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Amino Acid Supplementation of Low Protein Layer Diets

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AMINO ACID SUPPLEMENTATION OF LOW PROTEIN LAYER DIETS

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable as 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.

AMINO ACID SUPPLEMENTATION OF LOW PROTEIN LAYER DIETS
Abstract

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Under the supervision of Professor C. W. Carlson

Experiments were conducted at the South Dakota State University Poultry Research Center to study the effects of amino acid supplementation of low protein diets on performance of laying type hens. Hens, housed in 8-inch cages, were fed at 24 weeks of age typical 16% protein corn-soy diets diluted with glucose to 10.8% protein and supplemented with 0.15% DL-methionine, 0.19% L-lysine and 0.04% DL-tryptophan. After about 20 weeks of depletion, supplements of 0.05 and 0.1% DL-threonine and DL-valine and 0.1% DL-isoleucine alone and in all combinations were fed for a further period of 16 weeks using a factorial design.

Individual supplements of threonine and valine did not show any improvement in egg production, feed consumption, feed conversion or egg quality. Isoleucine at the 0.1% level showed a highly significant improvement ($P < .01$) in egg production. Both low and high levels of threonine and valine when supplemented in equal proportions showed improved egg production, but it was not superior to that observed with isoleucine supplementation alone. Egg size and egg quality were not influenced by the above dietary supplementations.

Based on the above performance, hens in a second study were fed the above basal diet further supplemented with 0.1% DL-isoleucine. They satisfactorily maintained egg production comparable to that of hens receiving 0.1% DL-isoleucine supplementation in Experiment 1. Supplemental

effects of single and combined 0.05 and 0.1% levels of threonine and valine with 0.25 or 0.5% glycine supplements were studied for a 16-week period, following a 16-week depletion period. Neither the low nor high level of glycine showed any improvement in egg production or feed efficiency. The combined low levels of glycine and valine were somewhat effective in improving egg production and feed efficiency. However, hens on the combined high levels of glycine and threonine laid at a 10% higher rate more efficiently than hens on the basal diet. Plasma levels of free amino acids somewhat reflected the effects of dietary supplementation upon egg production and indicated that 0.1% threonine effectively supplemented the basal diet. The excess dietary glycine probably caused more effective utilization of threonine for increased egg protein synthesis. Hens fed the 10.8% protein diet supplemented with amino acids produced eggs with less essential amino acids in the albumen than hens fed a 16% protein diet. This indicates that further work is needed on the low protein diet to produce eggs with more optimum amino acid levels.

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HC

TABLE OF CONTENTS

LIST OF TABLES

Table		Page

LIST OF APPENDIX TABLES

INTRODUCTION

Protein supplements account for approximately one-third of the cost of layer diets and are becoming increasingly more expensive and of limited supply. Increasing demand for high protein food products in human diets will further limit the availability of protein supplements for animal feeds. Scientists, therefore, are attempting to develop new ways of reducing and/or changing the need of high protein supplements for livestock and poultry.

There are several factors that affect the protein requirement of hens. These include the amino acid composition of the protein, the level of the energy in the diet, strain of birds, environmental temperatures and perhaps many other factors which all contribute to the nutritional requirements. Completely satisfactory answers to the questions of requirements have not yet been achieved.

Under practical conditions, feeds used for egg production are most commonly formulated to provide 16 to 18% protein derived primarily from corn and soybean meal. Most commonly the protein requirements or the amino acid needs of hens have been met by increasing the amounts of total protein in the diet. However, this procedure could be more expensive and less satisfactory from a nutritional standpoint than one using a minimum protein level and supplementing with amino acids (Scott, 1963). Studies at the South Dakota State University Agricultural Experiment Station have shown the effectiveness of glucose as an ideal diluent in a corn-soy diet. The methionine and lysine content of the ration was adequately supplied in this study to maintain fair

production (Britzman and Carlson, 1965). Novacek and Carlson (1969), using a factorial study of several amino acids and their combinations, observed that a corn-soy diet diluted to 9.4% protein with glucose could support 60% hen-day egg production for a period of 10 weeks when supplemented with 0.25% DL-methionine, 0.2% L-lysine and 0.04% DL-tryptophan.

Our work attempted to determine which amino acids besides methionine, lysine and tryptophan are further limiting for egg production for hens on the low protein corn-soy diet diluted to 10.8% protein by glucose. Based on the amino acid requirements of hens (N.R.C., 1971; Scott et al., 1969), factorial types of experimental plans were used in studies to investigate the requirement levels of essential and non-essential amino acids. The criteria for evaluating performance were hen-day egg production, egg weight, feed efficiency, Haugh units (Haugh, 1937), plasma levels of the free amino acids and amino acid composition of egg white.

REVIEW OF LITERATURE

The concept of the nutritive value of protein was pioneered by Voitt (1866), although the subject apparently remained closed until early 1914 when Osborne and Mendel introduced the term "biological value" of proteins. In later studies Osborne et al. (1919), while working with zein, tryptophan and lysine and glutein-lysine diets, demonstrated the indispensability of these amino acids for supporting normal growth. Mitchell (1924) modified the pioneer studies of Thomas (1909) concerning the biological value of proteins. Later Mitchell and Block (1946) suggested that the value of proteins be expressed on the basis of an amino acid chemical score. In a series of experiments, Rose et al. (1948) demonstrated the essentiality of eight amino acids in purified diets and contributed significantly in grouping the essential and nonessential amino acids. Studies involving crystalline amino acids in poultry rations were conducted by Johnson and Fisher (1956) to determine the amino acids essential for laying hens. They found arginine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine to be essential for egg production. With the exception of glutamic acid, omission of any one of the above amino acids resulted in immediate disruption of feed consumption and a 10-day pause in production. This occurred even when such incomplete diets were forced-fed for only 5 days. Although feed consumption was not affected by the deletion of glutamic acid, normal egg production could not be maintained. The hens in these studies failed to show any response in egg production to

glycine supplementation. This particular aspect of their study was in agreement with the results of Menge and Lillie (1956).

In later studies, Johnson and Fisher (1958) investigated the amino acid requirements of the laying hen. Lysine was chosen to be the base for calculating other essential amino acid requirements because it does not take part in transamination reactions and has no known function other than protein synthesis. After establishing the lysine requirement of the laying hen to be 0.5% of the diet, other essential amino acids were included in the diet in amounts proportional to the ratio of their content in the whole egg. Such a diet supported good production. The results of these studies have been the basis for setting up many subsequent experiments to study and evaluate different protein sources as sources of amino acids for egg production. Also of concern have been the several amino acid interrelationships in different poultry rations. In the meantime, new facets of information were sought to be correlated with the results of various growth studies for a better understanding of amino acid interrelationships. Longenecker and House (1959) devised a formula as follows to express the plasma amino acid ratio after feeding a test protein:

$$\text{Plasma amino acid ratio} = \frac{\text{Plasma concentration after feeding} - \text{Fasting concentration}}{\text{Amino acid requirement}} \times 100$$

Hill and Olsen (1963) used the concentrations of plasma amino acids after feeding a protein-free diet in place of the fasting values used in the above formula. This modified technique contributed to determining which were the first and second limiting amino acids in various

situations. From growth studies by Hill *et al.* (1961), it was predicted that the amino acids lysine, tryptophan and arginine were first limiting in diets in which the protein was supplied by soybeans plus zein, soybeans plus zein plus lysine and soybeans plus gelatin and casein, respectively. The predictions for the first limiting as well as the second limiting amino acids in the above diets were quite correct when compared with the growth studies. However, Hill and Olsen (1963) concluded that this approach was limited primarily to the detection of the first and second limiting amino acids. These early studies provided the basis for later studies which have used modern techniques and have contributed much knowledge concerning amino acids and protein nutrition. The approach in this review will be limited to discussing the amino acid requirements for egg production.

Amino Acid Requirements

It is well established that factors such as energy content of the diet, temperature and various stress conditions will alter feed intake and hence influence the level of dietary protein required for optimum performance. It is also quite clear that the protein requirement is equivalent to an adequate supply of amino acids for optimum growth, maintenance and production. In fact, amino acid requirements rather than protein requirements should be the primary consideration. Scientists in the past have used different methods to express amino acid and protein interrelationships in the diet. The requirements for amino acids sometimes have been given as a percentage of the total diet and sometimes as a percentage of the protein in the diet. When

expressed as a percentage of the protein in the diet, the amino acid requirement generally drops as the level of the protein in the diet is increased above that needed to furnish adequate amounts of other amino acids. However, as the percentage of dietary protein is increased, the minimum required levels of most essential amino acids also appear to be increased when they are expressed as a percent of the diet. Combs (1969) suggested that this was due to the effect of protein level in increasing voluntary food consumption.

Successful determination of amino acid requirements (Mitchell, 1950) for growth using carcass analysis led to investigations on the relationships between egg protein composition and amino acid requirements for egg production. Using information on the composition of hens' eggs, studies were conducted by Johnson and Fisher (1958) designed to reduce all amino acids to minimal levels for egg production. These scientists estimated the required minimum levels of the essential amino acids using the ratios of amino acids to lysine in whole egg protein as the basis. These levels of amino acids thus determined were fed to laying hens and compared with the same levels increased by 10%. The lower levels were found to maintain a high rate of egg production. Further studies (Johnson and Fisher, 1959) were conducted in attempts to relate the findings observed with synthetic amino acid diets with practical diets. Practical diets with only 10 to 11% protein did maintain a high rate of egg production in hens over a prolonged period. Later reports (Bray, 1960; Fitzsimmons <u>et al</u>., 1963) also supported the above findings.

Low Protein Concept

Nutrients which are most costly in laying hens' diets are energy and protein or amino acids. Since hens adjust their food intake according to energy need, protein and amino acid supplementations should be most critical with respect to cost. Therefore, it was important to determine the amount of these costly nutrients that should be supplied in order to have the most economical production system. Scott (1960) suggested that the high protein requirement of hens was correlated with the high content of certain essential amino acids in egg proteins, specifically methionine, lysine, isoleucine and valine. He further commented that to supply limiting amino acids by increased amounts of total protein was less satisfactory from a nutritional standpoint than using a minimum protein level and supplementing amino acids according to their requirements. Earlier studies (Thornton and co-workers, 1956, 1957) indicated no significant difference in egg production when four different "all vegetable" basal rations for laying hens containing 11, 13, 15 and 17% protein were studied. These workers suggested that the required protein level for egg production of caged layers may be only 13% or less. The results of a Wisconsin study (Miller et al., 1957) indicated optimum performance from a 12.9% protein level in supporting egg production. A protein level of 12% in a corn-soy diet also supported satisfactory egg production (Adams et al., 1958) when compared to that from hens on higher protein diets.

Frank and Waibel (1960), using 10.2, 12.4, 14.9, 19.9 and 29.9% protein diets, observed that diets of 12.4 and 14.9% protein, with

low and high energy levels, respectively, supported normal egg production. Previous research at South Dakota State University indicated that a 14% protein diet with methionine supplementation supported good egg production and was comparable to that of hens on a 16% protein diet without methionine (Carlson and Guenthner, 1969). Thus, reports discussed herein have favored a proposal that the protein requirement of the laying hen is less than 15% of the diet. A diet low in protein (10 to 11%) with ideal amino acid composition could support fairly normal egg production (Johnson and Fisher, 1969; Fisher et al., 1960).

Amino Acids Needed With Low Protein Diets for Egg Production

It seems logical to discuss the various amino acid interactions in diets where the protein level is minimal rather than maximal. Using an 11% protein corn-soy diet with 3% fish meal, Waldroup and Harms (1961) observed lysine to be first limiting and methionine second limiting when fed to commercial egg laying pullets. Results from their later studies (Harms and Waldroup, 1962) were in agreement with that reported earlier. Yates and Schaible (1969), using an 11% protein corn-soy diet, observed a positive effect from methionine supplementation but not with lysine. The combination of lysine with methionine was of no added benefit to rate of production. However, egg size was larger with the 16% protein control diet and the 11% protein ration containing both lysine and methionine than with the unsupplemented or singly supplemented diets. Stangeland and Carlson (1961) fed 11% protein corn-soy diets to laying hens and did not observe improvements in egg production from supplemental methionine, whereas the combination

of methionine and lysine consistently improved egg production and feed efficiency. Results of a similar study (Waibel and Johnson, 1961) indicated that methionine and lysine additions to a 10% protein diet improved egg production but not to the extent of that observed with the 16% protein control. Hens receiving 0.04% tryptophan in addition to methionine and lysine showed an increased rate of egg production of 3.2%, while 0.15% added valine depressed egg production. Lewis (1966) compared 12.5% and 14.5% protein diets and observed a response from methionine and lysine supplements in the 12.5% protein diet but not with the 14.5% protein diet.

In a further study by Bray and Garlick (1960), hens were fed a corn-soy diet with a 9% protein and the amino acids tryptophan, lysine, methionine, cystine, isoleucine and valine were supplemented to meet the minimal levels suggested by Johnson and Fisher (1958). The egg production resulting from these supplements was significantly improved over the 9% protein control but was not comparable to that observed with a 16% protein diet.

Research conducted at the South Dakota Experiment Station by Britzman and Carlson (1963, 1965) was designed to investigate the supplemental effects of the limiting amino acids for egg production in low protein diets having calorie-protein ratios identical to typical 16% protein diets made from corn and soybean meal. Methionine supplementing the 11% protein corn-soy diet allowed for improved egg production, but it was not equal to that observed with the 16% protein diet. Cumulative effects of methionine, lysine, glycine, valine,

arginine, isoleucine and tryptophan in 10 and 11% protein diets with approximately 2200 Cal. M.E./kg. were studied. Only methionine improved egg production, whereas the other amino acids did not affect laying hen performance.

Bray (1964a,b), using a diet with a 60:40 blend of protein from corn and soybean meal to supply 8.5% protein, found isoleucine and lysine to be the most limiting essential amino acids. The response to supplemental methionine decreased progressively as soybean protein was replaced by corn protein. The above diet was further studied (Bray, 1968) to evaluate the supplemental effects of methionine, tryptophan, lysine, isoleucine and valine. It was reported that, when tryptophan, lysine and isoleucine were individually deleted from the diet, the deletion of isoleucine alone caused the greatest decline in egg yields. The deletion of tryptophan and lysine caused lesser but similar declines. When the diet was made 125% adequate in all essential amino acids by the egg protein ratio requirement standard and thus brought up to a 1.92% nitrogen level (11.97% protein) with glutamic acid and fed to hens, egg production was observed to be similar to that of hens on an 18% protein corn-soy diet (Bray, 1969).

Further studies with similar objectives but using somewhat different diets have been carried out at South Dakota (Novacek and Carlson, 1969). Because it appeared to be an ideal diluent, the corn sugar glucose (Britzman and Carlson, 1965) was included in this work. Laying hens showed improved production due to supplementation with methionine, lysine and tryptophan, but cumulative supplements of

arginine, isoleucine, valine and diammonium citrate failed to give any additional improvement. Efforts were also made to improve production from this low protein (9.4%) diet by incorporating 3 or 6% protein equivalents from yellow corn, spring wheat, barley, soybean meal, soybean protein, fish meal, feather meal and meat and bone scraps. None of the supplements improved egg production over that observed with hens receiving the 9.4% protein diet with added methionine, lysine and tryptophan, although they increased egg size.

The absence of a response from diammonium citrate in this study was in agreement with the results of Moran et al. (1967). However, Young et al. (1965) and Reid et al. (1972) showed beneficial responses from the addition of nonprotein nitrogen to suboptimal protein diets.

The results reported from Cornell University (Young et al., 1965) indicated that egg production by hens on a 13% protein diet supplemented with diammonium citrate was comparable to that observed with a 16% protein diet. Using glutamic acid and glycine supplements, Shapiro (1968) reported that 55% of the total dietary protein could come from nonessential amino acid nitrogen for peak nitrogen retention. In a later study, Cornell workers (Manouks and Young, 1969) investigated the supplementation of a 12.5% protein diet which contained theoretically adequate amounts of essential amino acids for maximum egg production. When this diet was supplemented with a nonessential amino acid mixture of alanine, aspartic acid and glycine, a significant improvement in egg production was noted. In another phase of this experiment, hens fed a 12.5% protein diet supplemented with a 1.5%

nonessential mixture and 1.2% diammonium citrate produced eggs at a level equal to hens fed the 3% nonessential amino acids and equivalent to those fed a 15.5% protein diet. The results of these experiments also showed that egg production could be maintained in hens fed diets with an essential to nonessential amino acid ratio much lower than that found in egg protein.

In a recent study, Reid et al. (1972) observed that the minimum essential and nonessential amino nitrogen for optimum egg production could be in the range of 36 to 44% and 56 to 63% of the total protein nitrogen in the diet, respectively. They further commented that the conversion of essential amino nitrogen to nonessential amino nitrogen was not sufficient to meet the requirement of metabolic nitrogen. The nonprotein nitrogen additions in their study exerted a sparing effect on essential amino acid nitrogen.

Significance of Free Amino Acids in Plasma

Several studies in the past (Charkey et al., 1953; Richardson et al., 1953; Hill and Olsen, 1963) have reported on the relationship of free amino acids in blood plasma to the amino acid composition of the diet. Richardson et al. (1953), while studying the influence of amino acid supplements and protein on plasma amino acid levels, observed a relationship between these sources and the free amino acids of plasma, but any association of plasma levels and growth was not always apparent. The portion of this study which compared a synthetic diet to peanut meal and soybean meal showed that proteins as well as

free amino acids did have an influence on free amino acids of the plasma.

Because of the small size of the plasma amino acid pool, the usefulness of measuring plasma amino acid changes is contingent on the extent to which they reflect changes occurring in other tissues of the body. Reports (Richardson et al., 1965) indicated that a lysine deficiency could result in decreased plasma, liver and muscle lysine levels, increased threonine levels in these tissues and a decrease in plasma and muscle arginine. Aspartic acid was present in highest concentration in liver, while alanine was present in highest quantity in muscle. Based on these observations, it was suggested that alanine could be the principal carrier to the liver of amino nitrogen removed from nonessential and branched chain amino acids in muscle. Zimmerman and Scott (1965) have investigated the relationships between the amino acids required to give maximum growth of chicks and the amino acid content of the plasma. With suboptimal dietary levels of lysine, arginine and valine, they observed the plasma concentration of the limiting amino acid under consideration remained at a low constant level. Dietary levels in excess of the required amount to support maximum growth resulted in rapid accumulation of these amino acids in the plasma. These observations supported the use of the plasma amino acid titre for determination of the chick's amino acid requirements.

Taylor et al. (1970) have conducted studies to observe changes in plasma concentrations of free amino acids in relation to egg

formation in the hen. The following is an excerpt taken from the above report:

1. Amino acid concentrations are mostly higher at night than during the day.
2. Egg formation, in general, is associated with increases in the concentrations of nonessential and decreases in the concentrations of essential amino acids. Cystine and glutamic acid behave like the essential amino acids.
3. Voluntary food intake of hens may be influenced by changes in plasma levels of essential amino acids associated with the synthesis of egg albumen. Arginine possibly appears to be an amino acid influencing the voluntary food intake of hens.

Taylor et al. (1970) concluded that changes in the free amino acids of hen plasma might not provide enough information to study the amino acid requirements or the nutritive value of particular proteins for egg production. However, Bray (1970), while working on leucine imbalance in hen diets, observed a good correlation between dietary leucine and plasma leucine, isoleucine and valine levels.

Amino Acid Composition of Egg Protein

The protein of eggs has been regarded as superior in nutritional quality. Studies in the past (Sumner, 1938) demonstrated that the protein from eggs is apparently ideal for furnishing essential amino acids. Lewis et al. (1950), while working on the amino acid composition of egg proteins, also reported similar egg protein composition in their

analysis. Since then, several reports have been published on the amino acid composition of egg protein. This knowledge led scientists to suggest amino acid requirements of hens for optimum egg production.

In this review, the author wanted to discuss specific factors pertaining to amino acid supplementations of low protein diets. As a result, voluminous reports available in the area of protein and amino acid nutrition that did not contribute to this concern were carefully deleted so as to make this review thorough but brief.

EXPERIMENTAL PROCEDURE

Research to further elucidate the amino acids limiting egg production from hens fed a corn-soy low protein diet was continued. DeKalb 131 and 161 pullets for the first and second year studies, respectively, were housed two per cage in 8-inch cages. The pullets were reared under partially controlled environment and were fed the practical type, high energy starter and grower rations proposed by Carlson and Bonzer (1968). When transferred to laying cages at 20 weeks of age, the pullets were vaccinated against Newcastle Disease and debeaked. A standard corn-soy type laying ration diluted with cerelose (Tables 1 and 2) was fed for a 4-month depletion period or longer and until the pullets were placed on their experimental regime.

On the basis of individual records, those pullets not producing over 10 eggs during the month prior to the beginning of each experiment were removed. In the first experiment when production dropped to or below 60% on a hen-day basis, the pullets were randomly allotted to experimental diets containing 0, 0.05 and 0.1% levels of DL-threonine and DL-valine and 0 and 0.1% levels of DL-isoleucine in all combinations. Each treatment was replicated four times using two hens per replicate and fed for a 16-week period. Thus, the study was of a 3 x 3 x 2 x 4 factorial design. Mortality, feed consumption, weight gain and egg production were recorded. Eggs produced from each treatment were accumulated 3 days each week and the egg weights, Haugh units and egg shell thickness were recorded. At the end of the feeding trial four hens at random from each treatment were bled in the afternoon and a

Table 1. Composition of the cage layer basal diets used in Experiments 1 and 2[+]

Ingredients	Low protein basal	Practical laying diet
	%	%
Ground yellow corn	41.8	71.5
Cerelose	35.1	--
Soybean meal (47%)	13.0	14.0
Meat scraps	--	5.0
Alfalfa meal (17%)	--	2.0
Yellow grease	1.0	--
Limestone	5.0	5.0
Dicalcium phosphate	2.0	1.5
Salt mix[1]	0.5	0.5
Vitamin mix[2]	0.5	0.5
DL-methionine	0.15	0.05
L-lysine	0.19	--
DL-tryptophan	0.04	--
	99.28	100.00

[+] Contains 0.1% DL-isoleucine in addition to the above.

[1] Contains 97% NaCl, 0.3% S, 0.05% Cu, 0.17% Fe, 0.01% Co and 0.46% Mn.

[2] Provides per kg. of diet, 2370 I.U. vitamin A, 820 I.C.U. vitamin D_3, 11 I.U. vitamin E, 2.2 mg. vitamin K, 29 mg. niacin, 4.8 mg. pantothenate, 375 mg. choline, 4.8 mg. riboflavin, 9.7 mcg. cynocobalamine and 110 mg. ethoxyquin.

Table 2. Amino acid composition and requirement

Amino acids	N.R.C., 1971 15% protein	Scott *et al.*, 1969 16% protein	SDSU Basal 10.8% protein	Basal + isole. 0.1 Basal + val. 0.05 and 0.1 Basal + threo. 0.05 and 0.1 Basal + gly. 0.25 and 0.5	Balance required
Arginine	0.8	0.8	0.69	0.69	0.1
Cystine	0.25	--	0.14	0.14] 0.46	--
Methionine	0.53 (0.28)	0.58 (0.32)	0.32	0.32]	0.1
Glycine	--	--	0.55	0.80 and 1.050	--
Histidine	--	0.3	0.25	0.25	
Isoleucine	0.5	0.8	0.52	0.62	0.2
Leucine	1.2	1.2	0.97	0.97	--
Lysine	0.5	0.64	0.71	0.71	--
Phenylalanine	--	1.0 (0.7)	0.55	0.55	0.45
Tyrosine	--	--	0.21	0.21	--
Threonine	0.4	0.55	0.44	0.49 and 0.54	--
Tryptophan	0.11	0.16	0.18	0.18	--
Valine	--	0.8	0.51	0.56 and 0.61	0.2

pooled blood sample was saved to determine the free plasma amino acid concentration.

The pullets in the second study were reared under similar conditions. During the 24 to 40 weeks of age depletion period, the basal diet contained 0.1% DL-isoleucine in addition to the methionine, lysine and tryptophan supplement of Experiment 1. At the end of this period the hens were culled on the basis of their past production and were randomly assigned to 19 treatments containing 0, 0.25 and 0.5% of glycine and 0.05 and 0.1% of DL-threonine and DL-valine in all possible combinations. Each treatment was again replicated four times using two hens per replicate and fed for a further similar period of 16 weeks. Data on production and egg quality were collected in the same manner as for Experiment 1. After the hens had been on the experimental regime for 12 weeks, eggs from each replicate and treatment were saved for protein analysis of egg albumen by electrophoresis and for analysis of amino acids in the egg albumen. Eggs from hens of the same breed and age receiving 16% protein corn-soybean practical diets on another experiment at the Poultry Research Center served as positive controls for the latter analyses. Blood samples were collected at the termination of the experiment in a manner similar to that of the first study. After the end of the experimental regime, all hens received a practical 16% protein corn-soy diet for 2 weeks. Following this period, blood samples were obtained for amino acid analysis.

Procedure for Plasma Free Amino Acid Analysis

Pooled blood samples were collected in citrated tubes and centrifuged. In a separate tube, 8 ml. of plasma from these samples were deproteinized by adding 4 ml. of 20% sulfo saliycilic acid, 4 ml. of water and centrifuged. Plasma samples thus collected were kept in plastic vials and immediately frozen for future analysis. A 1 ml. aliquot of deproteinized plasma was analyzed on a Beckman 120 auto analyzer to determine the amounts of individual amino acids present.

Egg White Electrophoresis

One drop of fresh egg white was placed on a glass plate and a portion was transferred by blotter to Titan III cellulose acetate plates presoaked in the pH 8.8 tris buffer. The Titan III plates were then subjected to electrophoresis for 30 minutes at 180 volts. The acetate plates were then stained in Ponceau S and read in a densitometer at 760 nm after proper cleaning and drying procedures. The different protein fractions were calculated from the peak areas and expressed as a percentage of the egg white.

Amino Acid Composition of Egg White

One gram of egg white was hydrolyzed in 200 ml. of 6N HCl at 110° C. for 24 hours in an atmosphere of nitrogen gas to minimize oxidation. A 25 ml. aliquot of the hydrolyzed sample was centrifuged and evaporated to dryness in a rotary evaporator. Ten milliliters of distilled water were added to the flask and the sample was evaporated to dryness. This step was repeated twice to remove excess hydrochloric

acid. The dried sample was dissolved in 10 ml. of pH 2.2 acetate buffer and 1 ml. of the aliquot was analyzed in the Beckman 120 auto analyzer. The amino acid concentration was calculated and expressed as grams percent of the egg white.

Statistical Analysis

Data obtained during the entire experimental period were pooled and subjected to analysis of variance using a completely randomized block design. If a significant difference was observed among treatments for a particular measurement, the treatment combinations were then subjected to single degree of freedom orthogonal comparisons as described by Steel and Torrie (1960). The depletion periods in both experiments contributed to an inconsistency in rate of egg production for the earlier periods. As a result, data for all the individual periods were not subjected to statistical analysis.

RESULTS AND DISCUSSION

Experiment 1

Data on egg production, feed consumption and feed efficiency along with egg weights and Haugh units for Experiment 1 are presented by four week periods in Tables 3 to 6. Table 7 summarizes the data for the above variables and egg shell thickness, computed for the average of the four periods. Plasma levels of free amino acids analyzed at the end of the trial are presented in Table 8.

Hen-Day Egg Production. In that the 42-week old hens, after having received the basal diet for 16 weeks, were producing eggs at the rate of 61%, the results agree with those of Novacek (1969). The average egg mass produced per day ranged from 24 to 41 grams during the trial (Table 7). Threonine supplementation at 0.05% suppressed egg production as expressed both in percent and mass. Threonine addition at 0.1% on the other hand did not alter egg production. However, using the orthogonal analysis, the egg production response due to threonine supplementation (Table 9) was significantly ($P < .01$) lower compared to eggs produced by hens on different levels of valine and valine and threonine supplements. Some improvement in egg production was observed during the second period (Table 4) due to valine, but this did not persist. Egg production was somewhat improved by supplemental threonine and valine when both were either at the low or high level. Hen-day egg production observed with hens on diets with the 0.05% combined levels and 0.1% combined levels of threonine and

valine averaged 65% and 69%, respectively. Egg production in grams per hen-day averaged 40 and 39, respectively, for the above dietary supplements. Egg production changes for the hens receiving the diets with the other combinations of threonine and valine were inconsistent. The egg production as expressed both in percent and mass due to 0.05% threonine and 0.1% valine was significantly ($P<.01$, Table 9) lower contrasted to that of hens on high level supplements of threonine and valine.

Isoleucine at 0.1% had been added to the basal diet to study its potential single and supplemental effects on egg production. Results from the sole use of isoleucine indicated a superiority for the effects of this treatment. The hen-day egg production ranged as a percent from 67 to 74 with an average of 69.3 and as grams of egg mass from 39 to 44, averaging 41. This improvement was quite comparable to that observed with the diet containing the high levels (0.1% each) of threonine and valine and significantly ($P<.01$, Table 9) superior to egg production of hens receiving the isoleucine diet supplemented with threonine or valine or both. The responses observed due to threonine and valine with isoleucine were not superior, thus suggesting that isoleucine was probably more limiting than the other amino acids. Therefore, a trend toward improved egg production was noticeable with its use.

<u>Feed Consumption and Efficiency.</u> Feed consumption of hens in the first 4-week period as compared to later periods (Tables 3 to 6) was considerably lower for all the treatment combinations except for

Table 3. Effects of amino acid supplementation on egg production, feed efficiency and egg quality, Experiment 1, Period 1+

Treatments	Hen-day egg production		Hen-day feed cons.	Feed/ doz. eggs	Egg size	Haugh units
	%	g.	g.	kg.	g.	
Basal[1]	61.2	34.1	59.4	1.2	56.2	79.4
Threonine[2]	47.3	26.8	87.1	2.2	55.9	73.6
Threonine[3]	60.3	33.6	66.5	1.4	56.2	80.6
Valine[2]	62.9	36.6	54.5	1.2	58.6	76.4
Threo.[2], Val.[2]	69.6	42.2	80.4	1.4	60.5	80.4
Threo.[3], Val.[2]	63.4	36.1	62.9	1.2	57.1	74.7
Valine[3]	58.9	33.2	56.7	1.2	56.6	79.3
Threo.[2], Val.[3]	58.9	33.2	67.9	1.4	56.2	80.0
Threo.[3], Val.[3]	67.6	37.0	68.3	1.3	54.9	73.7
Isoleucine[3]	68.7	39.8	61.6	1.1	57.5	80.0
Threo.[2], Isole.[3]	62.9	35.9	49.6	1.0	56.9	74.0
Threo.[3], Isole.[3]	65.6	38.7	66.1	1.2	59.1	76.2
Val.[2], Isole.[3]	54.9	32.0	46.9	1.0	58.2	82.2
Threo.[2], Val.[2], Isole.[3]	59.4	34.7	51.8	1.1	58.5	77.9
Threo.[3], Val.[2], Isole.[3]	63.8	37.5	56.7	1.1	59.1	75.8
Val.[3], Isole.[3]	69.6	38.9	54.9	1.0	56.0	74.5
Threo.[2], Val.[3], Isole.[3]	59.8	36.5	50.9	1.2	61.7	78.9
Threo.[3], Val.[3], Isole.[3]	58.5	34.2	56.7	1.3	58.5	81.2

+ Hens 43 to 46 weeks of age, first 4 week portion of experimental period.

1 10.8% protein diet with 0.15% methionine, 0.19% lysine and 0.04% tryptophan.

2 0.05% threonine and valine.

3 0.1% threonine, valine and isoleucine.

(All amino acids added in DL form except for L-lysine.)

Table 4. Effects of amino acid supplementation on egg production, feed efficiency and egg quality, Experiment 1, Period 2+

Treatments	Hen-day egg production		Hen-day feed cons.	Feed/ doz. eggs	Egg size	Haugh units
	%	g.	g.	kg.	g.	
Basal[1]	62.1	36.1	78.6	1.5	58.7	77.8
Threonine[2]	42.4	24.6	120.1	3.4	56.3	72.4
Threonine[3]	63.9	35.7	87.5	1.7	56.2	74.0
Valine[2]	67.4	39.0	90.2	1.7	58.1	73.4
Threo.[2], Val.[2]	70.5	44.0	84.4	1.4	62.4	79.0
Threo.[3], Val.[2]	66.5	38.0	83.9	1.5	57.1	75.0
Valine[3]	64.3	37.1	84.4	1.6	57.4	77.1
Threo.[2], Val.[3]	57.6	32.8	82.6	1.8	57.4	78.7
Threo.[3], Val.[3]	74.6	41.4	85.7	1.4	55.6	69.2
Isoleucine[3]	74.1	43.6	91.1	1.5	58.7	77.1
Threo.[2], Isole.[3]	74.6	41.8	95.5	1.5	56.1	75.2
Threo.[3], Isole.[3]	71.9	42.0	85.6	1.4	58.5	74.6
Val.[2], Isole.[3]	65.2	39.4	98.7	1.8	60.5	73.6
Threo.[2], Val.[2], Isole.[3]	67.9	39.5	85.3	1.5	58.4	76.1
Threo.[3], Val.[2], Isole.[3]	68.3	40.2	83.0	1.5	59.2	75.4
Val.[3], Isole.[3]	72.3	41.4	93.7	1.6	57.3	72.8
Threo.[2], Val.[3], Isole.[3]	58.9	36.4	92.9	2.0	62.0	76.0
Threo.[3], Val.[3], Isole.[3]	69.2	41.8	89.7	1.6	60.5	77.9

+ Hens 47 to 50 weeks of age, second 4 week portion of experimental period.

1 10.8% protein diet with 0.15% methionine, 0.19% lysine and 0.04% tryptophan.

2 0.05% threonine and valine.

3 0.1% threonine, valine and isoleucine.

Table 5. Effects of amino acid supplementation on egg production, feed efficiency and egg quality, Experiment 1, Period 3[+]

Treatments	Hen-day egg production		Hen-day feed cons.	Feed/ doz. eggs	Egg size	Haugh units
	%	g.	g.	kg.	g.	
Basal[1]	61.2	35.6	90.6	1.8	58.3	76.7
Threonine[2]	38.2	23.0	86.3	3.1	57.8	68.1
Threonine[3]	49.6	28.3	79.9	2.2	57.6	74.9
Valine[2]	55.4	32.6	87.5	2.0	59.1	72.2
Threo.[2], Val.[2]	54.6	34.2	89.3	2.2	62.9	73.5
Threo.[3], Val.[2]	50.9	30.4	71.0	1.8	59.8	76.3
Valine[3]	57.8	33.3	79.9	1.7	57.5	75.6
Threo.[2], Val.[3]	52.2	30.4	80.8	2.0	59.0	77.2
Threo.[3], Val.[3]	65.8	37.9	87.6	1.6	57.2	69.3
Isoleucine[3]	66.5	38.8	87.1	1.6	58.2	75.5
Threo.[2], Isole.[3]	65.3	37.9	82.7	1.6	57.8	76.6
Threo.[3], Isole.[3]	65.2	39.3	100.4	1.9	60.4	72.6
Val.[2], Isole.[3]	61.5	37.3	85.2	1.8	60.5	74.7
Threo.[2], Val.[2], Isole.[3]	68.7	41.4	88.4	1.6	60.2	74.5
Threo.[3], Val.[2], Isole.[3]	62.1	37.8	76.3	1.5	61.2	78.1
Val.[3], Isole.[3]	68.3	39.0	81.7	1.4	57.2	72.4
Threo.[2], Val.[3], Isole.[3]	55.8	34.4	77.7	1.8	62.0	69.7
Threo.[3], Val.[3], Isole.[3]	61.6	37.4	71.0	1.4	60.7	74.8

[+] Hens 51 to 54 weeks of age, third 4 week portion of experimental period.

[1] 10.8% protein diet with 0.15% methionine, 0.19% lysine and 0.04% tryptophan.

[2] 0.05% threonine and valine.

[3] 0.1% threonine, valine and isoleucine.

Table 6. Effects of amino acid supplementation on egg production, feed efficiency and egg quality, Experiment 1, Period 4+

Treatments	Hen-day egg production		Hen-day feed cons.	Feed/ doz. eggs	Egg size	Haugh units
	%	g.	g.	kg.	g.	
Basal[1]	59.8	35.2	74.6	1.5	59.0	74.5
Threonine[2]	40.2	23.0	77.7	2.5	56.6	72.2
Threonine[3]	57.1	32.8	79.0	1.8	57.7	73.6
Valine[2]	53.6	31.2	75.9	1.8	58.2	67.2
Threo.[2], Val.[2]	63.4	38.8	82.1	1.6	61.3	75.5
Threo.[3], Val.[2]	57.1	33.8	78.6	1.7	59.1	77.4
Valine[3]	52.2	30.8	71.0	1.6	58.9	75.6
Threo.[2], Val.[3]	58.9	34.2	77.2	1.6	57.6	77.9
Threo.[3], Val.[3]	66.5	38.9	82.6	1.5	58.6	73.1
Isoleucine[3]	67.9	40.5	74.1	1.3	59.5	77.6
Threo.[2], Isole.[3]	65.2	37.5	96.4	1.8	57.9	72.5
Threo.[3], Isole.[3]	67.9	41.0	84.4	1.5	60.3	71.4
Val.[2], Isole.[3]	51.9	30.6	82.6	1.9	58.9	76.0
Threo.[2], Val.[2], Isole.[3]	65.6	38.8	82.1	1.5	59.3	78.5
Threo.[3], Val.[2], Isole.[3]	62.5	37.3	82.1	1.6	59.9	78.2
Val.[3], Isole.[3]	62.5	35.0	81.2	1.6	56.1	71.4
Threo.[2], Val.[3], Isole.[3]	63.8	40.0	82.1	1.7	62.8	77.2
Threo.[3], Val.[3], Isole.[3]	55.8	33.4	75.2	1.8	60.3	74.9

+ Hens 55 to 58 weeks of age, fourth 4 week portion of experimental period.

1 10.8% protein diet with 0.15% methionine, 0.19% lysine and 0.04% tryptophan.

2 0.05% threonine and valine.

3 0.1% threonine, valine and isoleucine.

Table 7. Effects of amino acid supplementation on egg production, feed efficiency and egg quality. Data on average of four periods, Experiment 1

Treatments	Hen-day egg production		Hen-day feed cons.	Feed per doz. eggs	Egg size	Haugh units	Egg shell thickness
	%	g.	g.	kg.	g.		mm.
Basal[1]	61.0	35.3	75.8	1.5	58.1	77.1	0.33
Threonine[2]	42.0	24.3	92.8	2.8	56.7	71.6	0.30
Threonine[3]	57.7	32.6	78.2	1.8	56.9	75.8	0.31
Valine[2]	59.8	34.8	77.0	1.7	58.5	72.3	0.31
Threo.[2], Val.[2]	64.5	39.8	84.1	1.7	61.8	77.1	0.32
Threo.[3], Val.[2]	59.5	34.6	74.1	1.5	58.3	75.9	0.34
Valine[3]	58.3	33.6	73.0	1.5	57.6	76.9	0.33
Threo.[2], Val.[3]	56.9	32.6	77.1	1.7	57.6	78.5	0.31
Threo.[3], Val.[3]	68.5	38.8	81.1	1.5	56.6	71.3	0.29
Isoleucine[3]	69.3	40.7	78.5	1.4	58.5	77.6	0.31
Threo.[2], Isole.[3]	67.0	38.3	81.5	1.5	57.2	74.6	0.33
Threo.[3], Isole.[3]	67.7	40.3	84.1	1.5	59.6	73.7	0.34
Val.[2], Isole.[3]	58.4	34.8	78.4	1.6	59.5	76.6	0.30
Threo.[2], Val.[2], Isole.[3]	65.4	38.6	76.9	1.4	59.1	76.8	0.31
Threo.[3], Val.[2], Isole.[3]	64.2	38.2	74.5	1.4	59.9	76.9	0.31
Val.[3], Isole.[3]	68.2	38.6	77.9	1.4	56.7	72.8	0.29
Threo.[2], Val.[3], Isole.[3]	59.5	36.8	75.9	1.7	62.1	75.5	0.29
Threo.[3], Val.[3], Isole.[3]	61.2	36.7	73.2	1.5	60.1	77.2	0.31

[1] 10.8% protein diet with 0.15% methionine, 0.19% lysine and 0.04% tryptophan.
[2] 0.05% threonine and valine.
[3] 0.1% threonine, valine and isoleucine.

Table 8. Effects of amino acid supplementation on plasma free amino acids, Experiment 1 (Micro Moles/100 ml. of plasma)

Treatments	Lysine	Arginine	Threonine	Serine	Glutamic acid	Glycine	Valine	Methionine	Isoleucine	Leucine
Basal[1]	34	13	14	193	28	42	12	9	6	7
Threonine[2]	43	17	42	154	30	40	17	9	9	21
Threonine[3]	52	26	42	217	43	53	17	14	10	23
Valine[2]	50	21	20	148	32	44	14	10	6	19
Threo.[2], Val.[2]	34	21	40	375	67	82	28	19	13	34
Threo.[3], Val.[2]	98	38	74	178	50	56	26	13	10	25
Valine[3]	42	18	30	133	48	39	21	11	8	20
Threo.[2], Val.[3]	41	26	21	160	52	44	14	10	6	18
Threo.[3], Val.[3]	100	55	43	159	54	44	21	11	9	17
Isoleucine[3]	36	18	24	195	49	54	15	12	10	20
Threo.[2], Isole.[3]	48	26	23	148	63	50	20	13	15	24
Threo.[3], Isole.[3]	47	25	24	129	55	44	23	11	13	22
Val.[2], Isole.[3]	37	17	26	177	47	61	16	13	13	20
Threo.[2], Val.[2], Isole.[3]	50	24	26	145	52	85	17	14	12	25
Threo.[3], Val.[2], Isole.[3]	38	21	18	121	46	40	18	10	8	18
Val.[3], Isole.[3]	40	20	32	127	43	49	13	14	8	18
Threo.[2], Val.[3], Isole.[3]	50	24	40	140	50	48	18	9	13	20
Threo.[3], Val.[3], Isole.[3]	58	22	67	122	53	48	28	10	13	25

[1] 10.8% protein diet with 0.15% methionine, 0.19% lysine and 0.04% tryptophan.
[2] 0.05% threonine and valine.
[3] 0.1% threonine, valine and isoleucine.

Table 9. Mean squares for hen-day egg production (Experiment 1)

Source of variation	df	Hen-day egg production Percent	Gram
Treatment	17	657.79**	966.93**
Basal[1] thru Threo. and Val. combinations vs. Isole. combinations	1	2454.09**	4720.03**
Basal[1] vs. Threo. and Val. combinations	1	99.88	105.90
Threo.[2,3] vs. Val. and Val. and Threo. combinations	1	3114.48	5040.20**
Threo.[2] vs. Threo.[3]	1	1968.78	2197.85**
Val.[2] and Val.[2], Threo.[2,3] vs. Val.[3] and Val.[3], Threo.[2,3]	1	0.07	183.04
Val.[2] vs. Val.[2], Threo.[2,3]	1	51.33	241.1736
Val.[2], Threo.[2] vs. Val.[2], Threo.[3]	1	205.03*	865.28**
Val.[3] vs. Val.[3], Threo.[2,3]	1	205.63*	194.71
Val.[3], Threo.[2] vs. Val.[3], Threo.[3]	1	1068.38**	1221.17**
Isole.[3] vs. rest of Isole.[3] combinations	1	408.03**	475.35*
Isole.[3], Threo.[2,3] vs. rest of Isole.[3] combinations	1	484.20**	373.51*
Isole.[3], Threo.[2] vs. Isole.[3], Threo.[3]	1	3.25	125.14
Isole.[3], Val.[2] and Isole.[3], Val.[2], Threo.[2,3] vs. Isole.[3], Val.[3] and Isole.[3], Val.[3], Threo.[2,3]	1	2.80	2.38
Isole.[3], Val.[2] vs. Isole.[3], Val.[2], Threo.[2,3]	1	436.05**	548.36**
Isole.[3], Val.[2], Threo.[2] vs. Isole.[3], Val.[2], Threo.[3]	1	12.00	4.50
Isole.[3], Val.[3] vs. Isole.[3], Val.[3], Threo.[2,3]	1	647.92**	138.82
Isole.[3], Val.[3], Threo.[2] vs. Isole.[3], Val.[3], Threo.[3]	1	20.48	0.41
Replicates	3	782.75**	837.05**
Error	216	53.13	78.56

[1] 10.8% protein diet with 0.15% methionine, 0.19% lysine and 0.04% tryptophan.

[2] 0.05% threonine and valine.

[3] 0.1% threonine, valine and isoleucine.

* $P < .05$.

** $P < .01$.

those hens receiving 0.05% threonine and 0.05% of both threonine and valine. They consumed 87 to 80 grams of feed, respectively, on a hen-day basis as contrasted to 59 grams of feed consumed by hens fed the basal diet. In later periods, egg production was increased and this was generally correlated with increased feed consumption. Considering all four periods together, the feed consumption was in the range of 70 to 80 grams on a hen-day basis for most of the treated groups. Hens receiving 0.05% threonine consumed 120 grams of feed in the second period and on the average about 93 grams on a hen-day basis. This high feed consumption might have been due to imbalance caused by the 0.05% threonine supplementation of the basal diet. However, threonine at 0.1% did not show higher feed consumption and on the average was comparable to that of the basal diet. Hens fed 0.1% isoleucine and laying significantly more eggs ($P < .01$) consumed 91 grams of feed during their peak production (Table 4) but on an average consumed 79 grams of feed, which was comparable to the 76 grams of feed consumed by hens fed the basal diet (Table 7). In general, feed consumption observed in Experiment 1 was not significantly different among the treated groups (Appendix Table 1).

Feed efficiency, expressed as kilograms of feed consumed per dozen eggs produced, for hens fed the basal diet ranged from 1.2 in the first period to 1.8 in the third period or 1.5 for the average of the four periods. Hens receiving 0.05% threonine showed poorer feed conversion, consuming 2.8 kg. of feed to produce one dozen eggs. Data available on feed conversion for other supplemental groups did

not show significant differences (Appendix Table 1) and were quite similar when computed on the average of four periods.

Egg Quality. The eggs produced by hens fed the basal diet weighed an average of 56 grams in period 1, 59 grams in period 4 or 58 grams for the average of all periods. The egg weights in general were comparable and appeared to be unaffected by amino acid supplementation. As an example, the 0.1% isoleucine-fed hens produced eggs averaging 58.5 grams.

Interior egg quality, as measured by the Haugh unit, was higher during the first two periods than was observed in the latter periods. Hens fed the basal diet and 0.1% isoleucine supplementation showed Haugh unit values of 75 to 79 and 76 to 80, respectively, or average Haugh unit values of 77 and 78, respectively (Table 7). Interior quality of eggs due to amino acids supplementing the basal diet appeared to be unaffected. Results presented in Table 7 showed a range of 0.29 to 0.34 mm. for egg shell thickness. Dietary supplementation did not appear to cause any consistent alteration of egg shell quality.

Plasma Levels of Free Amino Acids. Hens in this study received the basal diet for about 36 weeks which probably accounted, therefore, for the low levels of amino acids in their plasma pool. Supplementation of threonine, valine or isoleucine or their combinations resulted in elevated plasma levels of most of the free amino acids. These plasma amino acid levels were not consistent when comparing the effects of the dietary supplements. The levels of the nonessential amino acids,

serine, glutamic acid and glycine, were considerably elevated by the 0.05% threonine and valine supplements and were returned to that of the basal by the 0.1% levels. The beneficial effects on production of 0.1% threonine and valine over 0.05% threonine and valine indicated that the lower level of the amino acids was insufficient to give a proper balance of amino acids. The improved utilization of the protein at the 0.1% level of supplementation is reflected in part by the reduction of the plasma serine and glycine levels. Interestingly, however, the plasma lysine and arginine were elevated to very high levels and therefore this indicated a possible amino acid imbalance. Further supplementation of the basal diet with 0.1% isoleucine resulted in lowered plasma levels of lysine and arginine with very similar levels of the nonessential amino acids and isoleucine. Hens receiving the diet with only the 0.1% isoleucine supplement produced eggs at a maximum rate and probably utilized the total diet more effectively for egg protein synthesis.

Experiment 2

Egg production as expressed both in percent and mass on a hen-day basis along with feed consumption, feed efficiency and egg quality of Experiment 2 are presented in Tables 10 to 13. Table 14 summarizes the data for the above variables, averaged for the four periods. Data on plasma levels of free amino acids, electrophoretic pattern of egg white protein, and amino acid composition of egg white protein are presented in Tables 15, 16 and 17, respectively.

Table 10. Effects of amino acid supplementation on egg production, feed efficiency and egg quality, Experiment 2, Period 1[+]

Treatments	Hen-day egg production		Hen-day feed cons.	Feed/ doz. eggs	Egg size	Haugh units
	%	g.	g.	kg.	g.	
Basal[1]	71.0	41.9	84.4	1.5	59.0	81.3
Glycine[2]	68.3	38.2	83.9	1.5	55.8	72.5
Gly.[2], Threonine[2]	71.9	42.8	85.7	1.4	59.6	80.5
Gly.[2], Threo.[3]	64.3	37.5	84.4	1.6	58.2	79.3
Gly.[2], Valine[2]	75.4	42.6	84.0	1.3	56.3	77.3
Gly.[2], Threo.[2], Val.[2]	67.0	39.8	85.0	1.5	59.5	78.4
Gly.[2], Threo.[3], Val.[2]	73.2	41.8	83.5	1.4	57.1	74.9
Gly.[2], Valine[3]	69.6	40.1	84.4	1.5	57.6	75.4
Gly.[2], Threo.[2], Val.[3]	66.5	40.0	85.3	1.6	60.2	76.6
Gly.[2], Threo.[3], Val.[3]	68.7	41.7	84.4	1.5	60.7	75.8
Glycine[3]	69.2	39.8	84.8	1.5	57.5	79.8
Gly.[3], Threo.[2]	75.9	44.8	84.4	1.3	59.0	78.4
Gly.[3], Threo.[3]	73.7	41.4	84.8	1.4	56.2	76.7
Gly.[3], Val.[2]	75.9	45.0	83.0	1.3	59.4	76.5
Gly.[3], Threo.[2], Val.[2]	69.6	40.8	83.0	1.5	58.7	79.3
Gly.[3], Threo.[3], Val.[2]	69.2	39.5	83.5	1.5	57.2	78.5
Gly.[3], Val.[3]	68.3	39.1	84.0	1.5	57.1	78.1
Gly.[3], Threo.[2], Val.[3]	73.2	43.8	84.8	1.4	59.9	78.0
Gly.[3], Threo.[3], Val.[3]	65.6	38.1	83.5	1.6	58.0	82.8

[+] Hens 41 to 44 weeks of age, first 4 week portion of experimental period.

[1] 10.8% protein diet with 0.15% methionine, 0.19% lysine, 0.04% tryptophan and 0.1% isoleucine.

[2] 0.25% glycine and 0.05% threonine and valine.

[3] 0.5% glycine and 0.1% threonine and valine.

(All amino acids added in DL form except for L-lysine.)

Table 11. Effects of amino acid supplementation on egg production, feed efficiency and egg quality, Experiment 2, Period 2[+]

Treatments	Hen-day egg production		Hen-day feed cons.	Feed/ doz. eggs	Egg size	Haugh units
	%	g.	g.	kg.	g.	
Basal[1]	68.7	40.4	96.4	1.8	58.7	81.1
Glycine[2]	66.1	37.6	95.1	1.8	57.0	77.2
Gly.[2], Threonine[2]	67.0	40.6	96.4	1.8	60.8	87.4
Gly.[2], Threo.[3]	66.1	39.3	98.2	1.8	59.4	80.6
Gly.[2], Valine[2]	75.4	43.4	99.1	1.6	57.6	83.5
Gly.[2], Threo.[2], Val.[2]	67.4	41.2	98.7	1.8	61.1	76.0
Gly.[2], Threo.[3], Val.[2]	74.6	42.6	97.8	1.6	57.3	86.7
Gly.[2], Valine[3]	68.7	40.8	97.3	1.7	59.1	75.5
Gly.[2], Threo.[2], Val.[3]	63.4	38.6	98.2	1.9	61.2	77.0
Gly.[2], Threo.[3], Val.[3]	63.4	38.8	99.6	1.9	61.6	78.9
Glycine[3]	63.4	36.9	95.5	1.9	58.2	83.0
Gly.[3], Threo.[2]	67.4	40.0	96.4	1.7	60.8	79.7
Gly.[3], Threo.[3]	81.2	46.4	96.9	1.4	57.2	76.9
Gly.[3], Val.[2]	73.0	43.7	97.9	1.6	59.8	72.4
Gly.[3], Threo.[2], Val.[2]	65.6	39.8	96.0	1.8	60.7	84.1
Gly.[3], Threo.[3], Val.[2]	69.2	39.9	92.0	1.6	57.8	80.1
Gly.[3], Val.[3]	63.4	37.8	96.0	1.9	59.3	77.4
Gly.[3], Threo.[2], Val.[3]	72.8	43.7	97.8	1.6	60.1	80.1
Gly.[3], Threo.[3], Val.[3]	66.1	39.5	94.2	1.7	59.8	86.6

[+] Hens 45 to 48 weeks of age, second 4 week portion of experimental period.

[1] 10.8% protein diet with 0.15% methionine, 0.19% lysine, 0.04% tryptophan and 0.1% isoleucine.

[2] 0.25% glycine and 0.05% threonine and valine.

[3] 0.5% glycine and 0.1% threonine and valine.

Table 12. Effects of amino acid supplementation on egg production, feed efficiency and egg quality, Experiment 2, Period 3[+]

Treatments	Hen-day egg production		Hen-day feed cons.	Feed/ doz. eggs	Egg size	Haugh units
	%	g.	g.	kg.	g.	
Basal[1]	63.8	38.0	83.0	1.6	59.5	77.6
Glycine[2]	67.0	38.0	80.0	1.5	56.9	72.9
Gly.[2], Threonine[2]	58.5	35.7	80.4	1.9	62.5	77.2
Gly.[2], Threo.[3]	67.8	40.3	94.6	1.9	59.3	76.0
Gly.[2], Valine[2]	72.8	42.0	86.2	1.4	57.8	79.7
Gly.[2], Threo.[2], Val.[2]	67.9	41.1	83.5	1.5	60.6	76.9
Gly.[2], Threo.[3], Val.[2]	70.1	41.5	78.0	1.3	59.0	78.7
Gly.[2], Valine[3]	67.4	39.7	85.3	1.8	59.1	74.8
Gly.[2], Threo.[2], Val.[3]	64.3	41.0	88.4	1.7	64.0	73.8
Gly.[2], Threo.[3], Val.[3]	67.0	40.4	85.3	1.6	60.7	73.3
Glycine[3]	64.7	38.8	82.1	1.5	59.7	79.6
Gly.[3], Threo.[2]	69.6	42.0	81.2	1.4	60.4	77.4
Gly.[3], Threo.[3]	77.2	44.8	84.4	1.3	57.9	74.2
Gly.[3], Val.[2]	74.6	45.4	87.5	1.4	61.0	77.6
Gly.[3], Threo.[2], Val.[2]	63.4	37.3	73.7	1.4	58.9	75.3
Gly.[3], Threo.[3], Val.[2]	69.2	39.8	79.0	1.4	57.5	74.9
Gly.[3], Val.[3]	58.0	33.5	79.0	1.7	57.6	77.0
Gly.[3], Threo.[2], Val.[3]	71.0	43.8	87.1	1.5	61.8	74.2
Gly.[3], Threo.[3], Val.[3]	57.6	35.3	74.6	1.6	61.3	78.0

[+] Hens 49 to 52 weeks of age, third 4 week portion of experimental period.

[1] 10.8% protein diet with 0.15% methionine, 0.19% lysine, 0.04% tryptophan and 0.1% isoleucine.

[2] 0.25% glycine and 0.05% threonine and valine.

[3] 0.5% glycine and 0.1% threonine and valine.

Table 13. Effects of amino acid supplementation on egg production, feed efficiency and egg quality, Experiment 2, Period 4+

Treatments	Hen-day egg production		Hen-day feed cons.	Feed/ doz. eggs	Egg size	Haugh units
	%	g.	g.	kg.	g.	
Basal[1]	62.9	37.0	84.8	1.7	58.8	70.5
Glycine[2]	67.9	38.5	72.3	1.3	56.8	67.7
Gly.[2], Threonine[2]	54.0	32.4	67.4	2.6	61.0	72.7
Gly.[2], Threo.[3]	51.8	30.3	70.5	2.0	58.6	76.6
Gly.[2], Valine[2]	72.3	41.2	79.5	1.3	56.9	73.4
Gly.[2], Threo.[2], Val.[2]	68.3	41.8	80.8	1.4	61.3	73.6
Gly.[2], Threo.[3], Val.[2]	67.9	39.9	79.5	1.4	58.8	72.8
Gly.[2], Valine[3]	62.1	37.3	82.6	1.6	60.0	74.5
Gly.[2], Threo.[2], Val.[3]	56.7	35.1	79.9	1.8	62.1	70.3
Gly.[2], Threo.[3], Val.[3]	64.3	38.7	81.7	1.5	60.2	69.9
Glycine[3]	59.8	35.2	74.1	1.6	58.8	74.3
Gly.[3], Threo.[2]	51.8	32.1	71.4	1.8	61.7	72.9
Gly.[3], Threo.[3]	74.6	42.9	76.8	1.2	57.5	71.4
Gly.[3], Val.[2]	68.7	42.0	76.8	1.4	60.9	73.7
Gly.[3], Threo.[2], Val.[2]	60.9	35.9	73.1	1.5	58.9	73.0
Gly.[3], Threo.[3], Val.[2]	63.8	36.9	73.7	1.4	58.0	71.6
Gly.[3], Val.[3]	54.0	31.6	76.8	1.8	58.4	79.3
Gly.[3], Threo.[2], Val.[3]	67.4	40.6	80.8	1.5	60.4	71.6
Gly.[3], Threo.[3], Val.[3]	63.4	38.7	75.4	1.5	60.6	73.7

+ Hens 53 to 56 weeks of age, fourth 4 week portion of experimental period.

1 10.8% protein diet with 0.15% methionine, 0.19% lysine, 0.04% tryptophan and 0.1% isoleucine.

2 0.25% glycine and 0.05% threonine and valine.

3 0.5% glycine and 0.1% threonine and valine.

Table 14. Effects of amino acid supplementation on egg production, feed efficiency and egg quality. Data on average of four periods, Experiment 2

Treatments	Hen-day egg production		Hen-day feed cons.	Feed/ doz. eggs	Egg size	Haugh units
	%	g.	g.	kg.	g.	
Basal[1]	66.6	39.4	87.2	1.6	59.0	77.6
Glycine[2]	67.5	38.1	82.8	1.5	56.6	72.6
Gly.[2], Threonine[2]	62.9	37.9	82.5	1.9	61.0	79.5
Gly.[2], Threo.[3]	62.5	36.8	86.9	1.8	58.9	78.1
Gly.[2], Valine[2]	73.8	42.3	87.2	1.4	57.2	78.5
Gly.[2], Threo.[2], Val.[2]	67.6	41.0	87.0	1.6	60.6	76.2
Gly.[2], Threo.[3], Val.[2]	71.5	41.5	84.7	1.4	58.1	78.3
Gly.[2], Valine[3]	67.0	39.5	87.4	1.6	59.0	75.1
Gly.[2], Threo.[2], Val.[3]	62.7	38.7	88.0	1.7	62.0	74.4
Gly.[2], Threo.[3], Val.[3]	65.9	40.0	87.8	1.6	60.8	74.5
Glycine[3]	64.3	37.7	84.1	1.6	58.6	79.2
Gly.[3], Threo.[2]	66.2	40.0	83.4	1.6	60.5	77.1
Gly.[3], Threo.[3]	76.7	43.8	85.7	1.4	57.2	74.8
Gly.[3], Val.[2]	73.1	44.0	86.3	1.4	60.3	75.1
Gly.[3], Threo.[2], Val.[2]	64.9	38.4	81.5	1.5	59.3	77.9
Gly.[3], Threo.[3], Val.[2]	67.9	39.0	82.1	1.5	62.0	76.3
Gly.[3], Val.[3]	60.9	35.5	84.0	1.7	58.1	78.0
Gly.[3], Threo.[2], Val.[3]	71.1	43.0	87.6	1.5	60.6	76.0
Gly.[3], Threo.[3], Val.[3]	63.2	37.9	81.9	1.6	60.0	80.3

[1] 10.8% protein diet with 0.15% methionine, 0.19% lysine, 0.04% tryptophan and 0.1% isoleucine.

[2] 0.25% glycine and 0.05% threonine and valine.

[3] 0.5% glycine and 0.1% threonine and valine.

Table 15. Effects of amino acid supplementation on plasma free amino acids, Experiment 2 (Micro Moles/100 ml. of plasma)

Treatments	Lysine	Arginine	Threonine	Serine	Glutamic acid	Glycine	Valine	Methionine	Isoleucine	Leucine
Basal[1]	22	17	15	57	5	23	6	4	3	8
Glycine[2]	14	7	23	149	22	45	13	11	7	20
Gly.[2], Threonine[2]	31	16	42	253	28	91	24	14	14	25
Gly.[2], Threo.[3]	32	15	22	80	16	41	8	7	6	11
Gly.[2], Valine[2]	71	26	40	133	33	57	27	12	12	23
Gly.[2], Threo.[2], Val.[2]	59	27	27	176	24	64	12	10	8	19
Gly.[2], Threo.[3], Val.[2]	44	17	56	147	31	69	16	12	11	21
Gly.[2], Valine[3]	43	26	46	169	33	64	19	11	11	26
Gly.[2], Threo.[2], Val.[3]	30	12	16	69	14	27	9	5	5	10
Gly.[2], Threo.[3], Val.[3]	26	12	25	114	24	55	20	10	7	18
Glycine[3]	47	20	32	146	23	59	21	9	11	21
Gly.[3], Threo.[2]	29	16	56	246	51	175	18	18	15	30
Gly.[3], Threo.[3]	36	14	17	89	17	37	10	6	5	9
Gly.[3], Val.[2]	74	30	41	205	42	70	29	12	14	29
Gly.[3], Threo.[2], Val.[2]	44	23	36	144	27	54	24	11	11	25
Gly.[3], Threo.[3], Val.[2]	48	23	26	177	28	76	19	11	8	18
Gly.[3], Val.[3]	90	30	43	143	25	53	30	10	14	24
Gly.[3], Threo.[2], Val.[3]	49	30	22	147	24	91	29	15	12	24
Gly.[3], Threo.[3], Val.[3]	10	14	37	217	47	80	23	12	9	23
16% protein control	41	47	51	205	27	62	3	7	12	31
Post feeding[4]	41	39	52	156	27	49	7	10	15	38

[1] 10.8% protein diet with 0.15% methionine, 0.19% lysine, 0.04% tryptophan and 0.1% lysine.
[2] 0.25% glycine and 0.05% threonine and valine.
[3] 0.5% glycine and 0.1% threonine and valine.
[4] Basal fed 16% protein diet for 2 weeks after termination of Experiment 2.

Table 16. Effects of amino acid supplementation on distribution of egg white protein, Experiment 2 (% of egg white protein)

Treatments	Lysozyme	Ovomucin	Conalbumin	Ovomucoid + ovoglobulin	Ovoalbumin ($A_1 + A_2 + A_3$)
Basal[1]	5.0	0.60	22.0	7.2	65.4
Glycine[2]	3.3	0.49	19.5	8.7	68.1
Gly.[2], Threonine[2]	4.1	0.18	27.8	5.3	62.8
Gly.[2], Threo.[3]	3.8	0.17	30.3	7.9	57.9
Gly.[2], Valine[2]	3.4	0.24	25.7	5.4	65.3
Gly.[2], Threo.[2], Val.[2]	2.8	0.46	23.7	15.6	57.5
Gly.[2], Threo.[3], Val.[2]	4.0	0.43	24.8	11.7	61.1
Gly.[2], Valine[3]	4.6	1.70	24.7	11.7	57.4
Gly.[2], Threo.[2], Val.[3]	6.6	0.50	20.2	10.1	62.7
Gly.[2], Threo.[3], Val.[3]	3.5	0.62	18.8	11.2	66.0
Glycine[3]	3.2	0.16	26.4	5.6	54.7
Gly.[3], Threo.[2]	7.9	0.43	30.5	5.2	56.0
Gly.[3], Threo.[3]	5.9	0.47	22.2	10.4	61.1
Gly.[3], Val.[2]	5.3	0.73	19.7	12.5	61.8
Gly.[3], Threo.[2], Val.[2]	5.0	0.34	20.8	9.4	64.5
Gly.[3], Threo.[3], Val.[2]	5.0	0.45	23.1	10.5	61.0
Gly.[3], Val.[3]	4.1	0.24	19.6	10.6	65.5
Gly.[3], Threo.[2], Val.[3]	8.2	0.48	18.9	10.6	61.9
Gly.[3], Threo.[3], Val.[3]	4.9	0.40	20.1	12.5	62.1
16% protein control	5.6	0.50	14.3	6.8	72.0

[1] 10.8% protein diet with 0.15% methionine, 0.19% lysine, 0.04% tryptophan and 0.1% isoleucine.
[2] 0.25% glycine and 0.05% threonine and valine.
[3] 0.5% glycine and 0.1% threonine and valine.

Table 17. Effects of amino acid supplementation on amino acid composition of egg white protein, Experiment 2 (Micro Moles/g. of egg white protein)

Treatments	Lysine	Arginine	Threonine	Serine	Glutamic acid	Glycine	Valine	Methionine	Isoleucine	Leucine
Basal[1]	44	34	47	83	111	56	44	29	31	67
Glycine[2]	51	35	48	90	114	56	43	31	29	68
Gly.[2], Threonine[2]	39	28	31	60	74	39	41	19	30	56
Gly.[2], Threo.[3]	41	31	39	77	95	51	41	31	30	67
Gly.[2], Valine[2]	32	24	33	61	81	40	47	25	34	59
Gly.[2], Threo.[2], Val.[2]	42	32	35	63	82	42	44	24	32	58
Gly.[2], Threo.[3], Val.[2]	47	36	41	77	98	49	41	34	29	61
Gly.[2], Valine[3]	63	43	52	89	122	59	64	31	44	67
Gly.[2], Threo.[2], Val.[3]	61	45	44	88	119	56	55	25	32	58
Gly.[2], Threo.[3], Val.[3]	34	39	43	86	110	58	43	35	32	74
Glycine[3]	41	31	40	72	98	50	46	28	26	57
Gly.[3], Threo.[2]	33	25	32	60	78	40	31	21	22	47
Gly.[3], Threo.[3]	42	29	41	74	92	51	40	26	28	63
Gly.[3], Val.[2]	43	28	38	78	98	50	45	33	34	69
Gly.[3], Threo.[2], Val.[2]	15	11	12	27	35	18	15	9	10	21
Gly.[3], Threo.[3], Val.[2]	41	35	42	79	102	54	47	31	34	71
Gly.[3], Val.[3]	19	13	18	34	43	23	19	13	14	30
Gly.[3], Threo.[2], Val.[3]	23	18	23	41	55	27	24	15	17	34
Gly.[3], Threo.[3], Val.[3]	47	36	41	75	98	41	45	28	35	67
16% protein control	67	49	79	109	145	71	67	35	46	91

[1] 10.8% protein diet with 0.15% methionine, 0.19% lysine, 0.04% tryptophan and 0.1% lysine.
[2] 0.25% glycine and 0.05% threonine and valine.
[3] 0.5% glycine and 0.1% threonine and valine.

Hen-Day Egg Production. Markedly improved egg production was observed with the basal diet of Experiment 2 containing 0.1% isoleucine in addition to the methionine, lysine and tryptophan supplements of Experiment 1. Hens fed this basal diet produced eggs at the rate of 63 to 71% or 37 to 42 grams on a hen-day basis with an average of 67% or 39 grams (Table 14) during the 16 weeks of the trial period. It was interesting, however, that hens fed the basal diet in this study supported production which was very comparable to that of hens receiving the 0.1% isoleucine supplement in Experiment 1 (Table 7). Glycine at 0.25% effected no increase in egg production and further additions of 0.05 and 0.1% threonine resulted in significantly ($P<.01$) decreased production of eggs on a hen-day basis (Table 18). Hens receiving 0.05% valine and 0.25% glycine showed an apparent improved egg production. Further additions of threonine or the 0.10% level of valine with 0.25% glycine did not appear to improve egg production. As a result, the egg production responses from hens receiving 0.05% valine and 0.25% glycine combined with threonine were highly significant ($P<.01$, Table 18) when contrasted with similar supplements including 0.1% valine.

When glycine at 0.5% or with 0.05% threonine supplemented the basal diet, egg production was unaffected, but further supplementation of 0.1% threonine caused hens to produce eggs at a rate which was 10% higher than that of hens fed the basal diet. The percent hen-day egg production of hens fed the diets with 0.5% glycine and threonine was significantly ($P<.05$, Table 18) superior compared to that when

Table 18. Mean squares for hen-day egg production (Experiment 2)

Source of variation	df	Hen-day egg production	
		Percent	Gram
Treatment	18	290.95**	86.60**
Basal[1] vs. rest	1	4.68	2.12
Gly.[2] combinations vs. Gly.[3] combinations	1	42.54	12.95
Gly.[2] vs. rest of Gly.[2] combinations	1	8.42	36.58
Threo.[2,3] in Gly.[2] vs. rest of Gly.[2] combinations	1	740.06**	231.59*
Threo.[2] in Gly.[2] vs. Threo.[3] in Gly.[2]	1	1.09	8.71
Val.[2] and Val.[2], Threo.[2,3] vs. Val.[3] and Val.[3], Threo.[2,3] in Gly.[2]	1	798.56**	118.24
Val.[2] in Gly.[2] vs. Val.[2], Threo.[2,3] in Gly.[2]	1	190.52	12.52
Val.[2], Threo.[2] in Gly.[2] vs. Val.[2], Threo.[3] in Gly.[2]	1	117.50	2.06
Val.[3] in Gly.[2] vs. Val.[3], Threo.[2,3] in Gly.[2]	1	76.11	0.47
Val.[3], Threo.[2] in Gly.[2] vs. Val.[3], Threo.[3] in Gly.[2]	1	77.00	12.24
Gly.[3] vs. rest of Gly.[3] combinations	1	193.91	90.09
Threo.[2,3] in Gly.[3] vs. rest of Gly.[3] combinations	1	512.20**	121.17
Threo.[2] in Gly.[3] vs. Threo.[3] in Gly.[3]	1	877.39**	122.58
Val.[2] and Val.[2], Threo.[2,3] in Gly.[3] vs. Val.[3] and Val.[3], Threo.[2,3] in Gly.[3]	1	299.20	129.18*
Val.[2] in Gly.[3] vs. Val.[2], Threo.[2,3] in Gly.[3]	1	314.36	249.18**
Val.[2], Threo.[2] in Gly.[3] vs. Val.[2] Threo.[3] in Gly.[3]	1	71.4	2.78
Val.[3] in Gly.[3] vs. Val.[3], Threo.[2,3] in Gly.[3]	1	408.70*	200.99*
Val.[3] and Threo.[2] in Gly.[3] vs. Val.[3] and Threo.[3] in Gly.[3]	1	503.08**	205.39*
Replicates	3	1244.41**	432.85**
Error	282	97.26	34.00

[1] 10.8% protein diet with 0.15% methionine, 0.19% lysine, 0.04% tryptophan and 0.1% isoleucine.
[2] 0.25% glycine and 0.05% threonine and valine.
[3] 0.5% glycine and 0.1% threonine and valine.
* $P<.05$.
** $P<.01$.

threonine plus valine were used with 0.5% glycine. Further, supplementation of 0.5% glycine with valine and valine and threonine did not significantly improve egg production.

Feed Consumption and Efficiency. The data on feed consumption, Tables 10 to 13, indicated that all hens regulated their feed intake in part with the nutrient demand for egg production. Peak egg production for most groups was observed in the second period and feed consumption (Table 11) was greatest at that time. In general, feed consumption appeared to be unaffected by dietary amino acid supplements. For example, hens fed the basal diet ate at a rate of 83 to 96 grams per day and on the average 87 grams (Table 14) as compared to the high egg producing hens receiving 0.5% glycine and 0.1% threonine that consumed an average of 86 grams of feed per day.

Data on feed consumption per dozen eggs produced showed good correlation between feed intake and egg production. Hens producing eggs at an average rate of 61 to 65% converted 1.7 to 1.8 kg. of feed into one dozen eggs, whereas hens producing eggs at more than 65% required smaller amounts of feed to produce a dozen eggs (Table 14). The highest producing hens on the 0.5% glycine and 0.1% threonine supplements converted 1.4 kg. of feed into one dozen eggs.

Egg Quality. The data on egg weights, Tables 10 to 13, indicated that hens fed the basal diet produced eggs of a weight which was not significantly different (Appendix Table 2) from those of the rest of the treatments. However, hens receiving 0.25% glycine with

0.05% valine produced significantly smaller eggs ($P<.01$) than those receiving 0.1% valine. The highest producing hens appeared to produce smaller eggs when contrasted to those on the basal diet.

As reported in Experiment 1, Haugh unit values again appeared to be unaffected by amino acid supplements. Egg shell thicknesses and dry weights of egg shells were observed to range between 0.28 to 0.34 mm. and 5 to 7 grams, respectively, for all treatment groups and appeared to be unaffected by amino acid supplements. They were not, therefore, included in the tables. In general, the data on egg weights, Haugh units and egg shell quality suggest that hens effectively utilized low protein diets to produce apparently good quality eggs.

Plasma Levels of Free Amino Acids. The results shown in Table 15 indicated that hens receiving the low protein basal diet for a period longer than that of hens fed the 16% protein control diet showed lower plasma levels of several free amino acids. This may be due to depleted amino acid reserves or inadequate supplies of amino acids in the diet. Amino acids in the plasma of hens fed different dietary supplements were frequently observed to be at higher levels as compared to those of hens on the basal diet. Sometimes the changes closely reflected dietary amino acid supplementation. This appeared to be true for glycine, threonine and glycine and valine. Threonine at 0.05% with 0.25% glycine apparently reduced egg production (Table 14) and plasma threonine, serine and glycine were elevated to higher levels compared to the levels exhibited by hens on the basal and 0.25% glycine supplemented diets. Hens receiving additional threonine

showed markedly lower plasma levels of the above amino acids, whereas egg production was not changed. Plasma levels of most of the amino acids, except lysine, were observed to be identical when 0.25% glycine was fed with 0.05 or 0.1% valine. Egg production (Table 14), however, was superior on the diet with less valine, thus suggesting that probably both the lower levels of glycine and valine were adequate for maximum egg production. Threonine supplementation appeared to elicit similar plasma amino acid responses at both levels of glycine intake. Plasma glycine was elevated to a comparatively high level by the low level of threonine and decreased by the high level, yet egg production was unaffected. In that the higher supplemental levels of glycine and threonine caused hens to produce eggs at a maximum rate, the evidence suggests that 0.1% threonine was adequate and excess dietary glycine probably facilitated threonine utilization for increased egg protein synthesis.

Plasma amino acids shown by hens on the 16% protein diet, whether fed continuously or changed from the basal diet for 2 weeks after the termination of the experiment, were markedly similar, thus suggesting that plasma amino acids follow the dietary pattern.

<u>Distribution of Proteins in Egg Albumen.</u> Data on different protein fractions of egg white, Table 16, suggest that their distribution was not correlated with dietary amino acid supplements. Lysozyme and ovomucin fractions were comparatively consistent, irrespective of dietary supplements and were comparable to that observed in a 16% protein diet. Conalbumin and ovomucoid and globulin fractions, in

general, were higher in eggs from hens fed the basal and supplemental diets, whereas total ovoalbumin fractions were lower compared to those fractions reported for eggs from hens on a 16% protein diet.

Amino Acid Composition of Egg White Protein. Amino acid contents of albumen of eggs from hens fed the low protein diet or the several supplements were not consistent in this experiment but were comparatively low when compared with those produced by hens on a 16% protein diet (Table 17). Hens fed 0.05% threonine, with both low and high levels of glycine, showed almost identical egg white amino acid compositions. The increase of threonine to 0.1% appeared to generally increase the amino acid composition of egg white proteins. With the low level of glycine, valine tended to increase amino acid content of egg albumen, whereas with the 0.5% glycine level the opposite appeared to be true. The low level threonine supplement appeared to reduce amino acid composition of egg white protein, regardless of glycine levels.

GENERAL DISCUSSION

Hens fed a typical corn-soy diet diluted with glucose to 10.8% protein and supplemented with 0.15% methionine, 0.19% lysine and 0.04% tryptophan produced eggs at the rate of 61% over a 16-week period. Further supplements of threonine or valine at 0.05 and 0.1% levels did not improve egg production, feed consumption or feed efficiency and egg quality. With combined low levels of threonine and valine supplementing the basal diet, increased egg production was observed and the combination of both threonine and valine at the 0.1% levels showed the maximum response in egg production. This was comparable to that obtained by the sole supplementation of isoleucine at 0.1%. Feed consumption, feed conversion or egg quality were not markedly influenced in those instances where egg production was observed to be improved by amino acid supplements. Further responses in egg production from supplements of threonine and valine with isoleucine were not significantly superior ($P < .01$) to that observed from isoleucine alone. This suggested that isoleucine was probably more limiting for egg production than other amino acids in the low protein diet, which had already been adequately supplemented with methionine, lysine and tryptophan.

As a result, isoleucine at 0.1% was included in the basal diet and an approach was made to determine which amino acids are further limiting egg production in this low protein diet. Glycine, a two carbon dispensible amino acid, is required for maximum chick growth, whereas hens on a 16% protein practical diet have not been shown to require glycine. It was hypothesized, however, that because of its

ionic neutrality and its interrelationship with serine and threonine glycine supplementation could be beneficial in threonine utilization and by that means improved egg production could result. Hens fed either 0.25 or 0.5% glycine supplements alone failed to improve on the egg production obtained by feeding the basal diet. At this point it was interesting to observe that the egg production obtained with this basal diet was comparable to that observed in the previous experiment where 0.1% isoleucine supplemented the basal diet.

Threonine at 0.05% with either level of glycine did not show any improvement in performance. However, 0.05% valine, regardless of glycine levels, was effective in improving performance and hens fed the diets with 0.5% glycine and 0.10% threonine supplements showed maximum egg production and feed conversion. Further supplementation with valine showed no improvements in egg production or egg quality.

As discussed earlier, plasma levels of the free amino acids reported for Experiment 1 were most inconsistent, possibly because the amino acids already present or added as supplements to the low protein diet were not adequate to meet the requirement for metabolic nitrogen. In Experiment 2, plasma levels of most of the free amino acids as influenced by threonine or valine plus glycine supplements were observed to be more correlated with differences in the laying hen's performance. Plasma threonine, serine and glycine were influenced by these supplements and generally reflected the laying hen's performance. Hens fed 0.05% threonine, with either low or high glycine, showed increased plasma levels of these amino acids,

but egg production was not improved. Further increases of threonine to 0.1% caused a lowering of most of the plasma amino acid levels regardless of dietary glycine levels. The high level of dietary glycine (0.5%) with 0.1% threonine resulted in a significant improvement in egg production. These findings suggest that 0.1% threonine indeed improved the basal diet and that glycine in excess could facilitate utilization of threonine for increased egg protein synthesis.

Considering the metabolic interrelationships of threonine, glycine and serine (Meister, 1965), it was interesting to note that he reported that threonine could be degraded to glycine and that nonenzymatic reversion of this reaction is possible in the presence of pyridoxal and metal ions. Glycine, when metabolized, is readily converted to serine which then serves as a methyl donor and carbon donor for protein synthesis. Reid _et al_. (1972), using low protein layer diets supplemented with different levels of nonprotein nitrogen sources, commented that the conversion of essential amino acid nitrogen to nonessential amino acid nitrogen by hens fed low protein diets was relatively inefficient. They indicated that nonprotein nitrogen additions exerted a sparing effect on essential amino acid nitrogen to improve the performance of laying hens. These reports are supported by the findings of Experiment 2 in that dietary glycine, as an excess substrate, could effect more efficient threonine utilization, thereby resulting in a more positive nitrogen balance.

Data obtained on the distribution of protein types in egg white protein for hens on these low protein diets were not consistent when

compared to those observed for eggs from hens on a 16% protein diet. Amino acid composition of egg white protein indicated that hens receiving 16% protein diets deposit more essential and certain non-essential amino acids in eggs than hens receiving 10% protein diets. Threonine and valine supplements, regardless of glycine levels, influenced the amino acid composition of egg white protein, perhaps through the mechanisms discussed.

SUMMARY

Laying type pullets, housed in 8-inch cages under a controlled environment, were fed a typical 16% protein corn-soy diet diluted to 10.8% protein with glucose and supplemented with 0.15% DL-methionine, 0.19% L-lysine and 0.04% DL-tryptophan for a 20-week depletion period. Subsequently thereafter, individual supplementation of valine and threonine at 0, 0.05 and 0.1% levels and DL-isoleucine at 0 and 0.1% levels and all possible combinations were studied in a factorial design to determine their effects on the performance of laying hens. Each treatment was replicated four times using two hens per replicate fed for a 16-week period.

Hens fed the basal diet produced eggs at a rate of 61% or 35 grams of egg mass on a hen-day basis and consumed about 76 grams of feed per day. Threonine and valine did not influence performance. Combined supplements of 0.05% threonine and valine only slightly improved egg production, but the higher levels caused hens to produce 7.5% more eggs than hens fed the basal diet. However, hens fed the diet with only 0.1% isoleucine supplementation showed egg production comparable to that given the diet with 0.1% threonine and valine supplementation. Feed consumption, feed conversion or egg quality appeared to be unaffected where a response to improved egg production was observed from amino acid supplements.

Based on these results, hens in Experiment 2 were fed the 10.8% protein diet which contained 0.15% DL-methionine, 0.19% L-lysine, 0.04%

DL-tryptophan and 0.1% DL-isoleucine from 24 to 40 weeks of age. Further supplements of 0.05 and 0.1% DL-threonine and DL-valine with 0.25% or 0.5% glycine in all combinations were fed to hens in four replicated groups of two hens per replicate to determine their effects on laying hen performance. Hens fed the above basal diet maintained identical egg production to that reported with 0.1% isoleucine supplementation in Experiment 1. Feed intake and egg quality in this experiment were satisfactory regardless of the dietary supplementations. Glycine did not show improved egg production, whereas 0.05% valine with low or high glycine appeared to show some improvement in egg production. However, based on egg production data and on plasma levels of free amino acids, threonine at 0.1% effectively supplemented the basal diet and by feeding excess glycine hens were able to more efficiently utilize threonine for increased egg production. Data on the distribution of proteins in albumen were not conclusive. However, amino acid composition data indicate that hens fed low protein diets, even when supplemented, deposit less essential amino acids in their eggs than hens fed 16% protein diets. These results suggest that further investigations should be made to effectively maintain the increased egg production observed in this study and to provide the optimal dietary nitrogen for an improved amino acid make-up of egg white protein.

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APPENDIX

Table 1. Analysis of variance for feed consumption and feed per dozen eggs (Experiments 1 and 2)

Source of variation	df	Mean squares: Feed consumption	Mean squares: Feed/ doz. eggs
Experiment 1			
Treatment	17	1487.52	41.20
Replicates	3	43692.06	25.49
Error	216	2693.73	69.27
Experiment 2			
Treatment	18	92.14	
Replicates	3	2.09	
Error	282	119.87	

Table 2. Mean squares for feed per dozen eggs and egg weight (Experiment 2)

Source of variation	df	Feed per dozen eggs	Egg weight
Treatments	18	0.35*	35.14**
Basal[1] vs. rest	1	0.05	4.40
Gly.[2] combinations vs. Gly.[3] combinations	1	0.68	5.40
Gly.[2] vs. rest of Gly.[2] combinations	1	0.19	172.24**
Threo.[2,3] in Gly.[2] vs. rest of Gly.[2] combinations	1	2.39**	3.50
Threo.[2] in Gly.[2] vs. Threo.[3] in Gly.[3]	1	0.08	35.50
Val.[2] and Val.[2], Threo.[2,3] in Gly.[2] vs. Val.[3] and Val.[3], Threo.[2,3] in Gly.[2]	1	0.84*	107.80**
Val.[2] in Gly.[2] vs. Val.[2], Threo.[2,3] in Gly.[2]	1	0.05	51.18*
Val.[2], Threo.[2] vs. Val.[2], Threo.[3] in Gly.[2]	1	0.13	53.30*
Val.[3] in Gly.[2] vs. Val.[3], Threo.[2,3] in Gly.[2]	1	0.10	60.80*
Val.[3], Threo.[2] in Gly.[2] vs. Val.[3], Threo.[3] in Gly.[2]	1	0.08	9.68
Gly.[3] vs. rest of Gly.[3] combinations	1	0.13	19.25
Threo.[2,3] in Gly.[3] vs. rest of Gly.[3] combinations	1	0.12	33.19
Threo.[2] in Gly.[3] vs. Threo.[3] in Gly.[3]	1	0.44	105.80**
Val.[2] and Val.[2], Threo.[2,3] in Gly.[3] vs. Val.[3] and Val.[3], Threo.[2,3] in Gly.[3]	1	0.40	25.52
Val.[2] in Gly.[3] vs. Val.[2], Threo.[2,3] in Gly.[3]	1	0.07	1.40
Val.[2], Threo.[2] in Gly.[3] vs. Val.[2], Threo.[3] in Gly.[3]	1	0.03	57.78*
Val.[3] in Gly.[3] vs. Val.[3], Threo.[2,3] in Gly.[3]	1	0.37	48.60*
Val.[3], Threo.[2] in Gly.[3] vs. Val.[3], Threo.[3] in Gly.[3]	1	0.08	2.94
Replicates	3	1.69**	1.75
Error	282	0.19	10.51

[1] 10.8% protein diet with 0.15% methionine, 0.19% lysine, 0.04% tryptophan and 0.1% isoleucine.

[2] 0.25% glycine and 0.05% threonine and valine.

[3] 0.5% glycine and 0.1% threonine and valine.

* $P < .05$.

** $P < .01$.