks fed soybean meal, and the PAA decrease during fasting would not be expected to show the limiting amino acid. Chicks fed soybean meal did ingest almost twice as much protein as chicks fed milo, and PAA levels of these chicks compared with levels in chicks fed broiler starter produced ratios which indicated the sulfur amino acids to be limiting; these amino acids are calculated to be first limiting from standard table values (Table 15).

Chicks fed cottonseed meal showed a PAA level intermediate between those of chicks fed milo and broiler starter. The average essential PAA declined little during a 1 hour fast compared with chicks fed broiler starter (Table 13). More protein was ingested by chicks fed cottonseed meal than by chicks fed soybean meal in which PAA ratios to broiler starter did identify the most limiting amino acid. Lysine is calculated to be most limiting in cottonseed meal and it appears that birds consumed sufficient feed for the lysine level in the plasma to rise and then show the greatest decrease, 33%, during a 1 hour fast (Table 16). This data shows that when the limiting amino acid is allowed to increase in plasma concentration, it will show the most rapid decrease during a fasting period. The ratio of PAA levels of chicks fed cottonseed meal compared with chicks fed broiler starter was high for lysine, 1.14.

Chicks fed dried whole egg evidenced an elevated sum of non-fasting PAA levels and a decrease in these values during a 1 hour fast comparable to those of chicks fed broiler starter (Table 13). While chicks fed dried whole egg consumed 9.7 gms protein/chick/day, there is a 50% deficiency of glycine in egg which would make only 4.8 grams of protein utilizable for protein synthesis. However, it is suggested that serine was helping to meet the glycine requirement, allowing a normal feed consumption and drop in PAA level with fasting to occur. The level of plasma glycine did not become elevated to any extent, and PAA ratios comparing chicks fed dried whole egg to chicks fed broiler starter showed glycine to have the lowest ratio, 0.68, of the three amino acids capable of being detected by use of the broiler starter control diet (Table 17).

Summary. PAA ratios allowed detection of the limiting dietary amino acid in chicks fed ad libitum for 2 days provided feed consumption was adequate. A very low level of protein intake, as in chicks fed milo, resulted in PAA levels which responded little with feeding. Chicks fed soybean meal consumed larger amounts of protein and PAA levels were increased to an extent that reflected dietary amino acid composition, and comparison with the broiler starter diet proved to be the best means of estimating the limiting amino acid.

Chicks fed cottonseed meal consumed greater quantities of protein than did chicks fed soybean meal, and the decrease in PAA levels during a 1 hour fast identified the limiting amino acid as lysine. Chicks fed dried whole egg consumed amounts of protein so that the PAA level of the limiting amino acid increased only slightly during feeding and PAA ratios comparing chicks fed dried whole egg with chicks fed broiler starter predicted glycine as limiting.

Experiment 6: Effect of Dietary Amino Acid Composition on Plasma Amino Acid Levels in Chicks Fed for 1-3/4 Hours

This experiment attempted to determine protein quality under conditions of short-term feed consumption. Animals normally eat to satisfy energy requirements, but differences in individual protein sources, such as amino acid balance and protein level, can override energy needs and result in abnoramlly low consumption of nutrients. As seen in Table 3, PAA levels returned to fasting values approximately 2 hours after feeding. When a protein source was consumed in inadequate amounts, feeding for longer than this period allowed endogenous amino acids to mask those from dietary protein sources. Allowing chicks to feed ad libitum for 1-3/4 hours prior to sampling should allow PAA to reflect amino acid composition of the test diet while producing a minimal effect from the previous diet, endogenous amino acids and dietary amino acid balance.

Corn was calculated to be first limiting in lysine, followed by glycine (Table 18). PAA levels of chicks fed corn compared with levels of chicks fed broiler starter showed that of the detectable amino acids lysine had the lowest ratio followed by glycine. This pattern indicates that lysine is first limiting and glycine second limiting in corn.

Soybean meal was calculated to be first limiting in the sulfur amino acids and glycine second limiting (Table 19). Ratios comparing the PAA levels of chicks fed soybean meal with levels of chicks fed broiler starter showed the lowest value for methionine plus cystine. Glycine failed to exhibit the second lowest ratio, and the data suggest lysine as second limiting in soybean meal, although comparisons with composition data show a 26% excess in this amino acid.

Chicks were next allowed to consume cottonseed meal diets of three different protein levels varying from 41% protein to 15% protein. Cottonseed meal was calculated to be most deficient in lysine (Table 21). PAA levels of chicks fed the 41% protein cottonseed meal diet compared with levels of chicks fed broiler starter produced ratios for all amino acids above 1.00, indicating an overconsumption of protein in relation to broiler starter. Under conditions of excess protein consumption, this failure in detection of the limiting amino acid was not unreasonable. When the protein content was reduced to 21% or 15%, lysine showed the lowest ratio of the detectable amino acids in relation to broiler starter. The 15% protein level more accurately reflected the sequence of limiting amino acids than did the 21% protein level. Chicks fed the 21% protein diet consumed only 84% of the protein consumed by chicks fed broiler starter. Chicks fed the 15% protein diet consumed 70% of the amount of protein consumed by chicks fed broiler starter. That the lower protein diet (15%) more accurately reflected dietary amino acid deficiencies would seem to indicate that protein intakes must be maintained below the requirement in order for the plasma patterns to provide a meaningful ratio with the broiler starter control.

Although cottonseed meal was calculated to be deficient in leucine, the low PAA ratio for this amino acid can not be taken as an indication of deficiency due to the excess of this amino acid found in the broiler starter diet. Leucine and isoleucine were present in the broiler starter at approximately 35% above their requirement and in cottonseed meal at 73% and 92% respectively, of the required level. The PAA ratio to broiler starter was lower for isoleucine than for leucine, indicating the need for the lack of excesses in the standard reference diet.

Chicks were next fed commercial milo in two trials. Lysine was calculated to be first limiting with glycine present at 90% of its required amount (Table 22). The sulfur amino acids were calculated to be present in excess. In each trial lysine showed the lowest PAA ratio when chicks fed milo were compared with chicks fed broiler starter. Glycine showed a PAA ratio higher than that for lysine, and the sulfur amino acids introduced a ratio between the two, indicating that only the first limiting amino acid was determined under these conditions.

Chicks fed another variety of milo (NSA-940) showed PAA ratios for lysine and glycine which were 0.55 and 0.61 respectively, indicating that lysine was first limiting (Table 23).

With the present reference diet only lysine, glycine, and the sulfur amino acids could be shown to be limiting. A reference diet containing no excesses of essential amino acids would allow any essential amino acid to be detected as limiting from the lowest PAA ratio when a test protein was compared with it.

Summary. PAA levels of chicks fed corn, soybean meal, cottonseed meal or milo for 1-3/4 hours were compared with PAA levels of chicks fed broiler starter. As long as the protein content of the test diet was held below the requirement, this method of comparison allowed for the detection of the most limiting amino acid in these four protein sources.

CHAPTER 3

AMINO ACID IMBALANCE IN CHICKS

In prior experiments it has been shown that plasma amino acid (PAA) levels can reflect dietary composition. This experiment investigated whether or not amino acid values in plasma and also liver, thigh muscle or breast muscle could show altered amino acid patterns which might result in decreased appetite.

Experimental Procedures

Leghorn cockerels were raised under continuous lighting in electrically heated batteries having raised wire floors. Water and feed were supplied *ad libitum*. Chicks were fed a 22% protein broiler starter diet balanced in all nutrients (Table 1) from the time of hatching to the experimental period, during which they received diets supplemented with various amino acids to create either an imbalance or a toxicity (Table 24). Blood samples, taken by cardiac puncture using heparinized equipment, were taken from seven chicks per diet and pooled after chicks had been on the experimental diets for 1 day. Blood plasma was combined with 20% sulfosalicylic acid in a 2:1 ratio. Muscle and liver samples were taken from seven chicks per diet and pooled after being on the experimental diets for 1 week. These samples were prepared for analysis by combining 1.5 grams of wet tissue, 10 mls of distilled water and 1 ml of 20% sulfosalicylic acid and then homogenizing. All samples were mixed vigorously, allowed to stand overnight under refrigeration, and then centrifuged before analysis on a Beckman amino acid analyzer.

Results and Discussion

Chicks were fed diets supplemented with various amino acids to create either an imbalance or a toxicity (Table 24). Both situations will hereafter be referred to as an imbalance. The effects of these diets on feed consumption and changes in body weight are listed in Table 25. Chicks fed the methionine deficient diet consumed 62% of the amount consumed by chicks fed the 6% protein control diet, while the addition of 5% DL-leucine produced an even greater reduction in appetite. Chicks fed the 57% protein diet consumed slightly less than chicks fed the 16% protein diet over the 1 week period. Anderson, Benevenga and Harper (1968) found that rats fed high protein diets, following an initial marked decrease in appetite, consumed approximately normal amounts after being fed test diets for 1 week.

It appears that chicks were able to metabolize large amounts of nitrogen. Assuming chicks can utilize up to 23% protein in the diet, chicks fed the 57% protein diet received an excess of 34% protein or 0.56 grams of nitrogen per day to be metabolized for other than protein synthesis. Chicks fed either the methionine deficient or the high leucine diet received about 0.02 grams excess nitrogen per day. The problem therefore, as the name of the syndrome implies, concerns an imbalance, or toxicity, of amino acids. Changes in body weight paralleled feed consumption. The amounts consumed by chicks fed the imbalanced diets were insufficient to maintain body weight.

Chicks fed the imbalanced diets showed higher levels of liver free amino acids than chicks fed the 6% balanced protein diet (Table 26). Neither the leucine excess, nor the methionine deficiency, was reflected in liver amino acid levels. Chicks fed the high leucine diet generally showed higher liver amino acid levels than did chicks fed either the methionine deficient diet or the 16% balanced protein diet. Liver amino acid levels of chicks fed the 57% protein diets tended to approximate those of chicks fed the 16% protein diet. The elevated liver amino acid levels in chicks fed the imbalanced protein diets compared with chicks fed the 6% balanced diet indicate the expected reduction in protein synthesis.

While plasma amino acid levels of chicks fed imbalanced diets reflected amino acid deficiencies and supplementations, amino acid values mainly tended to reflect dietary protein level (Table 27). Plasma amino acid levels resulting from feeding the imbalanced diets were generally similar to levels of chicks fed the 6% protein control diet with the exception of lower methionine and higher glycine levels in the plasma of chicks fed the methionine deficient diet. In chicks fed the high leucine diet, the plasma level of leucine was greatly elevated while levels of valine and isoleucine were depressed. McLaughlan and Morrison (1968) noted relationships among the branched-chain amino acids. Administration of a single load of leucine resulted in an elevated level of leucine and depressed levels of amino acids such as valine and isoleucine in the plasma, and it was concluded that the observed changes in plasma were not readily explained as the result of competition in intestinal transport systems as the load was given on an empty stomach. Since amino acid levels in the urine and red blood cells paralleled those in plasma, it was suggested that an excess of a single amino acid stimulates common pathways of transport or of degradation.

Harper and Benevenga (1970), citing relationship between the activity of amino acid degrading enzymes of the liver and protein intake, postulated that reduced activities of enzymes that catabolize amino acids are the basis for the altered PAA patterns resulting from consuming low protein diets imbalanced in amino acid content.

The free amino acids in thigh muscle of chicks fed the imbalanced diets did not appear to vary greatly from the levels found in chicks fed the 6% protein control diet, except for lower levels of histidine (Table 28). This suggests limited tissue catabolism since protein that is necessary to maintain posture may be more essential than some other body proteins. Free amino acid levels in thigh muscle reflected both the methionine deficiency and the leucine excess in chicks fed those respective diets. However, the lower methionine level does not appear to be critical since even lower levels of that amino acid were noted in chicks fed the 57% and 16% protein diets.

The free essential amino acids in breast muscle of chicks fed the imbalanced diets were generally higher than the levels found in chicks fed the 6% balanced protein diet with the exception of lower levels of histidine (Table 29). The cause of these low levels is unknown. Of the two imbalanced diets the high leucine diet tended to produce greater concentrations of essential amino acids in breast muscle. The high level in chicks fed the imbalanced diets appears to indicate accelerated tissue catabolism to supply nutrients for more critical areas of the body. The high level of leucine, but not the deficiency of methionine, was reflected in the breast muscle of chicks fed the respective diets. Harper and Benevenga (1970) postulate that the effects of an amino acid imbalance or toxicity are due to the accumulation of amino acids in body fluids. The high levels of leucine noted in all tissues examined and the accompanying decrease in appetite appears to indicate a toxicity of leucine. However, the underlying basis for the adverse effects of not a single amino acid has been extablished.

Summary

The dietary deficiency of methionine was reflected in the free amino acid levels in plasma and thigh muscle. The dietary excess of leucine was reflected in the free amino acid levels in plasma, thigh muscle and breast muscle. The toxicity of high amounts of leucine may explain the reduced appetite accompanying ingestion of this diet. Free amino acid levels in livers of chicks fed imbalanced diets were higher than in chicks fed the 6% control diet, indicating the reduction in protein synthesis in these chicks. Plasma amino acid levels of chicks fed the high leucine diet demonstrated relationships among several branched-chain amino acids. In no liver or muscle tissue of chicks fed imbalanced diets did there appear to be an amino acid pattern variant enough to explain the decreased appetite, with the

exception of very low levels of histidine. With this exception, free amino acid levels in thigh muscle did not appear to vary greatly from the levels in chicks fed the 6% protein control diet. In breast muscle of chicks fed imbalanced diets, free amino acid levels were generally higher than the levels found in chicks fed the 6% balanced protein diet, suggesting increased tissue catabolism.

APPENDIX A

DATA -- CHAPTER 2

Table 1. Composition of broiler starter diet.

Ingredient	%
Milo, ground	56.55
Soybean meal, dehulled (48.5% protein)	31.25
Meat and bone scraps (50% protein)	2.50
Alfalfa Meal, dehydrated (17% protein)	2.00
Animal fat	2.50
Calcium carbonate	0.75
Dicalcium phosphate	1.25
Salt	0.50
Trace mineral mix[1]	0.10
Vitamin mix[2]	2.50
DL-methionine	0.10

1. Supplied the following in PPM of diet: 20 Fe, 60 Zn, 1 Mo, 60 Mn, 168 Ca, 4 Cu, 1.5 I, 1.5 Co.

2. Supplied the following per pound of diet: 4,500 IU vitamin A, 700 ICU vitamin D_3, 2.0 mg riboflavin, 12.5 mg niacin, 5.0 mg d-calcium pantothenate, 400 mg choline chloride, 6 mcg vitamin B_{12}, 2.5 IU vitamin E, 1.0 mg vitamin K.

Table 2. Amino acid composition of the broiler starter diet in comparison with NRC amino acid requirements.[1]

Amino acid	Percent of the protein		
	Broiler starter	Requirement	Broiler starter: Requirement ratio
Lysine	5.80	5.50	1.05
Histidine	2.44	2.00	1.22
Arginine	7.19	6.00	1.20
Threonine	3.95	3.50	1.13
Glycine	5.12	5.00	1.02
Valine	5.32	4.25	1.25
Methionine + cystine	3.72	3.75	0.99
Isoleucine	5.04	3.75	1.34
Leucine	9.58	7.00	1.36
Phenylalanine + tyrosine	9.70	6.50	1.49

1. National Research Council, Publication 1345, 1966.

Table 3. Fasting plasma amino acid levels of chicks previously fed broiler starter *ad libitum* (Experiment 1).

Amino acid	Nonfasting	Hours of fast			
		0.5	1.0	2.0	3.0
	micromoles/100 mls plasma				
Essential					
Lysine	56.8	31.2	26.3	27.2	40.8
Histidine	31.0	23.7	21.3	19.6	19.2
Arginine	50.8	34.5	28.7	24.0	23.6
Threonine	114.4	95.1	95.8	82.3	85.1
Glycine	90.9	60.2	62.4	53.8	45.0
Valine	35.7	21.3	20.8	16.3	18.9
Methionine	11.2	6.3	5.2	4.5	5.7
Cystine	14.5	9.5	12.2	10.3	13.4
Isoleucine	20.9	13.1	13.0	9.9	11.8
Leucine	36.6	25.3	24.3	21.2	20.6
Phenylalanine	17.7	11.4	11.0	11.8	11.2
Tyrosine	24.5	20.7	18.6	21.7	19.8
Total	505.0	352.3	339.6	302.5	315.1
Nonessential					
Aspartic acid	38.0	16.6	9.8	11.7	8.7
Serine	229.4	180.5	152.9	164.0	161.4
Glutamic acid	23.6	21.6	23.2	25.2	26.3
Proline	69.0	40.5	36.9	37.1	34.0
Alanine	110.2	72.0	60.1	64.5	62.1
Total	470.2	331.2	282.9	302.5	292.5

Table 4. Changes in plasma amino acid levels in chicks fed broiler starter and their relationship to dietary amino acid level (Experiment 1).

Amino acid	Ratio of amino acid level Nonfasting minus 2 hr plasma:Diet	% decline in plasma amino acid in 30 min
Lysine	5.1	45.1
Histidine	4.7	23.5
Arginine	3.7	32.0
Threonine	8.1	16.9
Glycine	7.2	33.7
Valine	3.6	40.3
Methionine + cystine	2.9	38.5
Isoleucine	2.2	37.3
Leucine	1.6	30.8
Phenylalanine + tyrosine	0.9	23.9

Table 5. Amino acid composition of the hen diet used in Experiment 1 in comparison with amino acid requirements.[1]

	Percent of the protein		
Amino acid	Diet	Requirement	Diet:Requirement ratio
Lysine	5.52	3.33	1.66
Histidine	2.35	1.33	1.77
Arginine	5.52	5.33	1.04
Threonine	4.10	2.67	1.54
Glycine	5.49	4.54	1.21
Valine	5.78	4.24	1.36
Methionine (excluding cystine)	2.22	1.87	1.19
Isoleucine	5.04	3.33	1.51
Leucine	10.65	8.00	1.33
Phenylalanine (excluding tyrosine)	5.04	3.33	1.55

1. Requirements are those of Smith (1965) for amino acids for which the National Research Council (NRC) lists no value. All other requirements are those given by NRC, Publication 1345, 1966.

Table 6. Plasma amino acid patterns in fasting hens (Experiment 1).

	Hours of fast		
	4	6	12
Ratio of the sum of the essential amino acids in the plasma (4 hour value taken as 1.00)	1.00	1.20	1.33
% of increase in plasma amino acids due to lysine	--	19.30	38.30

Table 7. Fasting plasma amino acid levels in chicks previously force-fed a soybean meal diet (Experiment 2).

Amino acid	Hours of fast								
	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.2	6.0
	micromoles/100 mls plasma								
Essential									
Lysine	43.2	46.2	52.3	46.2	48.4	48.2	36.7	49.1	54.2
Histidine	14.3	13.5	14.3	13.2	12.5	10.4	9.6	10.1	10.1
Arginine	37.9	30.6	37.7	32.8	53.3	30.3	29.4	34.5	35.1
Threonine	98.9	81.3	92.9	42.9	68.0	71.0	61.9	100.3	119.9
Glycine	50.0	49.4	47.8	35.8	50.9	46.5	46.9	50.8	47.3
Valine	21.1	17.5	17.5	11.5	19.4	18.7	18.4	21.6	19.6
Methionine	7.9	12.1	8.7	4.1	5.1	6.8	5.3	7.3	8.3
Cystine	15.7	9.9	9.0	3.9	6.4	7.3	7.1	9.5	8.4
Isoleucine	14.6	--	13.7	10.4	16.2	14.8	14.7	15.6	14.1
Leucine	21.2	--	19.0	13.2	20.6	19.0	19.1	19.4	17.5
Phenylalanine	9.0	--	9.4	6.7	10.5	9.0	8.5	9.1	8.1
Tyrosine	16.6	--	12.2	9.5	15.9	13.4	15.2	18.0	15.5
Total	350.3	--	334.5	230.2	309.2	295.4	272.8	345.3	358.1
Nonessential									
Aspartic acid	4.1	4.4	5.0	3.3	4.4	5.0	4.1	3.3	2.8
Serine	98.3	75.8	92.7	55.3	74.1	69.9	69.0	80.4	79.7
Glutamic acid	23.8	20.1	23.0	14.8	20.5	17.5	19.4	17.3	16.0
Proline	36.8	30.4	12.6	19.4	26.2	23.6	23.6	28.9	23.1
Alanine	55.1	55.5	55.2	37.3	52.6	43.4	44.3	50.2	54.5
Total	218.1	186.2	188.5	130.1	177.8	159.4	160.4	180.1	176.1

Table 8. Effect of methionine supplementation on correlation coefficients and regression equations of plasma amino acid levels (y) and dietary protein levels in chicks fed soybean meal (SBM, Experiment 3).

	Correlation coefficient		Regression equation y (micromoles/100 mls) =	
	SBM	SBM+met.	SBM	SBM+met.
Lysine	0.65	0.71	1.19X + 22.7	0.74X + 31.5
Histidine	0.84^{a}[1]	0.94^{a}	0.35X + 8.0	0.31X + 9.9
Arginine	0.80^{a}	0.99^{a}	1.36X + 15.6	1.51X + 15.7
Threonine	0.67	0.08	1.79X + 33.7	0.25X + 75.4
Glycine	0.90^{a}	0.98^{a}	1.11X + 33.7	0.88X + 30.8
Valine	0.91^{a}	0.90^{a}	0.62X + 13.2	0.66X + 9.7
Methionine + cystine	0.57	0.89^{a}	0.10X + 12.0	0.23X + 14.7
Isoleucine	0.93^{a}	0.95^{a}	0.45X + 9.3	0.52X + 7.9
Leucine	0.89^{a}	0.88^{a}	0.52X + 16.2	0.52X + 11.9
Phenylalanine + tyrosine	0.77^{a}	0.77^{a}	0.55X + 14.5	0.27X + 18.5

[1] Correlations showing a superscript are statistically significant at the 0.05 level of probability.

Table 9. Effect of fasting on plasma amino acid levels and ratios in chicks force-fed soybean meal (SBM, Experiment 4).

Amino acid	Plasma amino acid levels				Amino acids as a % of the protein
	Pretreatment		SBM:Broiler starter ratio		SBM:Requirement[1] ratio
	Nonfasted birds	Fasted birds	Nonfasted birds	Fasted birds	
	micromoles/100 mls plasma				
Essential					
Lysine	41.7	49.6	0.86	1.02	1.26
Histidine	17.1	18.9	0.84	1.05	1.37
Arginine	49.5	60.4	0.89	1.12	1.40
Threonine	86.7	101.7	0.75	0.99	1.17
Glycine	50.7	54.2	0.74	0.77	0.92
Methionine	7.7	8.3	0.50	0.56	0.70
Cystine	9.5	11.6	0.66	0.78	0.98
Methionine + cystine	17.2	19.9	0.58	0.67	0.85
Valine	27.4	28.1	0.73	0.85	1.25
Isoleucine	19.4	20.5	0.84	1.01	1.39
Leucine	26.6	25.7	0.74	0.84	1.20
Phenylalanine	12.2	11.8	0.81	0.92	1.44
Tyrosine	18.6	18.7	0.97	1.15	1.68
Phenylalanine + tyrosine	30.8	30.5	0.90	1.05	1.55
Nonessential					
Aspartic acid	3.4	4.2	--	--	--
Serine	110.4	131.4	--	--	--
Glutamic acid	22.7	22.9	--	--	--
Proline	38.3	39.9	--	--	--
Alanine	56.1	64.0	--	--	--

[1] National Research Council, Publication 1345, 1966.

Table 10. Effect of fasting on plasma amino acid levels and ratios in chicks force-fed cottonseed meal (CSM, Experiment 4).

Amino acid	Plasma amino acid levels				Amino acids as a % of the protein
	Pretreatment		CSM:Broiler starter ratio		CSM:Requirement[1] ratio
	Nonfasted birds	Fasted birds	Nonfasted birds	Fasted birds	
	micromoles/100 mls plasma				
Essential					
Lysine	48.1	45.9	0.99	0.94	0.71
Histidine	20.7	19.2	1.01	1.07	1.20
Arginine	70.8	67.4	1.28	1.24	1.22
Threonine	106.5	70.7	0.92	0.69	0.84
Glycine	53.1	58.4	0.78	0.83	0.93
Methionine	7.9	8.3	0.51	0.56	0.86
Cystine	10.8	14.4	0.75	0.97	1.39
Methionine + cystine	18.7	22.7	0.63	0.77	1.04
Valine	27.7	27.8	0.74	0.84	1.07
Isoleucine	14.6	13.9	0.63	0.68	0.92
Leucine	23.8	25.7	0.67	0.84	0.73
Phenylalanine	13.6	13.6	0.90	1.06	1.32
Tyrosine	19.0	20.5	0.99	1.26	1.26
Phenylalanine + tyrosine	32.6	34.1	0.95	1.17	1.29
Nonessential					
Aspartic acid	3.3	3.9	--	--	--
Serine	117.2	117.6	--	--	--
Glutamic acid	22.6	22.1	--	--	--
Proline	34.6	31.5	--	--	--
Alanine	60.3	63.2	--	--	--

1. National Research Council, Publication 1345, 1966.

Table 11. Effect of fasting on plasma amino acid levels and ratios in chicks force-fed commercial milo (Experiment 4).

Amino acid	Plasma amino acid levels				Amino acids as a % of the protein
	Pretreatment		Milo:Broiler starter ratio		
	Nonfasted birds	Fasted birds	Nonfasted birds	Fasted birds	Milo:Requirement[1] ratio
	micromoles/100 mls plasma				
Essential					
Lysine	29.4	31.4	0.60	0.64	0.51
Histidine	15.5	13.2	0.76	0.73	0.96
Arginine	33.0	26.0	0.60	0.48	0.67
Threonine	78.2	84.6	0.67	0.82	1.09
Glycine	43.8	37.6	0.64	0.53	0.90
Methionine	7.4	7.7	0.48	0.52	0.90
Cystine	11.6	10.5	0.80	0.70	1.22
Methionine + cystine	19.0	18.2	0.64	0.62	1.05
Valine	20.5	17.3	0.54	0.52	1.35
Isoleucine	12.9	11.8	0.56	0.58	1.37
Leucine	30.4	26.1	0.85	0.85	2.09
Phenylalanine	9.6	8.7	0.64	0.68	1.64
Tyrosine	12.8	15.9	0.67	0.98	1.46
Phenylalanine + tyrosine	22.4	24.6	0.65	0.84	1.56
Nonessential					
Aspartic acid	3.2	3.0	--	--	--
Serine	110.0	105.8	--	--	--
Glutamic acid	23.5	19.8	--	--	--
Proline	38.8	31.0	--	--	--
Alanine	54.9	51.5	--	--	--

[1] National Research Council, Publication 1345, 1966.

Table 12. Composition of test diets used in Experiment 5.

Ingredient	Diet: Dried whole egg	Diet: Soybean meal	Diet: Cottonseed meal	Diet: Milo
	%	%	%	%
Dried whole egg	45.6	--	--	--
Soybean meal	--	32.3	--	--
Cottonseed meal	--	--	35.8	--
Milo, ground	--	--	--	99.0
Glucose monohydrate	54.4	67.0	63.5	--
Animal fat	--	0.7	0.7	1.0
Diet				
Protein, %	21.4	15.7	14.7	8.9
Metabolizable energy, kcal/gm	4.02	3.08	2.90	3.30

Table 13. Nutrient consumption and resulting amounts of plasma amino acids in chicks fed various protein sources for two days (Experiment 5).

Protein source	Consumed/chick/day			Sum of essential amino acids in plasma		Av. % decline of essential amino acids in plasma during 1 hr fast
	Grams feed	Kcal M.E.	Grams protein	Nonfasting	1 hr fast	
				micromoles/100 mls plasma		
Broiler starter	--	--	--	505	340	35
Milo	37.1	122	3.3	292	269	7
Soybean meal	40.4	124	6.3	533	479	10
Cottonseed meal	55.0	160	8.1	366	300	6
Dried whole egg	45.4	182	9.7	538	424	28

Table 14. Plasma amino acid levels and ratios in chicks fed milo *ad libitum* for two days (Experiment 5).

Amino acid	Non-fasting	1 hour fast	1 hour fast:Non-fast ratio	Nonfasting plasma amino acid levels Milo:Broiler starter ratio	Amino acids as a % of the protein Milo:Requirement[1] ratio
	micromoles/100 mls plasma				
Essential					
Lysine	23.6	26.5	1.12	0.42	0.51
Histidine	17.3	16.1	0.93	0.56	0.96
Arginine	11.3	11.6	1.03	0.22	0.67
Threonine	72.2	70.9	0.98	0.63	1.09
Glycine	68.7	56.0	0.82	0.76	0.90
Methionine	4.4	4.0	0.91	0.39	0.90
Cystine	6.0	5.2	0.87	0.41	1.22
Methionine + cystine	10.4	9.2	0.88	0.41	1.05
Valine	15.7	13.3	0.85	0.44	1.35
Isoleucine	9.0	8.0	0.89	0.43	1.37
Leucine	28.1	23.1	0.82	0.77	2.09
Phenylalanine	11.6	10.4	0.90	0.66	1.64
Tyrosine	23.6	23.6	1.00	0.96	1.46
Phenylalanine + tyrosine	35.2	34.0	0.96	0.83	1.56
Nonessential					
Aspartic acid	5.7	4.3	0.75	--	--
Serine	235.4	202.2	0.86	--	--
Glutamic acid	26.1	25.8	0.99	--	--
Proline	46.7	37.3	0.80	--	--
Alanine	104.9	80.5	0.77	--	--

1. National Research Council, Publication 1345, 1966.

Table 15. Plasma amino acid levels and ratios in chicks fed soybean meal (SBM) *ad libitum* for two days (Experiment 5).

Amino acid	Non-fasting	1 hour fast	1 hour fast:Non-fast ratio	Nonfasting plasma amino acid levels SBM:Broiler starter ratio	Amino acids as a % of the protein SBM:Requirement[1] ratio
	micromoles/100 mls plasma				
Essential					
Lysine	146.3	138.0	0.94	2.58	1.26
Histidine	23.1	16.9	0.73	0.74	1.37
Arginine	24.4	18.7	0.77	0.48	1.40
Threonine	139.9	132.2	0.94	1.22	1.17
Glycine	91.8	73.0	0.80	1.01	0.92
Methionine	3.6	3.2	0.89	0.32	0.70
Cystine	5.2	6.0	1.15	0.36	0.98
Methionine + cystine	8.8	9.2	1.04	0.34	0.85
Valine	28.7	28.6	1.00	0.80	1.25
Isoleucine	17.3	16.1	0.93	0.82	1.39
Leucine	22.5	21.9	0.97	0.61	1.20
Phenylalanine	11.6	10.0	0.86	0.66	1.44
Tyrosine	18.6	14.8	0.80	0.76	1.68
Phenylalanine + tyrosine	30.2	24.8	0.82	0.72	1.55
Nonessential					
Aspartic acid	6.2	4.3	0.69	--	--
Serine	199.6	160.9	0.81	--	--
Glutamic acid	30.3	24.1	0.80	--	--
Proline	38.9	32.4	0.83	--	--
Alanine	83.8	59.7	0.71	--	--

1. National Research Council, Publication 1345, 1966.

Table 16. Plasma amino acid levels and ratios in chicks fed cottonseed meal (CSM) *ad libitum* for two days (Experiment 5).

Amino acid	Non-fasting	1 hour fast	1 hour fast:Non-fast ratio	Nonfasting plasma amino acid levels CSM:Broiler starter ratio	Amino acids as a % of the protein CSM:Requirement[1] ratio
	micromoles/100 mls plasma				
Essential					
Lysine	64.6	43.2	0.67	1.14	0.71
Histidine	22.9	19.4	0.85	0.74	1.20
Arginine	59.7	43.2	0.72	1.18	1.22
Threonine	59.3	50.8	0.86	0.52	0.84
Glycine	66.5	51.3	0.77	0.73	0.93
Methionine	2.6	3.6	1.38	0.23	0.86
Cystine	6.0	6.6	1.10	0.41	1.39
Methionine + cystine	8.6	10.2	1.19	0.33	1.04
Valine	29.3	25.9	0.88	0.82	1.07
Isoleucine	9.1	9.8	1.08	0.44	0.92
Leucine	16.9	18.1	1.07	0.46	0.73
Phenylalanine	12.2	9.6	0.79	0.69	1.32
Tyrosine	16.8	18.4	1.10	0.68	1.26
Phenylalanine + tyrosine	29.0	28.0	0.96	0.69	1.29
Nonessential					
Aspartic acid	6.1	4.0	0.65	--	--
Serine	173.3	137.8	0.80	--	--
Glutamic acid	32.8	24.9	0.76	--	--
Proline	29.9	23.4	0.78	--	--
Alanine	82.0	51.8	0.63	--	--

1. National Research Council, Publication 1345, 1966.

Table 17. Plasma amino acid levels and ratios in chicks fed dried whole egg (Egg) *ad libitum* for two days (Experiment 5).

Amino acid	Non-fasting	1 hour fast	1 hour fast:Non-fast ratio	Nonfasting plasma amino acid levels	Amino acids as a % of the protein
				Egg:Broiler starter ratio	Egg:Requirement[1] ratio
	micromoles/100 mls plasma				
Essential					
Lysine	70.6	53.7	0.76	1.24	1.14
Histidine	25.0	18.6	0.74	0.81	1.10
Arginine	25.0	14.9	0.60	0.49	1.13
Threonine	152.8	141.5	0.93	1.34	1.37
Glycine	61.5	49.9	0.81	0.68	0.50
Methionine	20.8	13.6	0.65	1.86	2.52
Cystine	23.0	20.2	0.88	1.59	1.48
Methionine + cystine	43.8	33.8	0.77	1.70	2.04
Valine	43.4	29.0	0.67	1.22	1.80
Isoleucine	19.8	12.5	0.63	0.95	2.31
Leucine	26.3	15.7	0.60	0.72	1.35
Phenylalanine	11.6	7.2	0.62	0.66	1.80
Tyrosine	57.8	47.6	0.82	2.36	1.65
Phenylalanine + tyrosine	69.4	54.8	0.79	1.64	1.73
Nonessential					
Aspartic acid	10.1	6.2	0.61	--	--
Serine	289.3	260.8	0.90	--	--
Glutamic acid	27.9	25.4	0.91	--	--
Proline	38.4	27.3	0.71	--	--
Alanine	133.6	90.5	0.68	--	--

[1] National Research Council, Publication 1345, 1966.

Table 18. Plasma amino acid levels and ratios in chicks fed corn for 1-3/4 hours (Experiment 6).

Amino acid	Plasma amino acid level		Amino acids as a % of the protein
	Trial 3 (9% prot.)	Corn:Broiler starter ratio	Corn:Requirement[1] ratio
	micromoles/100 mls plasma		
Essential			
Lysine	35.8	0.59	0.43
Histidine	15.9	0.74	1.25
Arginine	33.0	0.65	0.95
Threonine	68.0	0.72	1.01
Glycine	63.1	0.75	0.82
Methionine	7.4	0.61	1.02
Cystine	12.6	0.85	1.04
Methionine + cystine	20.0	0.74	1.03
Valine	19.6	0.54	1.15
Isoleucine	10.2	0.50	1.00
Leucine	27.4	0.80	1.54
Phenylalanine	8.3	0.68	1.36
Tyrosine	13.0	0.65	1.70
Phenylalanine + tyrosine	21.3	0.66	1.52
Nonessential			
Aspartic acid	10.3	--	--
Serine	109.1	--	--
Glutamic acid	22.1	--	--
Proline	47.8	--	--
Alanine	58.5	--	--

[1] National Research Council, Publication 1345, 1966.

Table 19. Plasma amino acid levels and ratios in chicks fed soybean meal (SBM) for 1-3/4 hours (Experiment 6).

Amino acid	Plasma amino acid level Trial 2 (21% prot.)	Plasma amino acid level SBM:Broiler starter ratio	Amino acids as a % of the protein SBM:Requirement[1] ratio
	micromoles/100 mls plasma		
Essential			
Lysine	44.6	0.75	1.26
Histidine	19.8	0.82	1.37
Arginine	44.8	0.83	1.40
Threonine	114.3	0.91	1.17
Glycine	55.0	0.82	0.92
Methionine	2.6	0.19	0.70
Cystine	10.8	0.84	0.98
Methionine + cystine	13.4	0.51	0.85
Valine	26.0	0.76	1.25
Isoleucine	15.0	0.78	1.39
Leucine	23.7	0.77	1.20
Phenylalanine	11.4	0.85	1.44
Tyrosine	19.9	1.02	1.68
Phenylalanine + tyrosine	31.3	0.95	1.55
Nonessential			
Aspartic acid	4.8	--	--
Serine	119.8	--	--
Glutamic acid	23.4	--	--
Proline	41.2	--	--
Alanine	57.5	--	--

1. National Research Council, Publication 1345, 1966.

Table 20. Plasma amino acid levels in chicks fed cottonseed meal for 1-3/4 hours (Experiment 6).

	Trial		
Amino acid	1 (41% prot.)	2 (21% prot.)	3 (15% prot.)
	micromoles/100 mls plasma		
Essential			
Lysine	67.8	44.6	32.0
Histidine	30.4	21.2	18.5
Arginine	82.4	69.6	51.2
Threonine	89.8	94.2	95.0
Glycine	67.6	57.7	61.0
Methionine	5.5	8.4	6.4
Cystine	19.7	12.2	11.8
Methionine + cystine	25.2	20.6	18.2
Valine	49.6	27.2	22.0
Isoleucine	17.0	14.0	10.6
Leucine	31.3	23.2	21.0
Phenylalanine	22.7	13.0	11.0
Tyrosine	19.4	17.1	13.5
Phenylalanine + tyrosine	42.1	30.1	24.5
Nonessential			
Aspartic acid	7.6	4.4	11.4
Serine	200.4	128.2	110.8
Glutamic acid	41.8	24.1	26.0
Proline	63.4	37.0	37.5
Alanine	58.3	56.8	61.5

Table 21. Plasma amino acid ratios in chicks fed cottonseed meal (CSM) for 1-3/4 hours (Experiment 6).

	Plasma amino acid level			Amino acids as a % of the protein
	CSM:Broiler starter ratio			
Amino acid	Trial 1 (41% prot.)	Trial 2 (21% prot.)	Trial 3 (15% prot.)	CSM:Requirement[1] ratio
Lysine	1.16	0.75	0.52	0.71
Histidine	1.49	0.88	0.86	1.20
Arginine	2.38	1.29	1.01	1.22
Threonine	1.04	0.75	1.00	0.84
Glycine	1.11	0.86	0.73	0.93
Methionine	0.82	0.62	0.52	0.86
Cystine	1.41	0.94	0.80	1.39
Methionine + cystine	1.22	0.78	0.67	1.04
Valine	1.49	0.80	0.61	1.07
Isoleucine	1.00	0.73	0.51	0.92
Leucine	1.05	0.75	0.62	0.73
Phenylalanine	1.49	0.97	0.90	1.32
Tyrosine	1.03	0.87	0.68	1.26
Phenylalanine + tyrosine	1.24	0.91	0.76	1.29

[1] National Research Council, Publication 1345, 1966.

Table 22. Plasma amino acid levels and ratios in chicks fed commercial milo for 1-3/4 hours (Experiment 6).

	Plasma amino acid level				Amino acids as a % of the protein
	Trial 1 (9% prot.)	Trial 2 (9% prot.)	Milo:Broiler starter ratio		Milo:Requirement[1] ratio
Amino acid			Trial 1	Trial 2	
	micromoles/100 mls plasma				
Essential					
Lysine	18.4	27.0	0.32	0.45	0.51
Histidine	11.5	14.8	0.56	0.61	0.96
Arginine	13.3	28.6	0.38	0.53	0.67
Threonine	62.5	78.4	0.72	0.63	1.09
Glycine	40.3	43.6	0.66	0.65	0.90
Methionine	2.3	5.4	0.34	0.40	0.90
Cystine	8.0	9.2	0.57	0.71	1.22
Methionine + cystine	10.3	14.6	0.50	0.55	1.05
Valine	17.4	17.2	0.52	0.50	1.35
Isoleucine	7.6	10.7	0.45	0.56	1.37
Leucine	26.3	27.3	0.88	0.89	2.09
Phenylalanine	10.9	8.1	0.72	0.60	1.64
Tyrosine	12.2	11.8	0.65	0.60	1.46
Phenylalanine + tyrosine	23.1	19.9	0.68	0.60	1.56
Nonessential					
Aspartic acid	4.1	3.8	--	--	--
Serine	136.4	103.8	--	--	--
Glutamic acid	22.0	22.5	--	--	--
Proline	42.1	36.0	--	--	--
Alanine	46.2	50.5	--	--	--

[1] National Research Council, Publication 1345, 1966.

Table 23. Plasma amino acid levels and ratios in chicks fed milo (NSA-940) for 1-3/4 hours (Experiment 6).

Amino acid	Plasma amino acid level		Amino acids as a % of the protein
	Trial 3 (13% prot.)	Milo:Broiler starter ratio	Milo:Requirement[1] ratio
	micromoles/100 mls plasma		
Essential			
Lysine	33.4	0.55	0.46
Histidine	16.6	0.78	1.08
Arginine	32.8	0.65	0.66
Threonine	84.8	0.89	0.85
Glycine	50.5	0.60	0.66
Methionine	6.6	0.54	0.54
Cystine	12.0	0.81	--
Methionine + cystine	18.6	0.69	--
Valine	18.6	0.52	1.28
Isoleucine	11.0	0.53	1.06
Leucine	26.4	0.78	2.06
Phenylalanine	9.0	0.74	1.66
Tyrosine	14.4	0.72	1.08
Phenylalanine + tyrosine	23.4	0.73	1.39
Nonessential			
Aspartic acid	9.2	--	--
Serine	132.9	--	--
Glutamic acid	24.4	--	--
Proline	40.2	--	--
Alanine	59.2	--	--

1. National Research Council, Publication 1345, 1966.

APPENDIX B

DATA -- CHAPTER 3

Table 24. Composition of diets used in amino acid imbalance studies.

Ingredient	6% prot. balanced	6% prot. met. defic.	6% prot. high leucine	57% prot.	16% prot. balanced
			%		
Isolated soybean protein	7.30	7.30	7.30	69.50	19.50
Cellulose	3.00	3.00	3.00	3.00	3.00
Glucose monohydrate	78.25	77.25	73.45	16.25	66.05
Vitamin mix[1]	4.00	4.00	4.00	4.00	4.00
Corn oil	1.00	1.00	1.00	1.00	1.00
Mineral mix[2]	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	4.25	4.25	4.25	4.25	4.25
DL-methionine	0.20	--	--	--	0.20
Glycine	--	1.00	--	--	--
L-tryptophan	--	0.20	--	--	--
DL-leucine	--	--	5.00	--	--

1. Supplied the following per kg of diet: 10,000 IU vitamin A, 960 ICU vitamin D_3, 8.8 IU vitamin E, 8.8 mg thiamine HCl, 12 mg riboflavin, 15.2 mg d-calcium pantothenate, 4.0 mg pyridoxine HCl, 20 mg p-amino benzoic acid, 1000 mg inositol, 88 mg niacin, 2206 mg choline chloride, 30 mcg vitamin B_{12}, 0.2 mg biotin, 2.0 mg folic acid, 6.6 mg menadione, and 50 mg ethoxyquin (as a preservative).

2. Supplied the following per kg of diet: 1969 mg Na, 5641 mg Cl, 5000 mg NaCl, 104 mg Mn, 289 mg Fe, 41 mg Cu, 77 mg Zn, 3114 mg K, 1.2 mg Co, 562 mg Mg, 3.2 mg Mo, 3.1 mg I, and 2907 mg SO_4.

Table 25. Feed intake and body weight of chicks consuming amino acid imbalanced diets for one week.

Treatment	Feed consumed/ bird/day	Body weight change/bird/day
	grams	
6% protein (balanced)	8.9	1.1
6% protein (met. defic.)	5.5	- 0.8
6% protein (high leucine)	3.6	- 1.5
57% protein	10.3	6.5
16% protein (balanced)	12.1	5.4

Table 26. Liver free amino acid levels of chicks consuming amino acid imbalanced diets for one week.

Amino acid	6% prot. balanced	6% prot. met. defic.	6% prot. high leucine	57% prot.	16% prot. balanced
	mg/100 gms liver				
Essential					
Lysine	25.8	67.3	80.9	46.5	56.9
Histidine	16.6	18.0	25.5	18.5	22.0
Arginine	15.8	30.2	53.3	40.3	32.3
Threonine	21.5	41.4	59.9	50.6	50.7
Glycine	32.0	41.4	57.9	54.2	51.8
Valine	18.9	29.4	48.8	40.0	36.9
Methionine	6.6	13.4	22.1	12.1	14.7
Cystine	14.9	16.0	19.6	8.1	16.2
Isoleucine	10.1	15.8	25.2	21.4	19.7
Leucine	27.8	42.3	76.0	48.6	51.2
Phenylalanine	13.4	21.6	34.7	21.9	25.5
Tyrosine	17.9	22.7	34.7	23.6	26.4
Nonessential					
Aspartic acid	31.6	75.7	102.8	85.1	77.9
Serine	119.9	170.1	147.5	73.4	124.0
Glutamic acid	93.2	138.6	157.8	185.7	149.4
Proline	20.8	28.4	50.0	49.2	41.5
Alanine	39.8	54.7	82.4	60.3	65.8

Table 27. Plasma free amino acid levels of chicks consuming amino acid imbalanced diets for one day.

Amino acid	6% prot. balanced	6% prot. met. defic.	6% prot. high leucine	57% prot.	16% prot. balanced
			micromoles/100 mls plasma		
Essential					
Lysine	111.4	76.6	120.1	154.7	123.3
Histidine	15.2	12.8	14.2	36.1	21.0
Arginine	18.2	24.6	27.7	75.3	30.6
Threonine	98.6	100.6	117.0	266.0	114.5
Glycine	63.3	133.4	74.6	149.8	63.9
Valine	27.1	24.3	14.7	124.1	37.6
Methionine	5.9	4.0	7.4	5.5	14.0
Cystine	20.7	9.8	17.3	16.2	10.6
Isoleucine	14.2	12.1	7.8	58.1	20.6
Leucine	22.0	17.9	112.1	79.4	30.0
Phenylalanine	13.7	14.6	11.4	28.6	19.1
Tyrosine	32.6	18.0	16.4	25.8	35.6
Nonessential					
Aspartic acid	13.3	14.8	13.2	42.8	13.0
Serine	252.2	279.6	175.9	274.2	244.3
Glutamic acid	27.6	28.8	34.2	60.5	38.2
Proline	30.6	33.0	25.7	191.8	57.5
Alanine	92.2	78.2	59.6	95.3	127.9

Table 28. Free amino acid levels in thigh muscle of chicks consuming amino acid imbalanced diets for one week.

Amino acid	6% prot. balanced	6% prot. met. defic.	6% prot. high leucine	57% prot.	16% prot. balanced
			mg/100 gms thigh muscle		
Essential					
Lysine	77.1	80.3	75.9	113.4	120.6
Histidine	24.5	5.6	13.9	38.3	47.5
Arginine	16.9	14.0	18.0	55.8	32.5
Threonine	13.8	18.6	21.5	28.8	38.6
Glycine	19.2	23.9	19.3	28.8	22.3
Valine	10.8	10.4	12.1	17.2	12.9
Methionine	6.9	5.1	6.4	2.8	4.6
Isoleucine	7.3	7.0	8.1	8.7	8.4
Leucine	14.4	14.0	24.7	16.5	17.2
Phenylalanine	8.8	7.4	9.2	9.9	10.0
Tyrosine	12.9	8.3	10.6	10.6	14.3
Nonessential					
Aspartic acid	20.6	23.8	26.0	19.0	23.2
Serine	105.0	119.2	85.8	98.7	134.7
Glutamic acid	47.5	47.6	43.9	39.1	45.7
Proline	11.6	9.1	13.7	35.6	17.1
Alanine	42.2	33.0	31.9	34.1	56.0

Table 29. Free amino acid levels in breast muscle of chicks consuming amino acid imbalanced diets for one week.

Amino acid	6% prot. balanced	6% prot. met. defic.	6% prot. high leucine	57% prot.	16% prot. balanced
			mg/100 gms breast muscle		
Essential					
Lysine	131.5	134.6	134.8	132.8	163.2
Histidine	28.9	3.8	8.5	67.3	100.2
Arginine	4.6	7.5	12.2	28.0	16.5
Threonine	5.6	11.8	16.2	25.0	22.7
Glycine	7.8	15.1	8.4	20.8	10.5
Valine	4.4	7.3	10.4	20.9	13.3
Methionine	3.5	4.2	9.8	5.6	5.0
Isoleucine	3.0	5.2	7.2	12.1	8.6
Leucine	7.7	13.2	28.7	26.3	20.6
Phenylalanine	4.8	5.7	9.2	13.1	10.6
Tyrosine	8.4	7.5	11.0	16.7	16.1
Nonessential					
Aspartic acid	8.7	11.0	12.9	15.1	13.8
Serine	39.8	56.7	54.6	63.5	59.4
Glutamic acid	14.6	19.6	26.3	38.6	30.9
Proline	3.5	4.2	6.5	25.5	7.8
Alanine	20.3	21.2	26.6	28.0	35.4

APPENDIX C

DATA -- CONTROL PLASMA AMINO ACID VALUES

Table 30. Plasma amino acid levels of chicks fed broiler starter.

Amino acid	Experiment				
	4		6		
	Nonfasted	Fasted	Trial 1	Trial 2	Trial 3
	micromoles/100 mls plasma				
Essential					
Lysine	48.6	48.7	58.2	59.7	60.9
Histidine	20.4	18.0	20.4	24.1	21.4
Arginine	55.4	54.1	34.6	54.0	50.5
Threonine	116.0	102.9	86.2	125.0	94.8
Glycine	68.0	70.5	60.9	67.1	84.0
Valine	37.6	33.2	33.2	34.0	36.0
Methionine	15.4	14.7	6.7	13.6	12.2
Cystine	14.4	14.8	14.0	12.9	14.8
Isoleucine	23.1	20.3	16.9	19.2	20.6
Leucine	35.7	30.6	29.8	30.8	34.0
Phenylalanine	15.1	12.8	15.2	13.4	12.2
Tyrosine	19.2	16.3	18.8	19.6	20.0
Nonessential					
Aspartic acid	3.7	4.4	4.7	5.3	12.8
Serine	125.2	145.1	207.6	131.0	137.2
Glutamic acid	22.9	21.6	25.6	23.0	25.0
Proline	57.2	53.0	64.1	57.8	68.5
Alanine	63.6	66.3	71.9	67.6	78.8

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