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Response of Strain to Amino Acid Supplementation and Lysine Requirements in Low Protein Layer Diets

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RESPONSE OF STRAIN TO AMINO ACID SUPPLEMENTATION AND LYSINE REQUIREMENTS IN LOW PROTEIN LAYER DIETS

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LYSINE REQUIREMENTS IN LOW PROTEIN LAYER DIETS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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JBC

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INTRODUCTION

Two primary means of meeting amino acid requirements of laying hens, and nonruminants in general, are the use of diets high enough in protein to supply the needed amounts of all amino acids along with some excesses or by feeding low protein diets supplemented with minimum levels of essential amino acids needed for maximum performance. Proper supplementation of these low protein diets requires knowledge of the laying hen's requirement for amino acids and the interrelationships among amino acids, along with information about the influence of factors such as strain and management on these requirements.

One purpose of the studies herein was to observe the influences of selected amino acid supplementations to a low protein layer diet on two strains of laying hens. A second purpose was to establish an optimum level of dietary lysine in low protein layer diets. Thirdly, some influences of supplementation of this diet with isoleucine and tryptophan on the hen's lysine requirement were examined.

As more data become available about amino acid supplementation of low protein diets, this information may be used in least-cost ration formulation programs. This will permit optimum utilization of natural feedstuffs and synthetic amino acids to more closely match the dietary amino acid content with minimum requirements.

REVIEW OF LITERATURE

Introduction

Johnson and Fisher (1956) found the amino acids arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine to be essential for laying hens. The amino acids alanine, cystine, glycine, hydroxyproline, proline, serine, tyrosine, asparagine, and citrulline were classified as nonessential. Although nonessential, glycine was needed for maximum production. These workers further classified glutamic acid as dispensable. Johnson and Fisher were also the earliest to develop a free amino acid diet to support egg production (Fisher and Johnson, 1956). This allowed researchers to begin establishing requirements for individual amino acids.

Protein Level and Other Factors Influencing Amino Acid Requirements

Prior to the work of Johnson and Fisher, some fundamental relationships between crude protein level and the requirement for specific amino acids had been observed. The requirement for lysine, in growing chicks, was shown to increase as the protein level increased (Grau, 1948). Later work showed that the requirements for arginine and lysine, in laying hens, depend on the total essential amino acid content of the diet (Anderson and Dobson, 1959). Environmental factors have been shown to influence the sulfur amino acid requirement. Reid and Weber (1973) demonstrated an increased requirement for sulfur amino acids when temperatures were high.

Early attempts to establish a protein requirement for laying hens were hampered by the influence of the amino acid pattern of the grain used in the experiment. The importance of protein quality or amino acid balance has been demonstrated by varying the relative amounts of various grains in experimental rations. Comparisons of wheat-based and corn-based rations show this effect. Johnson and Fisher (1959) found 10.4% protein wheat-based rations to support production equal to that of hens fed a 15.7% ration, but corn-based rations with a higher total protein content (11.3%) were inferior. Bray (1960) studied the effects of amino acid balance by varying the relative amounts of corn and soybean meal in a 10% protein diet. This study indicated that maximum production was attained when 55.5% of the protein was obtained from soybean meal.

Limiting Amino Acids and Established Requirements

Under practical situations, certain amino acids are more likely to be suboptimal than others. Studies to elucidate the most limiting amino acids in corn-soy diets show lysine, tryptophan, and methionine, respectively, to be most limiting in the presence of adequate isoleucine and valine (Bray, 1964). This work also showed that isoleucine could also become limiting in some cases. The limiting order of these amino acids is dependent on many factors. Sulfur amino acids, particularly cysteine, have been shown first limiting in both purified and practical diets (Baker and Bray, 1972).

Given that all other essential amino acids are present in adequate amounts, the requirements for a specific amino acid can be found. The earliest work in this area was in the establishment of the requirements for methionine and cystine. Leong and McGinnis (1952) found laying hens to require 0.28% methionine and 0.25% cystine. However, Johnson and Fisher (1956) did not classify cystine as essential. The chicken has the ability to derive cystine from methionine but not vice versa. In most practical situations therefore, the requirements for these amino acids can be given as total sulfur amino acids. Further considerations of these points will be presented in later sections of this review.

The requirements for lysine are set at 0.66 g./day (N.R.C., 1977). Recent work has shown this may be too little in that Halloran and Almquist (1978) found the requirement to be between 0.66 and 0.72 g./day. Lysine intakes beyond 0.72 and 0.93 g./day were not beneficial.

Amino Acid Supplementation Studies

The varying results reported from attempts to increase laying hen productivity via amino acid supplementation reflect the critical need for amino acid balance. Bray (1964) observed no benefit from supplements of various combinations of methionine, tryptophan, or lysine to a 60:40 blend of corn and soybean protein. Along with a critical balance of amino acids, consumption of the diet can become a determining factor in attaining the observed results (Bray and Garlich, 1960). The protein levels and protein qualities of the diets to which the amino acids are supplemented may influence their overall effect. By varying the ratio of corn protein to soy protein in a ration, Bray (1968) found the response to methionine supplementation increased as soy protein replaced that of corn. Mixtures of tryptophan, lysine, isoleucine, and valine gave decreasing results as soy replaced corn.

Methionine Supplementation of Laying Hen Diets

Supplementation studies involving only methionine have shown varied results. When supplemented in a 10% protein diet, Waibel and Johnson (1961) found methionine to improve production. No response to only methionine supplementation was reported using 11% protein diets (Stangeland and Carlson, 1961) or 8% protein wheat-based diets (Biely and March, 1964). Bray (1978) reported that the addition of methionine to an 80:20 corn:soy blend improved production. Carlson and Guenthner (1969) observed that no response to methionine supplementation was evident until the protein content of the diet was below 14%. These workers also noted an increase in utilization of protein due to methionine supplementation. Layers without methionine added to their diet required 17 g./day of protein, while those with supplemental methionine needed only 15 g./day of protein. These researchers felt that the requirement for lysine was under 671 mg./day and observed the requirement for methionine to be in excess of 300 mg./day. Others have found layers to respond to methionine supplementation when the protein content of the diet was 16% but found additional methionine detrimental in a 12% protein diet (Muller and Balloun, 1974). Additional methionine in both high and low energy, 12% protein diets was observed to improve production of laying hens (Scott et al., 1975). These workers also believed that the lysine requirement was higher than current recommendations. The amino acid makeup of the protein in these experimental rations probably greatly influenced the experimenters' observed results. If methionine was not one of the limiting amino acids in the ration or if another amino acid or amino acids were marginal, then the response to supplemental methionine would be reduced.

The presence of some other sources of sulfur have been shown to affect response of layers to methionine supplementation, also. Reid and Weber (1974) found that the presence of ammonium sulfate reduced the effectiveness of supplemental methionine, while the presence of sodium sulfate had no effect on the methionine requirement. Baker (1976) demonstrated that inorganic sulfur compounds are beneficial as supplements to poultry diets under rather rare circumstances. The sulfur amino acids must be suboptimal, with cystine more limiting than methionine. Under these conditions, sulfur compounds can presumably reduce the need for sulfur amino acids by donating sulfur to compounds normally requiring an amino acid to donate its sulfur molecule. This effect has been manifested in increased growth of broiler chicks. Only 165 p.p.m. SO_4 was necessary to bring about maximal responses. Practical poultry diets contain well beyond this level. Under practical situations, sulfate supplementation would not be beneficial. This does, however, demonstrate the ability of avian species to utilize sulfur from non-protein sources.

Lysine Supplementation of Laying Hen Diets

Response to supplemenation of laying hen diets with lysine is influenced by the amino acid balance as well as protein level of the diet. In low protein diets, lysine may not be the first limiting amino acid. If other amino acids are suboptimal, then their influence may overshadow any effect of lysine supplementation. Biely and March (1964) reported no improvement in egg production when an 8% protein wheat-based laying ration was supplemented with lysine. In studies with diets designed to be adequate in other amino acids, Boomgaardt and Baker (1970) observed the lysine requirement of young Leghorn chicks to remain constant, expressed as a percent of the protein, over the range of 14.5% to 20.0% crude protein.

Methionine and Lysine Supplementation of Laying Hen Diets

Many workers have reported improved production when both methionine and lysine are supplemented to laying rations (Stangeland and Carlson, 1961; Waibel and Johnson, 1961; Waldroup and Harms, 1961; Marin *et al*., 1971; Blair *et al*., 1976). Generally, the responses were greater at higher protein levels (12-14%), indicating that other amino acids may become limiting at low (8-11%) protein levels. Biely and March (1964) reported no improvement in production from supplementations of methionine and lysine to an 8% protein wheat-based ration, whereas Blair *et al*. (1976) found hens fed a 12.1% protein diet supplemented with methionine and lysine to produce at the same level as the controls fed a 15.8% protein diet.

Multiple Amino Acid Supplementations of Laying Hen Diets

Beyond supplementation of methionine and lysine, most work has involved the additional amino acids tryptophan, isoleucine, and valine. Work with only two or three amino acids has again demonstrated the critical balance necessary for proper performance. Additions of tryptophan and valine have been observed to be of no benefit in diets already supplemented with lysine (Waibel and Johnson, 1961). Using a isoleucine. In this study, he observed no response in production from supplementation of methionine, lysine, tryptophan, and isoleucine to the 12% protein diet. These amino acids and additional valine gave responses no better than the control 12% protein diet. An interaction was noted in supplementations of the 10% protein diet. Addition of isoleucine or valine singly had no effect, while supplementation of a combination of the two amino acids resulted in improved production.

In some instances, maintenance of body weight and production of eggs of adequate size have become important. Increasing the level of supplementation of a 6.24% protein basal diet 25% beyond the required or recommended level has been shown to allow for the maintenance of body weight and production of normal eggs (Waibel et al., 1961). This over-supplementation improved production but not to the extent of that of hens on a "standard laying ration." Omission of valine or tryptophan from a 13.4% protein ration supplemented with methionine, lysine, and isoleucine resulted in rapid weight loss (Fitzsimmons et al., 1963). Depletion or use of body stores of amino acids could cause this reduction in weight.

Other evidence of body stores being depleted has also been observed. When a 9% protein diet was supplemented with methionine, lysine, tryptophan, isoleucine, and valine, production was increased for a time but then fell off (Bray and Garlich, 1960). These workers felt that consumption of the diet was not satisfactory, which could also be a factor in maintenance of body weight. Several workers have shown that supplementation above the recommended levels for amino acids aids in

60:40 blend of corn and soy protein, Bray (1964) studied the supplementation of various combinations of methionine, tryptophan, lysine, isoleucine, and valine. This study revealed that supplementation with all five amino acids produced significant increases in egg production. He further noted that with any combination of four amino acids used in the study the addition of the fifth would improve performance. Britzman (1964) found methionine supplementation of a 16% protein diet to be of no benefit; but, when this diet was diluted to 10% protein with cellulose, supplemental methionine improved production. It was noted that on high protein and low energy diets the hens consumed an adequate amount of feed to meet their methionine requirements; but, when the diet was diluted to 10% protein, the hens did not consume enough feed to meet their needs for methionine. Another method used by Britzman to reduce the protein content of the diet was to replace soy with corn. This change resulted in changes in the amino acid makeup of the diet such that lysine and tryptophan also were limiting and required supplementation. In studying many different combinations of supplemental amino acids, Choudhury (1972) observed isoleucine to be most limiting after adequate amounts of methionine, lysine, and tryptophan were supplied. Also, the addition of both valine and threonine were observed to bring about the same response as isoleucine alone. Kashani (1975), using multiple supplements, found supplemental methionine and lysine to reduce production when added to an 11.8% protein diet, while additional tryptophan was slightly beneficial. He observed isoleucine to have no influence in his study. In a later study, Kashani (1978) supplemented both a 10% and a 12% protein diet with methionine, lysine, tryptophan, valine, and maintenance of body weight and egg production. Waibel and co-workers found that increasing supplementations of methionine, tryptophan, lysine, isoleucine, and valine to a 9% protein diet at a level 25% beyond the recommended level allowed hens to maintain body weight and produce a greater mass of eggs (Waibel et al., 1961). Supplementation of these amino acids to a 13.4% protein diet at 125% of the recommended level has been reported to support production near the control level in addition to maintaining body and egg weights (Fitzsimmons et al., 1963). This oversupplementation could, however, bring about imbalances which could essentially make some nonsupplemented amino acids limiting or suboptimal under conditions in which they normally would not be.

Lysine-Arginine Interactions

Many workers have observed a relationship in growing chicks between lysine content of the diet and the arginine requirement (Boorman and Fisher, 1966; Jones, 1964; Nesheim, 1968; O'Dell et al., 1962; O'Dell and Savage, 1966; Smith and Lewis, 1966; Snetsinger and Scott, 1961; and many others). With normal or marginal arginine intakes, excess lysine reduces growth and addition of arginine to the diet restores chick growth. Glycine also has been shown to partially alleviate the depressed growth (Snetsinger and Scott, 1961). Additions of other amino acids have been found to be harmful or of little benefit in correcting this growth reduction. Arginine has been shown to reduce growth under deficient lysine conditions (O'Dell and Savage, 1966). Plasma levels of lysine are increased when excess lysine is present and levels of arginine are reduced. Excesses of arginine, however, only alter the plasma levels of arginine (Jones, 1964).

A direct simple interaction apparently does not exist, as excesses of arginine are not corrected by additions of lysine to the diet (Smith, 1968). Boorman and Fisher (1966) concluded that the growth depression and related observations were not a unique phenomenon. Because lysine is detoxified slowly, they felt it produced greater metabolic influences when fed in excess. Others felt a specific metabolic interaction was causing the disorders (Smith and Lewis, 1966; O'Dell and Savage, 1966; Snetsinger and Scott, 1961; Jones et al., 1967; and others). The specifics of this relationship are still not clear. Along the lines of a detoxification process, Snetsinger and Scott (1961) suggested that the benefits observed from supplemental glycine are due to increased uric acid synthesis and that arginine was aiding nitrogen excretion via some type of latent urea cycle. Some evidence suggests that creatine per se may become limiting under intakes of excess lysine (Jones et al., 1967). In addition, some workers felt the specific makeup of the dietary protein was causing the imbalance (O'Dell and Savage, 1966), while others demonstrated the ill effects of excess lysine in a variety of diets (Snetsinger and Scott, 1961). Some evidence has suggested that the availability of arginine was altered by the excess lysine (Jones, 1964) and others found this not to be the case (Boorman and Fisher, 1966). The potassium content of the diet has been implicated (O'Dell et al., 1962), but this does not seem to be a major influence (Jones et al., 1967).

Excess lysine has been shown to alter the activities of kidney arginase and liver transamidinase (Jones et al., 1967), but the authors felt these changes occurred too slowly to account for the growth depression and related disfunction. Nesheim (1968) showed lysine, histidine, ornithine, and phenylalanine each to increase kidney arginase activity of growing chicks. Additions of α-amino isobutyric acid decrease kidney arginase activity as does L-threonine, Δ-hydroxy, α-amino valoric acid, and glycine (Austic and Nesheim, 1970).

Currently, there are at least three recognized means by which lysine and arginine interact--competition for reabsorption, decreased transamidinase activity, and increased arginase activity. All three of these factors plus the reduced feed intake of the imbalanced rations could account for nearly all reductions in chick growth under excess lysine intakes (Austic and Scott, 1975). These workers demonstrated the relative effects of each interaction at various lysine supplementation levels. In diets with 0.75% added lysine, increased arginase activity and reduced transamidinase activity accounted for nearly all the observed growth depression. At levels of supplemental lysine above 1.50%, reduced feed intake began to exert an influence on growth. Levels of supplemental lysine above 2.00% caused additional reductions in chick growth due to urinary excretion. Mechanisms for each of these interactions, however, remains unclear.

With strains selected for high and low arginine requirements, Nesheim (1968) was able to show that the differences between the strains existed in the ability of the chicks to metabolize lysine. He demonstrated that lysine, ornithine, and arginine are all excreted in the urine when excess lysine is fed. Lysine, ornithine, and arginine compete for reabsorption in the kidney. Nesheim believed that the excretion of these amino acids reflected their incomplete reabsorption and would therefore tend to increase the requirement for arginine.

Arginine has been shown to counteract, in varying degrees, growth depression of chicks fed excesses of lysine, tyrosine, histidine, methionine, cystine, valine, and leucine (Smith, 1968). Smith's data did not support the contention that arginine deficiency symptoms occurred because of reduced reabsorption, as he observed arginine to help alleviate toxicities of all classes of amino acids (dibasic, dicarboxylic, neutral, and imino). He further had doubts concerning arginine's role in uric acid synthesis and the latent urea cycle hypothesis. Smith reasoned that arginine or a metabolite was being used in some other fashion to dispose of the excess nitrogen.

Branched Chain Amino Acid Interactions

An interrelationship exists between the amino acids leucine, valine, and isoleucine. Growing chicks require supplements of both isoleucine and valine to counteract the growth depressing effects of excess leucine (D'Mello and Lewis, 1970). Laying hens also require supplements of this type (Muller and Balloun, 1976). D'Mello and Lewis (1970) found plasma levels of valine more sensitive to excess leucine than plasma levels of isoleucine. They also stated that at least two separate interactions were responsible for the growth depression they observed. The effects of leucine on isoleucine were different than the effects of leucine on valine. In laying hens, however, this interrelationship seems only to be present in low protein diets (Muller and Balloun, 1976). Diets of 10 to 13% protein were imbalanced by additions of as little as 0.75% L-leucine, while additions of 2.25% L-leucine to a 16% protein diet produced only slight effects and no reduced performance was observed from additions of L-leucine in diets of 19% protein.

The exact nature of this interaction is not known. There is no apparent competition among these amino acids for absorption or excretion (Smith and Austic, 1978) nor are there any notable changes in the activities of the catabolic enzymes of branched chain amino acids (Boldizsar *et al.*, 1973; Featherston and Horn, 1973; Smith and Austic, 1978). Smith and Austic (1978) suggest that the primary cause of a detrimental effect in the case of mild excesses of leucine in the chick is tissue sequestering of the amino acids isoleucine and valine.

Methionine-Cystine Relationships

Because methionine can be the first limiting amino acid in practical poultry rations, it is frequently supplemented in some form. Due to this fact, interrelationships between the sulfur amino acids are of more practical significance. That methionine may be used to synthesize cystine is established. On a weight basis, methionine is needed at a level 1.25 times greater than the amount of cystine needed to meet the chicken's requirement, due mainly to differing molecular weights of the two amino acids (Baker, 1976). Thus, a differing response may be observed in two proteins if only total sulfur amino acid content is considered. Cystine can constitute up to 50% of the total sulfur amino acids in chick diets (Baker, 1976). Featherston and Rogler (1978) found that in low methionine diets additional cystine would reduce chick growth. These workers speculated that this effect was due to competition for absorption of these amino acids.

Moderate deficiencies of methionine are overcome by increased feed consumption, which is coupled with reduced feed conversion. Severe deficiencies, however, reduce the productive energy values of the feed by increased heat production. The chicks lose weight and reduce feed consumption (Sekiz et al., 1975). The toxic effects of a severe deficiency may be due to a buildup of homocystine in the plasma and tissues, since cystathionine synthetase activity is reduced under these dietary conditions. Serine and threonine may also be influenced (Sekiz et al., 1975).

Influence of Diet on Plasma Free Amino Acids

The plasma free concentration of many amino acids has been shown to be directly affected by their level in the diet. Plasma concentrations of threonine, lysine, isoleucine, leucine, valine, methionine, tyrosine, and proline increased linearly in 5-month-old chickens fed casein diets of from 3% to 21% protein (Tasaki and Ohno, 1971). Of this group, threonine, lysine, and proline plasma levels were observed to be influenced the greatest.

The dietary adequacy of some amino acids may also be evaluated by their plasma concentration. Chi and Speers (1976) observed the plasma free lysine concentration to rise only slightly, when graded levels were fed, until the point that maximum production was attained. Following that, additional lysine resulted in large increases in the plasma free concentration of this amino acid.

Influence of Amino Acid Nutrition on Egg Parameters

Egg weight is readily influenced by amino acid or protein content of laying hen diets. The amino acids used in synthesis of egg proteins are directly from dietary sources (Wilcox, 1934). The protein level of the diet therefore would be expected to influence the total amount of egg proteins synthesized. The effect of inadequate protein manifests itself in reduced egg production and reduced egg size. Imbalanced diets which may contain more crude protein result in the production of eggs smaller than those produced from balanced lower protein rations (March and Biely, 1963). These workers noted changes in egg weights as early as 4 days following dietary modifications.

Lunven *et al* (1973) found no difference in protein content or amino acid composition between eggs produced by hens fed 11% or 20% protein diets.

MATERIALS AND METHODS

Experiment 1

In Experiment 1, a commercial strain of laying hens, DeKalb 271, and Cornell Randombred Control hens were used. The basal diet formulations are given in Table 1. Diet 1 was a standard 16% protein laying diet which served as the control, diet 2 was an unsupplemented 10% protein corn-soy diet. Diet 3 was the 10% protein diet with supplements of 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine. Diet 4 was as 3 with an additional 0.10% DL-methionine for a total of 0.40% supplemental DL-methionine. Diet 5 was as 4 plus 0.20% DL-valine, diet 6 was as 5 with 0.20% additional DL-threonine, and diet 7 was as 6 plus 0.20% L-arginine.

The birds were reared in cages and fed standard starting and growing rations. At 22 weeks of age, the birds were randomly housed in 8 in. by 12 in. laying cages with two birds per cage. Wing band numbers were recorded and the hens were weighed at this time. From housing until the initiation of the experimental treatments, all caged birds except those receiving the 16% diet were fed diet 3. This depletion phase was 14 weeks long and records were kept for the last 12 weeks in the same manner as described for later periods. This was done in order to deplete body reserves of amino acids and adapt the hens to a low plane of protein nutrition so that any later stimulus in production would not be overshadowed by the effects of sudden dietary protein level changes.

TABLE 1. Diet formulations of basal rations

Ingredient	Experiment 1		Experiment 2	
	16% ration	10% ration	12% ration	10% ration
	%			
Yellow corn	70.7	85.4	79.2	84.2
Soybean meal (47%)	19.8	4.1	9.0	4.0
Dehydrated alfalfa meal (17%)	2.0	3.0	2.0	3.0
Dicalcium phosphate	1.5	1.5	2.0	1.5
Ground limestone	5.0	5.0	5.0	5.0
Salt mix[a]	0.5	0.5	0.5	0.5
Vitamins[b]	0.5	0.5	0.5	0.5

[a] Supplied per kilogram of diet: NaCl, 4.8 g.; Zn, 18 mg.; Fe, 10 mg; Mn, 10 mg.; Mg, 7.5 mg.; Cu, 1.5 mg.; Co, 0.25 mg.; and I, 0.35 mg.

[b] Supplied per kilogram of diet: vitamin A, 5280 U.S.P.; vitamin D_3, 1375 U.S.P.; vitamin E, 22 I.U.; vitamin B_{12}, 0.0088 mg.; niacin, 44 mg.; choline chloride, 440 mg.; riboflavin, 6.6 mg.; D-calcium pantothenic acid, 8.8 mg.; vitamin K, 1.1 mg.; folic acid, 1.1 mg.; and biotin, 0.11 mg.

There were eight replicates of two birds of each of the two strains fed seven diets, resulting in 112 cages as experimental units. With this design, therefore, there was a total of 32 birds per diet. Experimental diets began when the hens were 36 weeks old on September 23, 1977, and continued for ten 28-day periods until July 7, 1978. Water and feed were offered to the birds <u>ad libitum</u>. Daily records were kept on egg production and mortality. Eggs were sampled in each period for average weight, Haugh units and shell thickness. Feed consumption was measured at the end of each period. The hens were weighed again in the last period.

Following each period, data were summarized and punched onto computer cards. A computer program was utilized to calculate and list performance parameters and daily nutrient intake for each cage on a per hen-day basis. Feed utilization was reported both as kilograms of feed consumed per dozen eggs produced and grams of egg produced per gram of feed consumed. Individual cage averages for percent hen-housed mortality, egg weight, Haugh units, and shell thickness were also computed. By means of this program, this information was punched onto a second set of computer cards which were used as the data set for analysis of variance.

In later periods, no eggs were produced in some cages. This resulted in missing values for average egg weight, Haugh units, shell thickness, and feed utilization expressed as kilograms of feed consumed per dozen eggs produced. Feed consumption and calculated daily nutrient intake values were not affected by this situation. Production as both percent and grams per hen-day and feed utilization expressed as grams of egg produced per gram of feed consumed were reported as zero. The above noted missing values made a least squares analysis of variance necessary.

<u>Experiment 2</u>

The objectives of Experiment 2 were to observe the influence of graded levels of lysine on laying hen performance in low protein diets and to observe the influence of further supplements of essential amino acids that are limiting in a 10% protein corn-soy diet. The diets used in this study were 10% protein and 12% protein corn-soy laying rations. Formulations for these rations are presented in Table 1. A third diet consisting of the 10% diet supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine was also included. Each of these rations was supplemented to supply six levels of total lysine (0.55%, 0.60%, 0.65%, 0.70%, 0.75%, 0.80%). This resulted in 18 dietary treatments. Commercial Babcock 300, Leghorn-type layers were used in the study. Rearing management was similar to Experiment 1. The hens were placed in the laying cages at 22 weeks of age and fed the 10% protein diet without supplemental amino acids from 24 to 40 weeks of age. Records for egg production and feed consumption were kept for 34 days prior to application of treatments.

Availability of cages necessitated the use of both 8 in. by 12 in. cages and 24 in. by 12 in. cages. Initially, two birds each were placed in 115 small cages and seven birds each in 76 large cages. Thirty-four days before the experimental treatments began, hens were selected and poor ones eliminated such that there remained 108 cages of two hens and 72 cages of six hens. Individual cage records were kept and during the next 7 days any nonproducing hen was replaced. Production records continued for 27 days until treatment diets were begun. The physical separation of the different cage types within the laying house made necessary the nesting of replicates of treatments in cage types, resulting in six replicates of the small cages and four replicates of the large cages per treatment. The hens were weighed at the beginning and the end of the experiment.

Initial plans were to conduct the experiment for six 28-day periods, but unavailability of sufficient DL-isoleucine forced termination of the study 9 days early. Thus, period 6 was only 19 days in length. The study began on February 15, 1979, and ended July 23, 1979. Data were summarized in the same manner as described for Experiment 1. A least squares analysis of variance procedure for nested factorial designs was used, all cages had hens that produced at least one egg in each period, and there were no missing data as in Experiment 1.

Egg White Hydrolysis

Sampling - Experiment 1. Pooled samples of five eggs from replicates 1 through 4 and replicates 5 through 8 were collected for each strain-diet combination during period 13.

Sampling - Experiment 2. Pooled samples of six eggs were collected from replicates 1 through 3 and replicates 4 through 6 in the small cages and replicates 1 and 2 and replicates 3 and 4 in the large cages during period 6. In order to reduce the total number of samples to be analyzed, only samples from lysine levels 1 and 6 in each protein group were initially hydrolyzed. If differences existed between these levels, the intermediate samples could be analyzed to allow more accurate characterization of these differences.

The albumen was separated from the yolks and frozen at 0^{o} F. prior to freeze-drying. In an effort to provide accurate sampling, these samples were freeze-dried in a VirTis Unitrap, 10-100 freeze-drier prior to mixing. Records were kept on amounts of water removed from the samples to allow the results to be reported on a raw albumen basis. After freeze-drying, the samples were crushed to a fine powder, mixed, and held at 0^{o} F. until analysis.

Aliquots of 0.5 g. of the dried albumens were hydrolyzed in 100 ml. of 6N HCl in boiling flasks under nitrogen at 110^{o} F. for 24 hours. Hydrolysates were then filtered and taken to a final volume of 250 ml. From these hydrolysates, 5 ml. were taken and extracted three times with glass distilled water in a rotary evaporator and taken to dryness. These samples were held at 32^{o} F. for a short time until analyzed. The dried hydrolysates were reconstituted with 0.2N sodium citrate buffer at pH 2.2 with internal standards of L-Norleucine and α-Amino-β-Guanidino Propionic Acid, Hydrochloride at 0.25 μM/ml. From these, 0.5 ml. was analyzed using a Beckman-Spinco Model 120 amino acid analyzer. Peak areas were calculated for all identifiable ninhydrin reactive compounds and compared to a standard for calculation of albumen content.

Diet Analysis

Samples of diet 1 and diet 2 from Experiment 1 and the 10% diet at 0.55% lysine and the 12% diet at 0.55% lysine from Experiment 2 were analyzed for amino acid content and Kjeldahl nitrogen for crude protein values. Hydrolysis procedure was the same as that for egg white analysis. Values reported for amino acids in Table 2 are presented as percent of Kjeldahl nitrogen.

TABLE 2. Diet analyses of basal rations

Amino acid	Experiment 1 16% ration	Experiment 1 10% ration	Experiment 2 12% ration[a]	Experiment 2 10% ration[a]
	%			
Lysine	0.81	0.38	0.58	0.55
Methionine	0.27	0.19	0.28	0.36
Half cystine	0.13	0.15	0.11	0.14
Valine	0.80	0.52	0.63	0.52
Arginine	1.04	0.47	0.77	0.59
Histidine	0.46	0.26	0.36	0.28
Phenylalanine	0.86	0.55	0.51	0.56
Tryptophan (calculated)	0.18	0.10	0.12	0.10
Isoleucine	0.72	0.40	0.44	0.39
Leucine	1.69	1.20	1.23	1.22
Threonine	0.61	0.39	0.42	0.39
Crude protein	15.68	10.10	11.57	10.57

[a] Analyses performed on samples of diets from 0.55% lysine level.

Plasma Free Amino Acid Analysis

Experiment 1. On the final day of the trial, two birds of each diet-strain combination were visually selected from replicates 1 through 4 and replicates 5 through 8. From each hen, 10 ml. of blood were drawn via cardiac puncture. These two samples were pooled in 50-ml. centrifuge tubes containing 0.5 ml. saturated sodium citrate solution and held on ice until sampling was complete. Collections began at 2:00 p.m. and continued until 5:00 p.m. Immediately after collections were completed, the blood samples were centrifuged at 5000 r.p.m. for 20 minutes. The plasma was placed in small vials and frozen at 0^{o} F. for 1 year prior to analysis. Plasma samples were prepared for free amino acid analysis by the method of Mondino *et al*. (1972). Prepared samples were frozen at 0^{o} F. for a short time until 50-μl. aliquots were analyzed using a Beckman 118 BL amino acid analyzer. Peak areas were calculated for compounds of interest and compared to a standard for calculation of the plasma free level in the sample.

Experiment 2. Plasma analysis in Experiment 2 differed from Experiment 1 only slightly in sampling procedure. On the final day of the experiment, 10-ml. blood samples were drawn from two birds via cardiac puncture and pooled from replicates 1 through 3 and replicates 4 through 6 in the small cages and from replicates 1 and 2 and replicates 3 and 4 in the large cages for each protein-lysine level combination. These samples were pooled in 50-ml. centrifuge tubes containing 0.5 ml. saturated lithium citrate solution and held on ice until sampling was complete. Sampling began at 10:00 a.m. and ended at 2:00 p.m. on 3 consecutive days. Subsequent preparation of these samples was the same as for Experiment 1. Analysis of these samples was completed within 6 months following collection.

RESULTS

Experiment 1

Missing Data. The previously described conditions that brought about missing data for some observations also results in a division of the data set into two groups. Discussion of means of parameters with missing information (average egg weight, average shell thickness, average Haugh unit score, and kilograms of feed consumed per dozen eggs produced) can refer only to hens in production. Discussion of other parameters must refer to all hens.

Egg Production Parameters. Means for percent hen-day production are presented in Table 3. Due to a high degree of variability, diets did not significantly influence this parameter. Table 4 contains means for hen-day feed consumption. This factor was not significantly affected by dietary alterations. Significant differences exist among the means for production of the hens expressed as grams of egg produced per hen-day. These values are presented in Table 5. As shown in the table, the differences in production between the 16% protein diet (diet 1) and the 10% protein unsupplemented diet (diet 2) or the 10% protein diet with the supplement containing the lower level of methionine (diet 3) were significant. Table 6 presents means for average egg weights for hens in production. A strain-by-diet interaction occurred which was significant at the 0.01 level of probability. Hens within the commercial strain fed 10% protein diets, except diet 6, produced significantly lighter eggs than did those fed the 16% protein diet

TABLE 3. Effects of diets and strain on percent hen-day production

Diet	Strain[a] 1	Strain[a] 2	Mean
	%		
1. Control (16%)	63.43	52.44	57.94
2. 10% crude protein	46.53	47.25	46.89
3. 10% + amino acid supplement[b]	40.11	53.30	46.71
4. As 3 + 0.10% DL-methionine	50.69	46.94	48.81
5. As 4 + 0.20% DL-valine	50.19	48.05	49.12
6. As 5 + 0.20% DL-threonine	53.59	49.89	51.74
7. As 6 + 0.20% L-arginine	54.53	48.64	51.59

No significant differences exist among the means.

[a] In this and all subsequent tables, strain 1 refers to the commercial strain and strain 2 refers to the random-bred strain.

[b] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 4. Effects of diets and strain on hen-day feed consumption

Diet	Strain 1	Strain 2	Mean
	g.		
1. Control (16%)	112.8	101.9	107.4
2. 10% crude protein	100.6	100.9	100.8
3. 10% + amino acid supplement[a]	95.3	104.4	99.9
4. As 3 + 0.10% DL-methionine	98.5	98.6	98.6
5. As 4 + 0.20% DL-valine	103.6	101.1	102.3
6. As 5 + 0.20% DL-threonine	105.9	106.3	106.1
7. As 6 + 0.20% L-arginine	104.4	101.2	102.8

No significant differences exist among the means.

[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 5. Effects of diets and strain on hen-day egg mass production

Diet	Strain 1	Strain 2	Mean
		g./HD	
1. Control (16%)	41.36	31.40	36.38[a]
2. 10% crude protein	27.31	26.80	27.06[b]
3. 10% + amino acid supplement[c]	24.80	30.21	27.51[b]
4. As 3 + 0.10% DL-methionine	31.24	27.22	29.23[ab]
5. As 4 + 0.20% DL-valine	30.73	27.89	29.31[ab]
6. As 5 + 0.20% DL-threonine	33.96	27.91	30.94[ab]
7. As 6 + 0.20% L-arginine	33.42	27.93	30.67[ab]

[a,b] Differing superscripts within a column denote significant differences ($P<0.05$).

[c] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 6. Effects of diets and strain on average egg weight

Diet	Strain 1	Strain 2	Mean
		g.	
1. Control (16%)	65.22[a]	59.71[a]	62.46
2. 10% crude protein	59.03[c]	56.73[b]	57.88
3. 10% + amino acid supplement[d]	62.28[b]	56.66[b]	59.47
4. As 3 + 0.10% DL-methionine	61.70[bc]	58.22[ab]	59.96
5. As 4 + 0.20% DL-valine	61.13[bc]	57.71[ab]	59.42
6. As 5 + 0.20% DL-threonine	63.50[ab]	56.01[b]	59.76
7. As 6 + 0.20% L-arginine	61.51[bc]	57.94[a]	59.73

[a,b,c] Differing superscripts within a column denote significant differences ($P<0.01$).

[d] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

(diet 1). Additionally, eggs produced by hens receiving diet 3 and diet 6 were significantly heavier than those produced by hens fed diet 2. The situation was quite different for the random-bred strain as diets 4, 5, and 7 were not different from the controls (diet 1). No significant differences exist among the means of the 10% diets (diets 2-7). Feed utilization (Table 7) was not significantly influenced by diets. Feed efficiency (Table 8) was significantly affected by diets for hens in production within the commercial strain but not within the random-bred strain. This interaction was significant at the 0.05 level of probability. Feed efficiency of commercial hens receiving diet 3 was significantly poorer than that of their counterparts receiving diet 1 and diet 6.

Average Haugh unit scores for hens in production are presented in Table 9. Commercial hens fed diet 2 produced higher quality eggs than those hens of this strain fed diet 1. None of the Haugh unit values for the eggs from hens on the other 10% diets were different from the controls. Diet 4 and diet 6 allowed for production of eggs of a score not different from feeding diet 2. Within the random-bred strain, eggs produced by hens receiving diets 2, 6, and 7 scored significantly higher than those produced by the control hens (diet 1). Haugh unit value of eggs from hens fed diets 3 to 7 were not significantly different. Diets 3, 4, and 5 each caused production of eggs scoring significantly lower than those produced by hens receiving diet 2. This strain-by-diet interaction was significant at the 0.05 level of probability. Total percent hen-housed mortality of the hens

TABLE 7. Effects of diets and strain on grams of egg produced per gram of feed consumed

Diet	Strain 1	Strain 2	Mean
	g. egg/g. feed		
1. Control (16%)	0.3642	0.3084	0.3363
2. 10% crude protein	0.2726	0.2653	0.2690
3. 10% + amino acid supplement[a]	0.2516	0.2847	0.2682
4. As 3 + 0.10% DL-methionine	0.3152	0.2761	0.2957
5. As 4 + 0.20% DL-valine	0.2896	0.2726	0.2811
6. As 5 + 0.20% DL-threonine	0.3185	0.2593	0.2889
7. As 6 + 0.20% L-arginine	0.3164	0.2694	0.2929

No significant differences exist among the means.

[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 8. Effects of diets and strain on feed consumed per dozen eggs produced

Diet	Strain 1	Strain 2	Mean
	kg.		
1. Control (16%)	2.42[a]	3.22	2.82
2. 10% crude protein	2.79[ab]	2.84	2.82
3. 10% + amino acid supplement[c]	4.98[b]	2.80	3.89
4. As 3 + 0.10% DL-methionine	2.91[ab]	2.91	2.91
5. As 4 + 0.20% DL-valine	4.00[ab]	3.79	3.89
6. As 5 + 0.20% DL-threonine	2.46[a]	3.10	2.78
7. As 6 + 0.20% L-arginine	2.54[ab]	3.24	2.89

[a,b] Differing superscripts within a column denote significant differences ($P<0.01$).

[c] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 9. Effects of diets and strain on average Haugh unit score

Diet	Strain 1	Strain 2	Mean
1. Control (16%)	80.62[b]	72.35[c]	76.48
2. 10% crude protein	86.17[a]	79.60[a]	82.88
3. 10% + amino acid supplement[d]	81.54[b]	74.14[bc]	77.84
4. As 3 + 0.10% DL-methionine	82.16[ab]	74.73[bc]	78.44
5. As 4 + 0.20% DL-valine	81.08[b]	75.38[bc]	78.23
6. As 5 + 0.20% DL-threonine	83.81[ab]	78.35[ab]	81.08
7. As 6 + 0.20% L-arginine	80.96[b]	78.12[ab]	79.54

[a,b,c] Differing superscripts within a column denote significant differences (P<0.01).

[d] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

was not significantly influenced by the diets (Table 10). Means in this table are a record of total cumulative mortality and are not period averages. Table 11 shows the means for average shell thickness of eggs for hens in production. Differences among the means were significant only for the random-bred strain. This strain-by-diet interaction was significant at the 0.01 level of probability. In this strain, shells of eggs produced by hens receiving diet 2 were significantly thicker than those of eggs produced by hens receiving diet 3 or diet 4. No other significant differences existed among the means.

Combined body weight data for both strains are presented in Table 12. No significant difference existed among the diet means for initial weight. The means for final weight for hens on all supplemented 10% protein diets (diets 3-7) were not different from the controls. Hens receiving diet 6 were significantly heavier at the

TABLE 10. Effects of diets and strain on total percent hen-housed mortality

Diet	Strain 1	Strain 2	Mean
	%		
1. Control (16%)	6.2	12.5	9.4
2. 10% crude protein	6.2	25.0	15.6
3. 10% + amino acid supplement[a]	0.0	12.5	6.2
4. As 3 + 0.10% DL-methionine	12.5	6.2	9.4
5. As 4 + 0.20% DL-valine	12.5	12.5	12.5
6. As 5 + 0.20% DL-threonine	12.5	18.7	15.6
7. As 6 + 0.20% L-arginine	6.2	18.7	12.5

No significant differences exist among the means.

[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 11. Effects of diets and strain on average shell thickness

Diet	Strain 1	Strain 2	Mean
	mm.		
1. Control (16%)	0.375	0.366[ab]	0.370
2. 10% crude protein	0.383	0.374[a]	0.378
3. 10% + amino acid supplement[c]	0.378	0.350[b]	0.364
4. As 3 + 0.10% DL-methionine	0.378	0.347[b]	0.363
5. As 4 + 0.20% DL-valine	0.375	0.362[ab]	0.369
6. As 5 + 0.20% DL-threonine	0.381	0.356[ab]	0.369
7. As 6 + 0.20% L-arginine	0.383	0.357[ab]	0.370

[a,b] Differing superscripts within a column denote significant differences ($P<0.01$).

[c] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 12. Effects of diets on body weights and body weight changes

Diet	Average of both strains: Initial weight	Final weight	Weight change
	kg.		
1. Control (16%)	1.65	1.82[ab]	0.17[ab]
2. 10% crude protein	1.62	1.64[b]	0.02[b]
3. 10% + amino acid supplement[c]	1.59	1.67[ab]	0.08[ab]
4. As 3 + 0.10% DL-methionine	1.63	1.69[ab]	0.06[ab]
5. As 4 + 0.20% DL-valine	1.62	1.90[ab]	0.28[a]
6. As 5 + 0.20% DL-threonine	1.61	1.91[a]	0.30[a]
7. As 6 + 0.20% L-arginine	1.60	1.81[ab]	0.21[ab]

[a,b] Differing superscripts within a column denote significant differences ($P<0.05$).

[c] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

end of the experiment than those fed the 10% protein unsupplemented diet (diet 2). An analysis of body weight change reveals the same significant differences, with diet 5 also allowing the hens to gain significantly more than the hens fed the 10% protein unsupplemented diet (diet 2).

<u>Plasma Free Amino Acid Analysis</u>. Means for plasma free levels of various amino acids are presented in Tables 13 through 23. The following amino acids were not significantly affected by diets: valine, isoleucine, leucine, lysine, arginine, phenylalanine, histidine, and tryptophan as well as total essential amino acids. Diets significantly influenced plasma free threonine levels (Table 13) and methionine levels (Table 14). Among the means for plasma free

TABLE 13. Effects of diets and strain on plasma free threonine levels

Diet	Strain 1	Strain 2	Mean
	μM/100 ml. plasma		
1. Control (16%)	44.40	38.05	41.33[abc]
2. 10% crude protein	36.35	32.25	34.30[bc]
3. 10% + amino acid supplement[d]	28.65	30.30	29.47[bc]
4. As 3 + 0.10% DL-methionine	27.45	22.90	25.17[bc]
5. As 4 + 0.20% DL-valine	17.75	31.20	24.47[c]
6. As 5 + 0.20% DL-threonine	48.20	45.85	47.02[ab]
7. As 6 + 0.20% L-arginine	58.25	67.85	63.05[a]

[a,b,c] Differing superscripts within a column denote significant differences ($P<0.05$).

[d] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 14. Effects of diets and strain on plasma free methionine levels

Diet	Strain 1	Strain 2	Mean
	μM/100 ml. plasma		
1. Control (16%)	6.70	7.65	7.17[b]
2. 10% crude protein	8.60	7.50	8.05[ab]
3. 10% + amino acid supplement[c]	6.90	14.55	10.72[ab]
4. As 3 + 0.10% DL-methionine	15.40	15.05	15.22[a]
5. As 4 + 0.20% DL-valine	12.05	10.15	11.10[ab]
6. As 5 + 0.20% DL-threonine	11.20	12.60	11.90[ab]
7. As 6 + 0.20% L-arginine	11.75	12.80	12.27[ab]

[a,b] Differing superscripts within a column denote significant differences ($P<0.05$).

[c] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 15. Effects of diets and strain on plasma free lysine levels

Diet	Strain 1	Strain 2	Mean
	μM/100 ml. plasma		
1. Control (16%)	17.20	24.00	20.60
2. 10% crude protein	11.00	14.95	12.97
3. 10% + amino acid supplement[a]	12.10	18.10	15.10
4. As 3 + 0.10% DL-methionine	28.60	18.90	23.75
5. As 4 + 0.20% DL-valine	14.15	17.15	15.65
6. As 5 + 0.20% DL-threonine	17.80	18.70	18.25
7. As 6 + 0.20% L-arginine	12.55	17.65	15.10

No significant differences exist among the means.

[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 16. Effects of diets and strain on plasma free leucine levels

Diet	Strain 1	Strain 2	Mean
	μM/100 ml. plasma		
1. Control (16%)	25.70	25.00	25.35
2. 10% crude protein	24.65	18.75	21.70
3. 10% + amino acid supplement[a]	13.55	27.20	20.37
4. As 3 + 0.10% DL-methionine	23.30	21.30	22.30
5. As 4 + 0.20% DL-valine	25.45	20.30	22.87
6. As 5 + 0.20% DL-threonine	19.20	25.70	22.45
7. As 6 + 0.20% L-arginine	19.10	23.50	21.30

No significant differences exist among the means.

[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 17. Effects of diets and strain on plasma free isoleucine levels

Diet	Strain 1	Strain 2	Mean
	µM/100 ml. plasma		
1. Control (16%)	9.55	11.10	10.32
2. 10% crude protein	8.25	5.75	7.00
3. 10% + amino acid supplement[a]	13.00	11.75	12.37
4. As 3 + 0.10% DL-methionine	10.10	9.80	9.95
5. As 4 + 0.20% DL-valine	10.80	8.80	9.80
6. As 5 + 0.20% DL-threonine	7.25	10.40	8.82
7. As 6 + 0.20% L-arginine	9.20	9.20	9.20

No significant differences exist among the means.

[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 18. Effects of diets and strain on plasma free phenylalanine levels

Diet	Strain 1	Strain 2	Mean
	µM/100 ml. plasma		
1. Control (16%)	14.30	13.85	14.07
2. 10% crude protein	15.05	15.45	15.25
3. 10% + amino acid supplement[a]	11.65	15.05	13.35
4. As 3 + 0.10% DL-methionine	14.60	12.90	13.75
5. As 4 + 0.20% DL-valine	13.80	12.85	13.32
6. As 5 + 0.20% DL-threonine	14.10	13.20	13.65
7. As 6 + 0.20% L-arginine	13.30	12.70	13.00

No significant differences exist among the means.

[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 19. Effects of diets and strain on plasma free histidine levels

Diet	Strain 1	Strain 2	Mean
	μM/100 ml. plasma		
1. Control (16%)	12.55	8.50	10.52
2. 10% crude protein	13.40	11.95	12.67
3. 10% + amino acid supplement[a]	10.70	10.95	10.82
4. As 3 + 0.10% DL-methionine	15.80	9.85	12.82
5. As 4 + 0.20% DL-valine	14.75	11.30	13.02
6. As 5 + 0.20% DL-threonine	11.50	12.40	11.95
7. As 6 + 0.20% L-arginine	11.90	10.10	11.00

No significant differences exist among the means.

[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 20. Effects of diets and strain on plasma free tryptophan levels

Diet	Strain 1	Strain 2	Mean
	μM/100 ml. plasma		
1. Control (16%)	6.75	8.55	7.65
2. 10% crude protein	7.20	6.20	6.70
3. 10% + amino acid supplement[a]	8.35	10.25	9.30
4. As 3 + 0.10% DL-methionine	7.85	8.30	8.07
5. As 4 + 0.20% DL-valine	9.60	11.30	9.72
6. As 5 + 0.20% DL-threonine	7.40	8.70	8.05
7. As 6 + 0.20% L-arginine	10.05	8.05	9.05

No significant differences exist among the means.

[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 21. Effects of diets and strain on plasma free valine levels

Diet	Strain 1	Strain 2	Mean
	μM/100 ml. plasma		
1. Control (16%)	22.00	21.75	21.87
2. 10% crude protein	18.00	11.95	14.97
3. 10% + amino acid supplement[a]	9.45	17.45	13.45
4. As 3 + 0.10% DL-methionine	14.90	12.95	13.92
5. As 4 + 0.20% DL-valine	24.75	21.85	23.30
6. As 5 + 0.20% DL-threonine	16.45	24.30	20.37
7. As 6 + 0.20% L-arginine	20.60	23.60	22.10

No significant differences exist among the means.

[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 22. Effects of diets and strain on plasma free arginine levels

Diet	Strain 1	Strain 2	Mean
	μM/100 ml. plasma		
1. Control (16%)	36.50	29.60	33.05
2. 10% crude protein	39.90	31.25	35.57
3. 10% + amino acid supplement[a]	29.00	27.25	28.12
4. As 3 + 0.10% DL-methionine	30.10	28.90	29.50
5. As 4 + 0.20% DL-valine	29.70	26.10	27.90
6. As 5 + 0.20% DL-threonine	46.30	27.65	36.97
7. As 6 + 0.20% L-arginine	32.85	29.10	30.97

No significant differences exist among the means.

[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 23. Effects of diets and strain on plasma free total essential amino acid levels

Diet	Strain 1	Strain 2	Mean
	μM/100 ml. plasma		
1. Control (16%)	197.25	187.90	192.57
2. 10% crude protein	182.35	156.20	169.27
3. 10% + amino acid supplement[a]	145.80	183.00	164.40
4. As 3 + 0.10% DL-methionine	188.25	160.95	174.60
5. As 4 + 0.20% DL-valine	172.45	169.60	171.02
6. As 5 + 0.20% DL-threonine	184.50	199.50	192.00
7. As 6 + 0.20% L-arginine	199.65	214.65	207.15

No significant differences exist among the means.
[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

threonine levels, none were significantly different from those observed for hens fed diet 1. Levels of threonine in plasma samples from hens fed diet 7 were significantly higher than those in samples from hens receiving diets 2 to 5. The free threonine in samples from birds fed diet 6 were significantly higher than that in samples from those fed diet 5. Methionine plasma free levels are presented in Table 14. Averaged over strains, plasma levels of free methionine in hens fed diet 4 were significantly higher than those of hens receiving diet 1. No other significant differences existed among the means.

Egg Albumen Amino Acid Analysis. Diets did not significantly influence the amino acid content of albumen of eggs produced in the later part of the study. Values for mean albumen contents of amino acids are presented in Table 24.

TABLE 24. Effects of diets on amino acid content of egg albumen

Diet	Average of both strains								
	Amino acid								
	Lysine	Histidine	Arginine	Threonine	Serine	Aspartic acid	Proline	Glycine	Alanine
	%								
1. Control (16%)	0.62	0.20	0.54	0.41	0.63	0.99	0.35	0.32	0.55
2. 10% crude protein	0.66	0.20	0.56	0.46	0.68	1.03	0.32	0.35	0.60
3. 10% + amino acid supplement[a]	0.65	0.21	0.55	0.45	0.63	1.00	0.33	0.32	0.55
4. As 3 + 0.10% DL-methionine	0.60	0.20	0.54	0.41	0.62	0.94	0.32	0.32	0.56
5. As 4 + 0.20% DL-valine	0.67	0.20	0.54	0.43	0.66	1.06	0.38	0.34	0.60
6. As 5 + 0.20% DL-threonine	0.64	0.24	0.54	0.45	0.68	1.18	0.31	0.35	0.60
7. As 6 + 0.20% L-arginine	0.65	0.21	0.52	0.44	0.68	1.01	0.33	0.34	0.58
Average	0.64	0.21	0.55	0.44	0.66	1.03	0.33	0.33	0.58
Cotterill *et al.* (1977)	0.66	0.21	0.56	0.42	0.64	1.09	0.45	0.33	0.58

No significant differences exist among the means.

[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

TABLE 24 Continued

Diet	Half cystine	Valine	Methio-nine	Iso-leu-cine	Leucine	Glu-tamic acid	Tyro-sine	Phenyl-ala-nine
	Average of both strains							
	Amino acid							
	%							
1. Control (16%)	0.24	0.63	0.37	0.49	0.80	1.26	0.38	0.56
2. 10% crude protein	0.26	0.68	0.38	0.50	0.83	1.31	0.39	0.59
3. 10% + amino acid supplement[a]	0.21	0.65	0.34	0.46	0.77	1.23	0.36	0.55
4. As 3 + 0.10% DL-methionine	0.24	0.63	0.36	0.48	0.79	1.22	0.36	0.54
5. As 4 + 0.20% DL-valine	0.25	0.71	0.38	0.50	0.84	1.29	0.40	0.58
6. As 5 + 0.20% DL-threonine	0.25	0.73	0.39	0.51	0.85	1.34	0.41	0.59
7. As 6 + 0.20% L-arginine	0.34	0.65	0.37	0.48	0.82	1.28	0.38	0.58
Average	0.26	0.67	0.37	0.49	0.82	1.27	0.38	0.57
Cotterill *et al.* (1977)	0.30	0.67	0.39	0.51	0.83	1.32	0.41	0.55

No significant differences exist among the means.

[a] Amino acid supplement contained 0.20% L-lysine, 0.15% DL-tryptophan, 0.25% DL-isoleucine, and 0.30% DL-methionine.

Experiment 2

Protein-period Interactions. A statistically significant interaction existed between periods and proteins in percent hen-day egg production, production as grams per hen-day, feed consumption, grams of egg produced per gram of feed consumed, average egg weight, and kilograms of feed consumed per dozen eggs produced. These interactions are of a minor nature in that relationships among proteins remain the same in all periods, but relative differences change and result in the interaction being statistically significant. The degree of these interactions is such that they are of minor practical significance and averaging proteins over periods will not mask any relationships among proteins.

All means presented, unless otherwise stated, are averaged over periods and cage type.

Percent Hen-day Egg Production. Means for percent hen-day egg production are presented in Table 25. Additionally, means for each protein averaged over lysine levels are calculated. Comparisons among means were done on a row-by-row basis using Tukey's hsd procedure (Steele and Torrie, 1960). This procedure was applied in the same manner to means of all parameters listed in subsequent tables.

Although contrasts were not made among lysine levels within a protein, production was numerically greatest for hens fed the unsupplemented 10% protein diet at the 0.65% lysine level. For those fed the 12% protein diet, the greatest value for percent hen-day egg production appears to be between the 0.65% and 0.70% lysine levels. No clear

TABLE 25. Effects of proteins and lysine levels on percent hen-day egg production

Lysine level	Means of six periods and two cage types		
	Protein[a]		
	10%	10% +[b]	12%
		%	
0.55%	54.98	54.27	59.48
0.60%	54.13	52.47	64.17
0.65%	59.05	52.61	69.33
0.70%	54.71	49.41	70.54
0.75%	50.84	51.59	67.64
0.80%	53.57	53.12	70.32
Proteins averaged over lysine levels	54.55[d]	52.25[d]	66.91[c]

[a] All diets supplemented to 0.55% total sulfur amino acids with DL-methionine.

[b] Additionally supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.

[c,d] Differing superscripts within a row denote significant differences ($P<0.01$).

maximization of production was observed among the levels of lysine in the 10% protein supplemented diet. Lysine levels averaged over all periods did not significantly influence percent hen-day egg production. Comparisons among the protein means averaged over lysine levels reveal that production for the 12% protein diet was significantly greater than with either 10% protein diet. There was no significant difference between the 10% diets.

Production Expressed as Grams Per Hen-day. Means for production expressed as grams per hen-day are presented in Table 26. Comparisons among the various classes of means were carried out in the same manner as previously described. Significant differences among these means follow the exact same pattern as those described for percent hen-day egg production.

TABLE 26. Effects of proteins and lysine levels on egg mass per hen-day

Lysine level	Means of six periods and two cage types		
	Protein[a]		
	10%	10% +[b]	12%
	g./HD		
0.55%	32.80	33.22	36.84
0.60%	33.00	32.39	39.78
0.65%	35.81	31.92	43.32
0.70%	33.43	30.56	43.29
0.75%	30.71	30.64	41.68
0.80%	33.18	32.08	43.68
Proteins averaged over lysine levels	33.16[d]	31.80[d]	41.43[c]

[a] All diets supplemented to 0.55% total sulfur amino acids with DL-methionine.

[b] Additionally supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.

[c,d] Differing superscripts within a row denote significant differences ($P<0.01$).

<u>Hen-day Feed Consumption</u>. Hen-day feed consumption means are presented in Table 27. The hen-day feed consumption of hens fed the 12% protein diet was significantly greater than that of hens fed the 10% protein supplemented diet and the 10% protein unsupplemented diet. Lysine levels averaged over proteins did not significantly influence feed consumption.

TABLE 27. Effects of proteins and lysine levels on hen-day feed consumption

Lysine level	Means of six periods and two cage types		
	Protein[a]		
	10%	10% +[b]	12%
	g./HD		
0.55%	95.33	95.11	105.79
0.60%	98.96	92.83	106.15
0.65%	99.94	88.98	103.56
0.70%	97.02	89.31	107.51
0.75%	93.35	88.33	104.49
0.80%	95.33	89.03	107.25
Proteins averaged over lysine levels	96.65[d]	90.60[d]	105.79[c]

[a] All diets supplemented to 0.55% total sulfur amino acids with DL-methionine.

[b] Additionally supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.

[c,d] Differing superscripts within a row denote significant differences (P<0.01).

Period-Cage Type-Protein Interaction in Feed Conversion. A significant interaction existed between periods, cage type, and proteins in feed conversion. Means for this factor are presented in Table 28. As shown in the table, no differences existed between the hen's ability to convert either 10% protein diet in both cage types in any period. The interaction existed due to the fact that a significantly reduced conversion of the 10% protein unsupplemented diet was occurring only in certain periods in each cage type. In periods 3 and 6 in the small cages, the 10% protein diet supplemented with isoleucine and tryptophan was not converted by the hens as well as the 12% protein diet

TABLE 28. The influence of periods, cage type, and proteins on feed conversion

Cage type	Period	Protein[a] 10%	10% +[b]	12%
		g. egg/g. feed		
Small	1	0.3182	0.3118	0.3449
	2	0.3233[d]	0.3406[cd]	0.3884[c]
	3	0.3740[d]	0.3411[d]	0.4307[c]
	4	0.3071	0.3495	0.3248
	5	0.3671[d]	0.3980[cd]	0.4285[c]
	6	0.3611[d]	0.3757[d]	0.4772[c]
Large	1	0.2999	0.3122	0.3320
	2	0.3502	0.3359	0.3897
	3	0.3374[d]	0.3466[cd]	0.3980[c]
	4	0.3609	0.3635	0.4022
	5	0.3598	0.3715	0.3831
	6	0.3583[d]	0.3852[cd]	0.4198[c]

[a] All diets supplemented to 0.55% total sulfur amino acids with DL-methionine.

[b] Additionally supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.

[c,d] Differing superscripts within a row denote significant differences (P<0.01).

Influence of Diets on Body Weight and Weight Change. Means for body weights and weight changes of the hens as influenced by protein are presented in Table 29. Hens receiving the 12% protein diet had significantly higher body weights at the end of the experiment and thus a significant weight gain. No differences existed between the 10% diets. The hens on both actually lost weight.

TABLE 29. Influence of proteins on body weights and body weight changes

	Protein[a]		
	10%	10% +[b]	12%
	kg.		
Initial weight	1.52	1.53	1.50
Final weight	1.44[d]	1.40[d]	1.60[c]
Weight change	-0.08[d]	-0.13[d]	+0.10[c]

[a] All diets supplemented to 0.55% total sulfur amino acids with DL-methionine.

[b] Additionally supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.

[c,d] Differing superscripts within a row denote significant differences ($P<0.01$).

Factors Not Influenced by Diets. No significant differences existed among any class of means in average egg weight (Table 30), kilograms of feed consumed per dozen eggs produced (Table 31), Haugh units (Table 32), percent hen-housed mortality (Table 33), or shell thickness (Table 34).

TABLE 30. Effects of proteins and lysine levels on average egg weight

Lysine level	Means of six periods and two cage types		
	Protein[a]		
	10%	10% +[b]	12%
		g.	
0.55%	59.69	61.20	61.90
0.60%	60.79	61.51	61.86
0.65%	60.51	60.52	62.31
0.70%	61.08	61.68	61.17
0.75%	60.27	59.52	61.48
0.80%	61.76	60.34	62.12
Proteins averaged over lysine levels	60.68	60.79	61.81

No significant differences exist among these means.

[a] All diets supplemented to 0.55% total sulfur amino acids with DL-methionine.

[b] Additionally supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.

TABLE 31. Effects of proteins and lysine levels on feed consumed per dozen eggs produced

Lysine level	Means of six periods and two cage types		
	Protein[a]		
	10%	10% +[b]	12%
	kg./dozen		
0.55%	2.18	2.43	2.27
0.60%	2.43	2.16	2.04
0.65%	2.06	2.06	1.83
0.70%	2.20	2.28	1.85
0.75%	2.40	2.25	1.89
0.80%	2.20	2.09	1.87
Proteins averaged over lysine levels	2.25	2.21	1.96

No significant differences exist among these means.

[a] All diets supplemented to 0.55% total sulfur amino acids with DL-methionine.

[b] Additionally supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.

TABLE 32. Effects of proteins and lysine levels on average Haugh unit score

Lysine level	Means of six periods and two cage types		
	Protein[a]		
	10%	10% +[b]	12%
0.55%	78.52	76.39	75.88
0.60%	77.81	75.03	76.48
0.65%	76.06	73.92	75.57
0.70%	76.04	75.04	74.44
0.75%	77.91	76.77	76.86
0.80%	77.88	77.22	74.93
Proteins averaged over lysine levels	77.37	75.73	75.69

No significant differences exist among these means.

[a] All diets supplemented to 0.55% total sulfur amino acids with DL-methionine.

[b] Additionally supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.

TABLE 33. Effects of proteins and lysine levels on total percent hen-housed mortality

Lysine level	Means of two cage types		
	Protein[a]		
	10%	10% +[b]	12%
	%		
0.55%	18.30	1.68	6.66
0.60%	11.64	18.30	8.34
0.65%	11.64	3.30	3.30
0.70%	6.66	16.62	23.28
0.75%	11.64	9.96	4.98
0.80%	11.64	15.00	0.00
Proteins averaged over lysine levels	11.94	10.92	7.74

No significant differences exist among these means.

[a] All diets supplemented to 0.55% total sulfur amino acids with DL-methionine.

[b] Additionally supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.

TABLE 34. Effects of proteins and lysine levels on average shell thickness

Lysine level	Means of six periods and two cage types		
	Protein[a]		
	10%	10% +[b]	12%
	mm.		
0.55%	0.3804	0.3828	0.3837
0.60%	0.3724	0.3723	0.3825
0.65%	0.3811	0.3808	0.3836
0.70%	0.3770	0.3723	0.3770
0.75%	0.3796	0.3732	0.3811
0.80%	0.3845	0.3727	0.3751
Proteins averaged over lysine levels	0.3792[cd]	0.3757[d]	0.3805[c]

[a] All diets supplemented to 0.55% total sulfur amino acids with DL-methionine.
[b] Additionally supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.
[c,d] Differing superscripts within a row denote significant differences ($P<0.05$).

<u>Plasma Free Amino Acid Analysis</u>. Table 35 presents means for plasma free essential amino acid levels of the hens as they were influenced by proteins. As shown in the table, the bird's plasma free threonine, valine, isoleucine, leucine, lysine, and arginine levels were each significantly influenced by proteins. Free levels of methionine, phenylalanine, tryptophan, and histidine in the plasma of the hens were not significantly influenced by proteins. Free threonine plasma levels of hens receiving the 10% protein diet with supplemental isoleucine and tryptophan were significantly lower than those of hens fed the 10% unsupplemented or 12% protein diets. The difference between the free threonine plasma levels of hens fed the 10% protein unsupplemented diet and the 12% protein diet was also significant. The same significant differences were observed among plasma free leucine levels of the hens. Plasma free valine and isoleucine levels followed like trends in that no significant difference existed for plasma levels of either free amino acid between hens receiving the 10% diets. Hens fed both 10% protein diets had significantly lower plasma free amino acid levels than hens fed the 12% diet. Hens fed the 10% diet supplemented with isoleucine and tryptophan had significantly lower plasma free lysine levels than those fed the 10% diet without additional supplementations. Neither 10% protein diet produced plasma free lysine levels significantly different from those of hens receiving the 12% diet. Plasma free arginine levels of hens receiving the 10% protein diet supplemented with isoleucine and tryptophan were significantly lower than that from hens on any other diet. No

TABLE 35. Influence of proteins on plasma free essential amino acid levels

Amino acid	Protein[a] 10%	10% +[b]	12%
	µM/100 ml. plasma		
Threonine	35.71[d]	25.67[e]	42.91[c]
Valine	21.56[d]	18.08[d]	28.50[c]
Methionine	18.94	18.47	21.93
Isoleucine	11.01[d]	12.29[d]	15.24[c]
Leucine	34.23[d]	27.28[e]	39.62[c]
Phenylalanine	24.16	18.67	22.63
Tryptophan	7.40	9.35	10.63
Lysine	47.47[c]	32.38[d]	40.92[cd]
Histidine	25.06	19.77	23.16
Arginine	44.26[c]	34.44[d]	46.95[c]

[a] All diets supplemented to 0.55% total sulfur amino acids with DL-methionine.
[b] Additionally supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.
[c,d,e] Differing superscripts within a row denote significant differences ($P<0.05$).

significant differences existed between plasma free arginine levels of hens fed the 10% protein diet without additional supplementation or the 12% protein diet. A significant interaction exists between cage type and proteins in plasma free total essential amino acid levels of the hens. In both cage types, hens fed the 10% protein diet supplemented with isoleucine and tryptophan had significantly lower plasma free total essential amino acid levels than those on either of the other diets. The interaction is significant due to the fact that the reduction in plasma free total essential amino acid levels of hens fed the 10% protein diet without additional isoleucine and tryptophan in the small cages did not occur among hens in the large cages (Table 36). Table 37 presents means for the influence of dietary lysine levels on plasma free lysine and arginine levels of the hens. Hens fed diets containing 0.80% lysine had significantly higher plasma free lysine levels than those fed diets containing 0.55% or 0.60% lysine. No other significant differences existed among the means for the hens' plasma free lysine levels. The influence of dietary lysine levels on plasma free arginine levels of the hens approached significance.

Egg Albumen Amino Acid Analysis. Analysis of egg albumen from hens receiving the highest and lowest dietary lysine level in each protein and cage type revealed no dietary or cage type influences on albumen content of amino acids. Means for egg albumen amino acid contents are presented in Table 38.

TABLE 36. Influence of cage type and proteins on plasma free total essential amino acid levels

Cage type	Protein[a]		
	10%	10% +[b]	12%
	μM/100 ml. plasma		
Small	253.73[d]	207.01[e]	311.05[c]
Large	285.87[c]	226.04[d]	273.96[c]

[a] All diets supplemented to 0.55% total sulfur amino acids with DL-methionine.

[b] Additionally supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.

[c,d,e] Differing superscripts within a row denote significant differences ($P<0.01$).

TABLE 37. Influence of dietary lysine levels on plasma free lysine and arginine levels

Amino acid	Dietary lysine level					
	0.55%	0.60%	0.65%	0.70%	0.75%	0.80%
	μM/100 ml. plasma					
Lysine	34.61[b]	34.84[b]	41.28[ab]	38.67[ab]	43.31[ab]	49.12[a]
Arginine	46.80	47.61	40.79	38.65	39.48	37.97

[a,b] Differing superscripts within a row denote significant differences ($P<0.05$).

TABLE 38. Influence of proteins and lysine levels on egg albumen amino acid content

Protein	Lysine level	Amino acid								
		Lysine	Histi-dine	Argi-nine	Aspar-tic acid	Threo-nine	Serine	Glu-tamic acid	Pro-line	Gly-cine
		%								
10%	0.55%	0.68	0.23	0.58	0.93	0.40	0.62	1.19	0.32	0.32
10%	0.80%	0.63	0.22	0.54	0.97	0.41	0.61	1.18	0.35	0.32
10% +[a]	0.55%	0.66	0.25	0.58	1.09	0.42	0.64	1.25	0.35	0.33
10% +[a]	0.80%	0.62	0.21	0.50	0.98	0.35	0.66	1.26	0.34	0.33
12%	0.55%	0.60	0.22	0.57	1.00	0.43	0.64	1.29	0.34	0.34
12%	0.80%	0.62	0.25	0.56	0.97	0.42	0.65	1.26	0.34	0.32
Mean		0.64	0.23	0.56	0.99	0.41	0.64	1.24	0.34	0.33
Cotterill *et al.* (1977)		0.66	0.21	0.56	1.09	0.42	0.64	1.32	0.45	0.33

No significant differences exist among the means.

[a] Further supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.

TABLE 38 Continued

Protein	Lysine level	Amino acid: Ala-nine	Half cystine	Valine	Methio-nine	Leucine	Tyro-sine	Phenyl-ala-nine	Iso-leu-cine
		%							
10%	0.55%	0.55	0.24	0.63	0.31	0.78	0.36	0.55	0.47
10%	0.80%	0.55	0.24	0.64	0.35	0.79	0.36	0.56	0.47
10% +[a]	0.55%	0.57	0.25	0.63	0.32	0.81	0.38	0.57	0.50
10% +[a]	0.80%	0.57	0.23	0.64	0.34	0.81	0.38	0.57	0.49
12%	0.55%	0.59	0.25	0.66	0.35	0.83	0.39	0.59	0.51
12%	0.80%	0.57	0.23	0.67	0.39	0.82	0.38	0.58	0.50
Mean		0.57	0.24	0.64	0.35	0.81	0.38	0.57	0.49
Cotterill *et al.* (1977)		0.58	0.30	0.67	0.39	0.83	0.41	0.55	0.51

No significant differences exist among the means.

[a] Further supplemented with 0.075% L-tryptophan and 0.25% DL-isoleucine.

DISCUSSION

Experiment 1

Significant strain-by-diet interactions existed in average egg weight, kilograms of feed consumed per dozen eggs produced, average Haugh unit score, and average shell thickness. From these and other nonsignificant trends in other parameters, it was apparent that the strains differed in their amino acid requirements or at least in their degree of response to supplementation of these low protein diets. The first major difference in the strains was in their methionine requirement. Percent hen-day production of the random-bred birds receiving the lower level of methionine (diet 3) was numerically greater than their controls (Table 3). The apparent adequacy of this methionine level was also reflected in increased feed consumption, hen-day egg mass production, grams of egg produced per gram of feed consumed, and kilograms of feed consumed per dozen eggs produced (Tables 4, 5, 7, and 8, respectively). Plasma free methionine levels in this strain at this methionine level were also a great deal higher than those of the commercial strain (Table 14, diet 3). It appeared that the commercial strain required a higher dietary level of methionine than the random-bred strain in that their percent hen-day production, grams of egg produced per gram of feed consumed, and, significantly, kilograms of feed consumed per dozen eggs produced did not increase until the higher level of methionine (diet 4) was fed. Response of the two strains to the inclusion of threonine in the amino acid supplement did not appear to differ greatly. Both production measures, feed consumption, feed utilization, and efficiency followed similar trends for both strains. A difference did exist in average egg weights (Table 6). Within the commercial strain, additional threonine (diet 6) allowed for the production of eggs of a weight not different from the controls. The random-bred hens, however, produced eggs significantly lighter than their controls when they received diet 6. Plasma free threonine levels, Table 13, reflected a similar response to threonine supplementation for both strains and therefore they apparently do not differ greatly in their threonine requirement.

Although a different requirement for the aforementioned amino acids is the primary reason given for the different responses of the strains, the true situation may not be obvious or even interpretable. The differing requirements need not be only those of methionine. If the requirements for other amino acids added in later supplements (diets 5-7) differ, then similar results could be observed. That is to say that, if threonine, valine, or arginine or any combination of these amino acids were limiting in the 10% diet for the commercial strain but not the random-bred strain, then methionine supplementation would make the diet relatively more balanced for the random-bred strain than the commercial strain. The commercial strain would require supplementation of this amino acid or combination of amino acids before the same balance could be realized. Comparison of diet 3 in the random-bred strain with diets 5 to 7 in the commercial strain reveals similar values for percent hen-day production, feed consumption, hen-day egg mass production, and grams of egg produced per gram of feed consumed.

This point is made as a possible alternative interpretation of these data. It is felt by the author, however, that the major difference between the strains was in their requirement for methionine.

Setting the methionine requirements aside, some other interesting influences of diets showed up when they were averaged over both strains. Primarily, it was the apparent necessity for valine in maintenance of body weight or rather providing for additional body weight gain. This influence of valine in maintenance of body weight supports the findings of Fitzsimmons _et al_. (1963). Differences among means for average Haugh unit score and average shell thickness follow classic trends in that higher producing birds laid lower quality, thinner shelled eggs than those producing at a lower rate. Values observed for albumen amino acid content followed closely those reported by Cotterill _et al_. (1977) and showed no effects of diet.

Experiment 2

Since there were no differences between the 10% protein diets, apparently the additions of tryptophan and isoleucine were not adequate in supplying essential amino acids. Either an imbalance was created or another amino acid was limiting or both. If it is assumed that the lysine requirement remained relatively the same for both the 10% diets, then, as lysine levels were increased, some numerical trends in egg production reflected this situation (Table 25). In the lowest levels of lysine, there was very little numerical difference between the 10% means. At approximately the 0.65% lysine level, the situation changed in that lysine is no longer a limiting amino acid in the 10% diet.

The unsupplemented (i.e., without additional tryptophan or isoleucine) 10% protein diet showed an apparent peak in production at this point. In the 10% protein supplemented diet, the apparent imbalance caused a reduction in egg production. At higher levels of lysine (0.75%-0.80%), it appeared that the excess lysine created an imbalance of some type in the 10% unsupplemented diet, also. The imbalance created when lysine became adequate in the 10% protein diet was not observed in the 12% protein diet. As lysine became adequate in the 10% protein diet, another amino acid or group of amino acids may have become limiting. This situation was not corrected by additions of isoleucine and tryptophan. If these amino acids had any effects, they were negative in this situation. This does not mean that these amino acids were not limiting in the 10% protein diet, rather it means that other amino acids were more or equally limiting and would require supplementation. Results of Experiment 1 indicate that the primary amino acid that may be needed in the 10% protein diet would be threonine. The fact that the hen's plasma free threonine levels were lowered in the 10% protein diet by the addition of isoleucine and tryptophan also indicated that this amino acid was needed also before the imbalance could be corrected (Table 35). Valine apparently was needed in the 10% diet as the hens fed these diets had lower plasma free amino acid levels. The diet analysis revealed that the 10% protein diet contained much less valine than the 12% protein diet and slightly less threonine (Table 2). The 10% protein diet would probably require supplementation of both of these amino acids to correct an imbalance. The limiting order of these amino acids cannot be established from these data, only that the hens had differing plasma free amino acid levels and that the diets differed in their content of valine and threonine.

The methionine requirement of the hens was apparently met by all diets, as no differences were observed in their plasma free methionine levels (Table 35). Due to a high degree of variability, the plasma free tryptophan levels of the hens were not significantly influenced by the proteins. The supplementation of isoleucine and tryptophan to the 10% protein diet did cause a numerical increase in the hens' plasma free tryptophan levels. Had the 10% protein diet with supplemental isoleucine and tryptophan been more balanced, the hens' plasma free tryptophan levels when they were fed the 10% protein diet supplemented with isoleucine and tryptophan may not have been lower than the level observed when they received the 12% protein diet. The hens' plasma free lysine and arginine levels were both reduced by supplementation of the 10% protein diet with isoleucine and tryptophan. The exact cause of this reduction is not clear, but this may be due to the reduced consumption of the 10% protein diet with supplemental isoleucine and tryptophan. Reduced consumption of the diet may also explain the reduction in the hens' plasma free leucine levels. Failure of the hens to consume adequate amounts of the 10% protein diet supplemented with isoleucine and tryptophan may also explain the hens' reduced plasma free total essential amino acid levels when they were fed this diet (Table 36). Only in the small cages was the plasma free total essential amino acid level lower for hens fed the 10% protein diet without supplemental isoleucine or tryptophan than that of hens fed the 12% protein diet. The important fact is that in all cages the supplementation of the 10% protein diet with isoleucine and tryptophan served to create an imbalance such that the hens' plasma free total essential amino acid levels when they received this diet were lower than that of those receiving either the 10% protein diet without supplemental isoleucine and tryptophan or the 12% protein diet.

The fact that the diet analysis revealed only a 1% difference in crude protein between the 10% and 12% protein diets (Table 2) and such great differences in responses were obtained with the diets suggests that the requirements for many amino acids are between the levels of these amino acids in each diet. The apparent adequacy of the 12% protein diet compared to the 10% protein diet was reflected in the final body weights of the hens and in their weight change (Table 29). Hens fed either of the 10% protein diets lost weight, probably because of the amino acid imbalance for protein synthesis. However, hens fed the 12% protein diet were able to gain weight, thus reflecting a balanced protein.

As seen in previous studies, consumption of a diet is readily influenced by its amino acid balance. There is a consistent but not significant trend for reduced consumption of the supplemented 10% protein diet compared to the nonsupplemented 10% protein diet (Table 27). The consumption of the 10% protein supplemented diet was lower than that of the 12% protein diet at and above the 0.65% level of lysine. This is the point where the lysine requirements were met in all diets. The greatest change in the hens' plasma free lysine levels occurred between the 0.60% and 0.65% dietary lysine level. Statistical evidence suggested that the requirement was between 0.60% and 0.80% dietary lysine, in that the hens' plasma free lysine levels were significantly higher when they received diets with these lysine levels (Table 37). It was apparent, though, from the great increase in the plasma free lysine levels of the hens when the dietary lysine level increased from 0.60% to 0.65% that this was the point where the lysine requirement was met. The influence of dietary lysine levels on plasma free arginine levels of the hens approached significance and it appears that an excess of lysine may have been provided in diets containing higher lysine levels. Generally, as dietary lysine levels were increased, the hens' plasma free arginine levels decreased. This follows the general trend described in earlier sections of this report concerning the inter-relations of lysine and arginine.

The effects of protein level on grams of egg produced per gram of feed consumed were significant only in some periods (Table 28). The trend for improved utilization of the 12% protein diet was present but not significant in all periods. The failure of these differences to be significant when utilization was measured as kilograms of feed consumed per dozen eggs produced (Table 31) may reflect the relatively more limited accuracy of this statistic. The same numerical trends were present, however.

Average egg weights were not significantly different for any treatment. Any numerical differences were inconsistent (Table 30) and of no practical importance. All weights were adequate such that the eggs produced would be classified large. No significant differences existed among means for Haugh units (Table 32). All means were above a score of 72 which is required for grade AA.

Hen-housed mortality means (Table 33) showed no consistent trend and the numerical differences probably reflect the relatively low sensitivity in measuring mortality with the small numbers of hens on each diet. That is to say in a group of 36 hens, one death represented 2.78% hen-housed mortality in that period. The mortality for the entire flock was 10.14%, which can be considered below normal.

Differences in egg albumen content due to dietary factors had been noted in other experiments (Choudhury, 1972), but the characterization of these influences was uncertain. The means for egg albumen content of all amino acids (Table 38) agreed closely with those of Cotterill et al. (1977) and no dietary or cage type influences existed.

In Experiment 2, some information was available on the influence of cage type or group size on performance of the hens. However, this was not designed to be an experiment to investigate these influences and any information applies only to hens fed low protein diets. The primary significant effects of cage type were upon feed consumption, body weight, and average egg weight. Hens housed six per cage consumed 5 g. more feed per hen-day, laid eggs that were 1 g. heavier, and weighed 0.07 kg. heavier at the end of the study than did those housed two per cage (Table 39). No significant effects of cage type existed

TABLE 39. Influence of cage type on production parameters

Cage type	Parameter: Hen-day production (%)	Hen-day feed consumption (g.)*	Average egg weight (g.)*	Final body weight (kg.)*
Small	57.35	95.7	60.7	1.45
Large	58.74	100.7	61.7	1.52

* Difference in means is significant (P<0.01).

among means for any other parameters except those already discussed for feed conversion and plasma free total essential amino acid levels. A direct interpretation of these differences might not be applicable to a normal situation, but some differences nevertheless exist. The cause of the observed influences are not discernible from the observations taken in this study. All hens had an equal amount of floor and feeder space. There was one water cup per three hens in the large cages and one water cup per four hens in the small cages. The major differences noted between the cage types were in group size and feeder height. The small cages held two birds and the large cages held six birds. The feeder trough, although of identical design, was 3 in. higher for the large cages than for the small cages. Overall depth of the cages was the same in each type. The fact that the hens in the large cages had greater final body weights reflects their increased feed consumption.

Research on the influence of cage design on laying hen performance has only recently become quite active. The influences observed in this study tend to follow those reported by Hill and Hunt (1977). These workers found hens in shallow cages to produce heavier eggs and consume more feed than those in deep cages. Lee and Bolton (1976), however, observed hens in shallow cages to consume less feed than those in deep cages. The cages used in this study do not fall into the categories of shallow and deep. Discussions of cage influences on layer performance point out feeding pattern and bird orientation as possible causes of the observed differences (Hughes and Black, 1976). Also included as possible factors are mortality, cannibalism, and antagonistic behavior. All aspects of cage design which may influence performance of laying hens need further and more precise investigation.

SUMMARY

Two experiments were undertaken to examine the influence of supplementation of essential amino acids to low protein layer diets. Criteria used to evaluate the performance of the layers were egg production, egg mass, feed consumption, egg quality, mortality, plasma-free amino acids, and albumen amino acid content. In the first study, commercial and random-bred strains of layers were used. Birds of treatment 1 were fed a standard 16% protein layer diet and hens in treatment 2 received a 10% protein diet. The diet used for treatment 3 was the 10% protein diet supplemented with 0.20% L-lysine, 0.25% DL-isoleucine, 0.15% DL-tryptophan, and 0.30% DL-methionine. The diets of treatments 4 through 7 were as treatment 3 with cumulative supplements of 0.10% DL-methionine, 0.20% DL-valine, 0.20% DL-threonine, and 0.20% L-arginine, respectively.

Performance of the laying hens was highly variable. Results indicated a differing requirement in the strains for methionine. Both strains responded to threonine supplementation in terms of increased egg mass and valine was needed for maintenance of body weight.

In Experiment 2, another strain of commercial layers was fed 10% protein and 12% protein diets. All diets were supplemented to 0.55% total sulfur amino acids with DL-methionine. A third diet was created by supplementation of the 10% protein diet with 0.25% DL-isoleucine and 0.075% L-tryptophan. Within each of these proteins, diets were mixed to contain six levels of lysine (0.55%, 0.60%, 0.65%, 0.70%, 0.75%, and

REFERENCES

Anderson, J. O., and D. C. Dobson, 1959. Amino acid requirements of the chick. 2. Effect of total essential amino acid level in the diet on arginine and lysine requirements. Poultry Sci. 38:1140-1150.

Austic, R. E., and M. C. Nesheim, 1970. Role of kidney arginase in variations of the arginine requirement of chicks. J. Nutr. 100:855-868.

Austic, R. E., and R. C. Scott, 1975. Involvement of food intake in the lysine-arginine antagonism in chicks. J. Nutr. 105:1122-1131.

Baker, D. H., 1976. Nutritional and metabolic interrelationships among sulfur compounds in avian nutrition. Fed. Proc. 35:1917-1922.

Baker, D. H., and D. J. Bray, 1972. Comparison of supplemental sulfur amino acid sources for growing chicks fed a corn-soybean meal diet. Poultry Sci. 51:1982.

Biely, J., and B. E. March, 1964. Protein level and amino acid balance in the laying ration. Poultry Sci. 43:98-105.

Blair, R., D. J. W. Fisher, G. Fisher, and Caroline C. McCorgnodale, 1976. Responses of laying hens to a low protein diet supplemented with essential amino acids, L-glutamic acid and/or intact protein. Brit. Poultry Sci. 17:427-440.

Boldizsar, H. K., K. N. Boorman, and P. J. Buttery, 1973. The effect of excess leucine on valine catabolism in the chick. Brit. Poultry Sci. 30:501-510.

Boomgaardt, John, and D. H. Baker, 1970. The lysine requirement of the chick at five protein levels. Poultry Sci. 49:1369.

Boorman, K. N., and Hans Fisher, 1966. The arginine-lysine interaction in the chick. Brit. Poultry Sci. 7:39-44.

Bray, D. J., 1960. Studies with corn-soya laying diets. 2. Optimum combinations of corn and soybean protein. Poultry Sci. 39:1541-1545.

Bray, D. J., 1964. Studies with corn-soya laying diets. 7. Limiting amino acids in a 60:40 blend of corn and soybean protein. Poultry Sci. 43:396-401.

0.80%). Birds were housed in two types of cages, small cages with two birds per cage and large cages with six birds per cage.

The results indicate that the lysine requirement of the hens was met by diets containing 0.65% lysine. The 10% protein diet was imbalanced by the supplementation of isoleucine and tryptophan and apparently required further supplementations of valine and threonine.

The hens kept in large cages consumed more feed, laid heavier eggs, and had heavier final body weights than those kept in small cages.

Bray, D. J., 1968. The effect of the ratio of corn to soybean protein in layer diets upon response to supplemental amino acids. Poultry Sci. 47:815-821.

Bray, D. J., and J. D. Garlich, 1960. Studies with corn-soya laying diets. 1. Amino acid supplementation of low protein diets. Poultry Sci. 39:1346-1349.

Britzman, D. G., 1964. Studies on the limiting amino acids in laying hen diets. Ph.D. Thesis. South Dakota State University, Brookings.

Carlson, C. W., and E. Guenthner, 1969. Response of laying hens fed typical corn-soy diets to supplements of methionine and lysine. Poultry Sci. 48:137.

Chi, M. S., and G. M. Speers, 1976. Effects of dietary protein and lysine on plasma amino acids, nitrogen retention and egg production in laying hens. J. Nutr. 106:1192-1201.

Choudhury, H., 1972. Amino acid supplementation of low protein layer diets. Ph.D. Thesis. South Dakota State University, Brookings.

Cotterill, O. J., W. M. Marion, and E. C. Naber, 1977. A nutrient re-evaluation of shell eggs. Poultry Sci. 56:1927-1934.

D'Mello, J. P. F., and D. Lewis, 1970. Amino acid interactions in chick nutrition. 2. Interrelationships between leucine, isoleucine, and valine. Brit. Poultry Sci. 11:313-323.

Featherston, W. R., and G. W. Horn, 1973. Dietary influences on the activities of enzymes involved in branched chain amino acid catabolism in the chick. J. Nutr. 103:757-765.

Featherston, W. R., and J. C. Rogler, 1978. Methionine-cystine interrelations in chicks fed diets containing suboptimal levels of methionine. J. Nutr. 108:1954-1958.

Fisher, Hans, and D. Johnson, Jr., 1956. The amino acid requirement of the laying hen. I. The development of a free amino acid diet for maintenance of egg production. J. Nutr. 60:261-274.

Fitzsimmons, R. C., P. E. Waibel, and D. C. Snetsinger, 1963. Studies on the amino acid and soybean protein requirements of white leghorn hens fed diets composed principally of corn. Poultry Sci. 42:202-206.

Grau, C. R., 1948. Effect of protein level on the lysine requirement of the chick. J. Nutr. 36:99-108.

Halloran, H. R., and H. J. Almquist, 1978. Lysine requirement for laying hens. Feedstuffs 50(32):18-19.

Hill, A. T., and J. R. Hunt, 1977. Cage depth effects on layer performance. Poultry Sci. 51:1722.

Hughes, B. O., and A. J. Black, 1976. Battery cage shape: its effect on diurnal feeding pattern, egg shell cracking, and feather picking. Brit. Poultry Sci. 17:327-336.

Johnson, D., Jr., and Hans Fisher, 1956. The amino acid requirements of the laying hen. II. Classification of the essential amino acids required for egg production. J. Nutr. 60:275-282.

Johnson, D., Jr., and Hans Fisher, 1959. The amino acid requirement of laying hens. 4. Supplying minimal levels of essential amino acids from natural feed ingredients. Poultry Sci. 38:149-152.

Jones, James D., 1964. Lysine-arginine antagonism in the chick. J. Nutr. 84:313-321.

Jones, J. D., S. J. Petersburg, and P. C. Burnett, 1967. The mechanism of the lysine-arginine antagonism in the chick: Effect of lysine on digestion, kidney arginase, and liver transamidinase. J. Nutr. 93:103-115.

Kashani, A. B., 1975. Amino acid additions to a low protein corn-soy diet for egg production. M.S. Thesis. South Dakota State University, Brookings.

Kashani, A. B., 1978. Effects of strain on response of laying hens to low protein diets and amino acid supplements. Ph.D. Thesis. South Dakota State University, Brookings.

Lee, D. J. W., and W. Bolton, 1976. Battery cage shape: The laying performance of medium and light body weight strains of hens. Brit. Poultry Sci. 17:321-326.

Leong, K. C., and J. McGinnis, 1952. An estimate of the methionine requirement for egg production. Poultry Sci. 31:692-695.

Lunven, P., C. LeClement De St. Moreg, E. Carnovale, and A. Fratoni, 1973. Amino acid composition of hen's egg. Brit. J. Nutr. 30:189-194.

March, B. E., and J. Biely, 1963. Control of egg size in chickens through dietary amino acid balance. Nature 200:702-703.

Marin, O. M., J. W. Bradley, and J. H. Quisenberry, 1971. Amino acid supplementation of low protein laying diets. Poultry Sci. 50:1600.

Mondino, Angelo, Gianni Bongiovanni, Silvano Fumero, and Lucia Rossi, 1972. An improved method of plasma deproteination with sulphosalicylic acid for determining amino acids and related compounds. J. Chromatography 74:255-263.

Muller, R. D., and S. L. Balloun, 1974. Response to methionine supplementation of leghorn hens fed low protein corn-soy meal diets. Poultry Sci. 53:1463-1475.

Muller, R. D., and S. L. Balloun, 1976. Leucine imbalance in corn-soybean meal diets for laying hens. Poultry Sci. 55:487-496.

National Research Council, 1977. Nutrient Requirements of Poultry. 7th Ed. National Academy of Science, Washington, D. C.

Nesheim, M. C., 1968. Kidney arginase activity and lysine tolerance in strains of chickens selected for a high or low requirement of arginine. J. Nutr. 95:79-87.

O'Dell, B. L., C. L. Limbaugh, and J. E. Savage, 1962. Arginine-lysine antagonism and deficiencies of casein for the chick. Fed. Proc. 21(2):8.

O'Dell, B. L., and J. E. Savage, 1966. Arginine-lysine antagonism in the chick and its relationship to dietary cations. J. Nutr. 90:364-370.

Reid, B. L., and C. W. Weber, 1973. Dietary protein and sulfur amino acid levels for laying hens during heat stress. Poultry Sci. 52:1335-1343.

Reid, B. L., and C. W. Weber, 1974. Lack of sulfur amino acid sparing effect with ammonium sulfate and sodium sulfate in laying hen diets. Poultry Sci. 53:964-969.

Scott, J. T., C. R. Creger, and John W. Bradley, 1975. Supplementary methionine and energy in low protein diets for laying hens. Nutrition Reports International 12(4):221-225.

Sekiz, S. S., M. L. Scott, and M. C. Nesheim, 1975. The effect of methionine deficiency on body weight, food and energy utilization in the chick. Poultry Sci. 54:1184-1188.

Smith, R. E., 1968. Effect of arginine upon the toxicity of excesses of single amino acids. J. Nutr. 95:542-553.

Smith, T. K., and R. E. Austic, 1978. The branched-chain amino acid antagonism in chicks. J. Nutr. 108:1180-1191.

Smith, G. H., and D. Lewis, 1966. Arginine in poultry nutrition. 3. Agent and target in amino acid interactions. Brit. J. Nutr. 20:621-631.

Snetsinger, D. C., and H. M. Scott, 1961. Efficacy of glycine and arginine in alleviating the stress induced by dietary excesses of single amino acids. Poultry Sci. 40:1675-1681.

Stangeland, V. A., and C. W. Carlson, 1961. The effect of supplemental methionine, lysine, and furazolidone in low protein laying rations. Poultry Sci. 40:1460.

Steele, R. G. D., and G. H. Torrie, 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York.

Tasaki, I., and T. Ohno, 1971. Effect of dietary protein level on plasma free amino acids in the chicken. J. Nutr. 101:1225-1232.

Waibel, P. E., R. C. Fitzsimmons, and D. C. Snetsinger, 1961. Amino acid supplementation of corn meal when used as the sole source of protein in laying rations. Poultry Sci. 40:1466.

Waibel, P. E., and E. L. Johnson, 1961. Effect of low protein corn-soybean oil meal diets and amino acid supplementation on performance of laying hens. Poultry Sci. 40:293-298.

Waldroup, P. W., and R. H. Harms, 1961. Amino acid supplementation of diets for commercial egg production type laying pullets. Poultry Sci. 40:1466-1467.

Wilcox, J. S., 1934. The nitrogen balance of laying hens. J. Agr. Sci. 24:636-648.