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BY

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FOR LAYING HENS AND TURKEY POULTS

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, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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REPLACEMENT OF PROTEIN WITH AMINO ACIDS IN DIETS FOR LAYING HENS AND TURKEY POULTS

Abstract

EDWIN J. NOVACEK

Under the supervision of Professor C. W. Carlson

Low protein diets were formulated for experiments with laying hens and turkey poults. The diets were supplemented with equal amounts of protein from different feedstuffs while maintaining equal calculated levels of selected amino acids. Single trials were also reported for factorially applied amino acid supplements to turkey starter and cage-layer diets.

The three-year studies indicated that a typical corn-soy layer diet diluted with glucose monohydrate to 9.4 percent protein was deficient in methionine, lysine and trytophan. Attempts to further improve the rate of egg production by additional supplements of arginine, isoleucine, valine, or diammonium citrate were not successful.

The low protein diet for layers was then supplemented with 3 or 6 percentage equivalents of protein from common feedstuffs in an effort to improve egg production from approximately 55 percent, on a hen-day basis, to 65 percent or better for 10 months of production. It was apparent that the 9.4 percent protein layer diet was deficient in a substance which could be supplied by several feedstuffs. Supplements of three percentage equivalents of protein from yellow corn, spring wheat, barley, soybean meal, soybean protein and fish meal elicited a response in production.

Supplements of 6 percentage equivalents of protein from soybean meal, soybean protein or a mixture consisting of a 40:60 ratio of protein from corn and soybean meal did not further improve production. Supplements of 3 percentage equivalents of protein from hydrolyzed feather meal, oats, meat and bone scraps, or milo or of nf-180 did not stimulate marked improvement over that obtained from the 9.4 percent protein amino acid supplemented basal diet.

Studies were also made with turkey poults fed low protein starter diets supplemented with amino acids to 40, 60, or 80 percent of the feeding standard proposed by Dunkelgod et al. (1961). Growth rates of poults fed to four weeks of age on 20 percent protein diets were slower for poults fed diets containing 13 percent protein equivalents from hydrolyzed blood meal, corn gluten or meat and bone scraps than were growth rates of poults fed diets with soybean meal, safflower meal or fish meal as the supplement. Growth rates were improved for all diets except fish meal by supplementing the diet with amino acids to 60 percent of the standard. Further supplements to 80 percent of the standard did not affect growth rates.

Studies were also made with turkey poults which involved factorial application of treatments. The 20 percent protein diet was supplemented with amino acids to 100 percent of the amount calculated for the 28 percent protein corn-soy control diet. The results of the factorial expressed as effect means of body weight indicated that the effects were consistent for five consecutive weeks. The effects indicate that methionine, lysine, and tryptophan were deficient in the 20 percent protein corn-soy starter diet. Further indications of a deficiency of valine and a detrimental effect of excess isoleucine were observed. A similar type of study was made with a 9.4 percent protein diet for caged layers. Supplements of methionine, lysine, valine and inositol were used. The results indicated that variation between groups was not consistently correlated with time or with the four factors in the test. The effect means of hen-day egg production may not have been an adequate measure of response to dietary supplements in this limited study.

Free amino acid levels in serum from turkey poults and electrophoretic patterns of egg protein and plasma protein on polyacrylamide gel were found to be poor indicators of the nutritional adequacy of the diets used in this study. Free amino acid levels were not consistent among birds treated alike. Serum protein electrophoretic patterns were essentially the same for all hens and poults regardless of the diet being fed.

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INTRODUCTION

In the past decade, competition between animals for food has greatly increased. This is due to an increase in population of both man and animals (Bishop, 1964) and to methods of feeding which use potential human food for animal production (Weiss and Leverton, 1964). The continuation of the trend will result in a greater competition for feed and food because more animals will be fed to provide sustenance for an increased population and more people will eat more food. As competition increases, substitutes for the dietary components must be found either as naturally occurring or synthesized materials.

Several sources of feedstuffs which supply primarily energy are abundant. Fats are available in liberal amounts from the animal packing plant industry as well as a by-product from processing seed proteins and starch. Fat is also discarded from deep-fat-fryers, as trim and melted fat in commercial food preparation centers, in institutions as well as in the home. The cereals, milo, oats, and corn, are also extensively used in poultry rations as a source of energy. These and other low protein feedstuffs are available in liberal amounts. Production and quality may be improved by developing new varieties and by implementing improved farming techniques.

Minerals and vitamins are also plentiful. Synthesis of vitamins by the pharmaceutical industry could be increased to supply almost unlimited amounts of all vitamins. Minerals are available in quantity, as by-products or primary products of the mining and chemical industry.

The feedstuffs which are already limiting in most parts of the world are those rich in good quality protein (Weiss and Leverton, 1964). According to Wokes (1968) the human requirement for protein will increase from 68 million tons in 1960 to 112 million in 1975. Natural sources such as fish in the sea (Shapiro, 1964) can, conceivably, be exhausted. It may become necessary to develop substitutes for protein to supply both the human and animal population if the world population continues its rapid increase. The larger human population will require more protein, and less will be available for animal feeds. Fewer animals fed will yield less good quality protein, already a limiting factor.

Fortunately, animals, including poultry, have no requirement for protein _per se._ Feeds used for poultry production may be formulated from low protein feedstuffs and supplemented with combinations of amino acids to satisfy the animal's amino acid requirement. A protein shortage could be made less acute by replacing a portion of the natural protein with a protein substitute from a synthetic source. A substitute for protein in diets for poultry could be the free amino acid. Before satisfactory utilization of the substitute can be achieved, minimum requirements for each amino acid must be more completely understood.

Although many studies have been made, knowledge about minimum levels of amino acids in poultry diets has been limited. Several factors probably have influenced the delay, only one of which has been the high cost and limited production of amino acids. Also most experimental units were investigating what were considered to be more pressing problems, and funds were generally scarce for protein and amino acid studies (Keith, 1963). Another important factor was the confusion resulting from limited knowledge about vitamins. The effect of vitamin B_{12} and its association with animal protein is an example of the confusion which has not been overcome although twenty years have passed since vitamin B_{12} was synthesized in a laboratory. The confusion has yielded experimental data which have further complicated the already complex subject of protein nutrition.

OBJECTIVE

The objective of this work was to gather information on the influence of amino acid supplements to low protein diets for poultry. The turkey and the laying hen together consume large quantities of good quality protein. The level of protein required could be reduced by substitution of a synthetic source of the amino acids which are limited at the lower protein level. Before low protein diets can be used successfully under commercial conditions, more information must be obtained about requirements for amino acids.

To ascertain the amino acid requirement of laying hens and turkeys it is necessary to obtain diets adequate in all other nutrients except the amino acid being studied. It is and will also be necessary to select, even arbitrarily, criteria which reflect the response and which can be measured quantitatively. Information should be obtained concerning the amino acids which are deficient in a low protein diet and about methods of measuring the deficiency. Until these simple situations are more fully understood, the effect of amino acids and their interactions cannot be intelligently studied.

LITERATURE REVIEW

Studies concerning the nutritive value of proteins have been made for over 100 years. According to Sahyun (1944), Albanese (1959) and Mitchell (1962), Voit (1866) was the first to publish information on the comparative values of different proteins for dogs as related to nitrogen balance. The subject apparently remained closed from 1866 until Thomas (1909) introduced the term Biological Value. This system fell into disuse until it was modified by Mitchell (1924) to consider endogenous urinary and metabolic fecal nitrogen losses. During the interim, Willcock and Hopkins (1912) and Osborne and Mendel (1916) found that a relationship existed between the amino acid patterns of the protein and performance of animals fed the protein. Several years were to pass for vitamins to be discovered. Shortly after the discovery of most of the fat soluble and water soluble vitamins, Mitchell and Block (1946) presented data on "protein score". The "protein score" was a comparison of the amino acid pattern of a protein with the pattern found in whole egg protein. The amino acid with the lowest value relative to that in egg protein determined the protein score which was expressed on a percent basis. Since this work, several different methods of expressing the nutritive value of proteins which use growth rate in the equation have been suggested, but except for plasma amino acid studies, they will not be reviewed.

Attempts to quantitatively assess the nutritive value of proteins have been rather sporadic since the late 1800's and early 1900's. The early work evidently was stimulated by the published works of Thomas, who was concerned with nitrogen balance and introduced the term Biological Value, Willcock and Hopkins, who provided information on the influence of tryptophan in deficient diets, Osborne and Mendel, who published several papers on the nutritive value of proteins, and work with the cooperation of Ferry that introduced the term protein efficiency ratio. The Thomas equation for Biological Value was modified by Mitchell (1924) for use in comparative studies with proteins. Many papers were published on the Biological Value of feeds, but the major discrepancy was that the Biological Value of combinations of proteins was not predictable. Later Mitchell and Block (1946) suggested that the value of proteins be expressed on the basis of an amino acid chemical score. The amino acid content of several feedstuffs was determined chemically by hydrolysis. Because the hydrolysis procedure was more severe than the processes of digestion, the chemical score and nutritive value were not always in agreement. During the succeeding 13 years, several reports were made concerning free amino acid concentrations in plasma and their relation to pathological conditions. Concern was expressed about the reliability of the levels and ratios when the patient consumed random food during the period before the analysis was made. It was suggested (Re, 1940) that previous protein intake would affect the relative level of amino acids in plasma and that under certain conditions this effect could either mask a pathological state or simulate it.

Interest in the nutritional aspects of plasma-free amino acids was stimulated when Man et al. (1946) showed a relationship between low plasma amino acid levels and a poor post-operative nutritional status. This report was supported by the data of Bonsnes and Brew (1947) that pregnant women have lower plasma levels than non-pregnant women. Actually, the effect of diet on levels of nonspecific amino acids was first reported in 1906 by Howell, but the results of his efforts were not understood by his co-workers, if indeed he understood them himself, because it was not until 1908 that the amino acids were recognized as being physiologically active. Failure to recognize the importance of amino acids was due at least in part to growth trials that tested the effect of a single amino acid, namely asparagine. Tests with a single amino acid were proven invalid after Cohnheim (1908) reported that an enzyme, erepsin, was found in the small intestine which was active in reducing the peptones to what was called the "amido acids". It was then recognized (Cohnheim, 1908) that the physiological reconstruction of the protein was dependent upon the presence of several amino acids and not just asparagine. These data were evidently the added evidence to support the growing interest in the physiological activities of amino acids indicated by Howell (1906).

Today it is generally agreed that there are at least eight amino acids essential for growth of simple stomached animals. Flexible standards of requirements for these amino acids for most domestic animals are available (NRC, 1966). Requirement standards are flexible because they are affected by specific conditions including species, degree and type of production, expression of requirement on a per day basis or percent of protein or diet and many other factors.

Free Amino Acids in Plasma

Because of lack of agreement and the difficulty involved in testing for a gross response from several amino acids, plasma studies were welcomed as a tool. The animal under test was usually fed according to a specific schedule and blood samples were taken into heparinized tubes. They were centrifuged to precipitate cells, and a chemical was added to denature protein. After the protein was precipitated, free amino acids remained in solution and analysis was made by paper and column chromatographic, or microbiological procedures.

Application of free amino acid studies to nutrition research was to some extent a result of publicity given to the work of Lotspeick (1949) who reported that the administration of insulin resulted in a decrease of amino acid levels in the blood of mongrel dogs. This work was adapted and reoriented by Denton and Elvehjem (1954a) who fed dogs different levels of amino acids in synthetic diets. Their results indicate that the level of amino acids in plasma was changed for both dogs after feeding but that the level of any specific amino acid was as dependent upon the specific animal as the diet being fed. This work was extended by the same authors, 1954b. They investigated the effect of casein, beef, zein and nitrogen-free diets on free amino acid patterns in dogs. The study again showed a difference between dogs at zero hour and that the source of protein resulted in a different degree of response. It was stated that beef and casein were evidently digested more rapidly than zein, thus a more full utilization of beef should be obtained. The statement was probably made because it had been suggested that all amino acids must be at the site of protein synthesis at the same time or they are lost. Life expectancy in the amino acid pool was thought to be about one hour.

Several research groups have reported an effect of protein on the free amino acid pattern in poultry plasma. Without exception the results of studies have been obtained on groups of chicks and the variation between individual animals was not apparent. Richardson, et al. (1953) evidently had been working on the effects of vitamin B_{12} and Charkey et al. (1950) reported that vitamin B_{12} influenced the free amino acid patterns of plasma. The data of Charkey et al. (1950) were in agreement with Richardson et al. (1953a), and in addition, other vitamins affected amino acid levels. The only amino acid affected by all vitamins was methionine. It was increased by all factors except that it was reduced by vitamin B_{12}. Richardson et al. (1953b) further reported on the influence of amino acid supplements and protein on plasma levels. Although both sources affected plasma levels, an 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 plasma free amino acids.

Longenecker and Hause (1959) used free amino acids in plasma in an attempt to classify proteins in a manner similar to the method of Mitchell and Block (1946). The difference here was that the protein under test would be subjected to the physiological conditions of digestion rather than acid hydrolysis. The results showed that even though different dogs had different levels of free amino acid, in plasma all responded in such a way that an estimate of the relative deficiency of an amino acid could be made. The plasma amino acid score obtained indicated that wheat gluten was limiting in lysine, methionine, arginine and valine in that order.

McLaughlin et al. (1967) have given a list of proteins on which they have determined the first limiting amino acid by the use of plasma studies. The standard error of all plasma values was seldom greater than ±5 percent of the tabled value and often ±0.0. Even though the lysine plasma value for rye was 24 mg. percent, compared to 16 for wheat, it was reported to be the first limiting amino acid for both grains. It was of considerable interest to note the agreement between the plasma score and rat growth. There were no exceptions to the agreement between their values for a first limiting amino acid and that obtained by rat growth studies. The rat growth studies had been previously reported by several other authors.

Factors Affecting Levels of Plasma Free Amino Acid

These results show that although plasma values have serious inconsistencies, they can yield useful information. One principal inconsistency is that shown between animals on the same feeding schedule. This is particularly unfortunate for studies involving small animals. For studies with large animals the same individual can be used for a series of tests. With small animals such as the chick, poult or rat the amount of blood required for analysis may produce such a physiological response that the animal is no longer useful for the study. Because of these individual variations when small animals are tested, the analyses must be made on pooled samples with fifteen or twenty animals for each replicate sample to overcome the effect of one or two animals with unusual plasma levels.

Large animals were used in studies reported by Theurer et al. (1968) on work with free amino acids in the plasma of lambs. With lambs the effect of diet must be mediated by the rumen microorganisms as well as that of the more soluble portion of the diet which can pass through the rumen before being degraded by the organism. Although it is unlikely that diet would influence plasma levels in the ruminant, an exception may be observed for lysine and perhaps leucine. The concentrations of these amino acids in plasma were known to be different for dietary corn gluten compared to that of soybean meal. The increased levels of leucine and lower levels for lysine in the plasma of the lambs fed corn gluten meal would be expected. However, the high value for lysine in plasma of lambs fed a urea diet was not expected.

Besides the dietary factors which are related to protein, several other factors have been shown to influence plasma amino acid levels. Alvarado (1968) showed that galactose inhibited the absorption of amino acids in the hamster. No specific inhibition was suggested. The results were given only for the amino acid mixture used.

Shinwari and Lewis (1968) have reported that when arginine was added to a chick diet, L-amino acid oxidase activity was increased. The increase was even greater when excess lysine and arginine were both added, though lysine alone had no effect. If L-amino acid oxidase activity was increased under these conditions, then all amino acids would be expected to be more rapidly oxidized and thus change a specific pattern depending upon the relative concentration and affinity for the enzyme.

The ratio of plasma amino acids has been used to identify nutritional deficiencies, particularly those where low protein-calorie intakes result in Kwasiorkhor and Marasumus. Heard and co-workers (1969) as well as Saunders et al. (1967) suggest that plasma albumen or plasma protein levels are more reliable as an indicator of nutritional adequacy. They showed that the correlation of plasma protein and plasma amino acid was approximately 0.20. Thus, only about four percent of the variation in the amino acid pattern was due to, or associated with, a change in plasma protein levels, and they probably are reflections of different physiological factors. If the two are so poorly correlated they both cannot be efficient measures of nutritional status.

De Bodo and Altszuler (1957) reportd that the anabolic effect of growth hormone was dependent upon the ability of the animal to secrete an adequate amount of insulin. In their work, when growth hormone and insulin were administered, levels of free amino acid in plasma were reduced.

Knopf and five co-workers (1965) have reported that specific L-amino acids injected intravenously affected levels of growth hormone and blood sugar. L-leucine, L-lysine, L-phenylalanine and L-arginine promoted a reduction in blood glucose. L-lysine, L-arginine, and L-methionine affected blood levels of growth hormone. It was of more than passing interest to note that Fajans et al. (1967) reported that both arginine and leucine increased the release of insulin in human subjects.

Noall et al. (1957) suggested that several endocrine secretions influence the ability of the cell to receive amino acids from the surrounding medium. The influence of stilbestrol was reported by Oltjen and Lehmans (1968). The effect of stilbestrol (DES) on plasma values for a corn diet was different than its effect with wheat. Most amino acid values were decreased for animals fed DES in corn diets whereas they were increased in the animals fed DES in wheat diets. The plasma amino acids in the ruminant are mediated by the rumen microbial population. If a dietary treatment affects the plasma levels, the change can be due either to a change in the microbial population or the dietary protein must bypass fermentation and be acted upon by the digestive enzymes. The relationship of microbial tissue and growth was considered by Bergen and Purser (1968) in a study with rats. The only difference observed between bacterial and protozoal protein was that protozoa were deficient in histidine for the rat. No amino acid deficiencies were observed in the bacterial protein by the plasma free amino acid study.

Zimmerman and Scott (1965) have presented data about the effect of specific supplements on the level of free amino acids in chicks. All amino acids tend to vary at random and it is difficult to interpret the data. They have suggested that the point of reference will affect the interpretation of a study and that the reference point should be from a group of animals fed a diet with an amino acid balance as close to the requirement as possible. Earlier, the data of McLaughlan et al. (1967) as well as Longnecker (1959) showed fasting amino acid levels as the point of reference. Oltjen and Lehman (1968) and Oltjen and Putman (1966) used each test value against another test value with no fixed point of reference. In view of an observation that the first limiting amino acid will remain at low levels in plasma until the requirement is met before it will increase, a fixed point of reference at that level seems desirable. The data of McLaughlan et al. (1967) contradict this theory. Although they used fasting levels as a point of reference, it is difficult to present an argument against a method shown to be 100 percent accurate for predicting the first limiting amino acid.

It is also apparent that the site selected for collecting blood is important. Collections have been made from the jugular vein, by heart puncture, portal vein cannulation and by decapitation. The data of Theurer et al. (1966) showed that free amino acids at the jugular collection site were different than at the portal site. Evidently, they did not believe that the difference was important or that the two varied together, or, in the interest of being consistent with other work reported, the jugular site was used in later work. The data of Bergen and Purser (1968) support the theory that plasma levels vary with collection site and further indicate that endogenous secretions, loss of mucosal tissue and residual protein can mask the amino acid pattern within the gut in less than two hours after feeding. These data were supported by Williams (1969) who showed that the amino acid ratios supplied to the liver from the intestine do not necessarily reflect the ratios in the intestine and are markedly different between individuals infused with equimolar concentrations of amino acids.

Nasset et al. (1963) investigated the effect of the corn protein, zein, on free amino acid levels in gut contents and plasma. Two hours after being fed specific quantities of zein, the dogs were anesthetized and blood samples collected from the carotid, mesenteric, portal and jugular veins. Amino acid levels were generally increased over fasting levels for all sites of collection, especially in the mesenteric vein. Lysine and tryptophan were found in liberal quantities in the gut contents even though these amino acids are virtually absent in zein. Plasma levels of lysine and tryptophan were not affected by the low dietary levels. The data were supported by an extensive report by Elwyn (1968) on amino acid movements between the intestine, liver and peripheral areas in the dog. Plasma samples were obtained by catheters implanted into the portal vein, hepatic vein and hepatic artery. Although it was stated that the "composition of gut outputs of essential amino acids was similar to the meal" it was also evident that gut output of amino acid was not consistent with time or between dogs. During a twelve-hour collection period, lysine varied randomly between one and 42 micromoles per 100 milliliters of blood. A major peak of 62 micromoles occurred six hours after a single feeding of horsemeat. Splanchnic outputs of amino acids varied from 900 micromoles to 2000 between dogs, and amino acid output from the gut varied from approximately 200 percent of increase over the gut intake to less than an eight percent increase. One reason for the difference in absorption rates between dogs may have been related to the length of the small intestine (Williams, 1969). Intestines with shorter length were shown to have markedly slower rates of absorption.

Protein and Amino Acids for Hens

Studies on the protein requirements of laying hens have also received considerable attention. Many of the early studies which were in one way or another important in the development of present feeding methods were covered in a review by Heuser (1941). Certainly at that early date, several factors besides protein affected the rate of lay. The confounding of the effects of these factors with those of protein level *per se* was unavoidable. Vitamins were supplied entirely from the feedstuffs and minerals were supplied from bone meal and limestone. Also, level of production was itself limiting and 45 percent production for a 12-month period was considered good. It may be expected that the loss of protein in the egg when hens are laying at a higher rate of production would be greater than the extra cost of energy when production increases from 45 to 70 percent. On the basis of the review it was concluded that the diet for hens should contain 15 percent protein, but it was suggested that consideration be given to expressing the nitrogen requirement in terms of amino acids as a function of metabolic size, plus an amount for growth or production. Continuing his line of thought, the protein requirements should be equivalent to the limiting amino acids provided by the 15 percent diet plus the extra cost of increasing the rate of lay from 45 to 70 percent. In order to establish requirement levels it would also be necessary to consider total feed intake, especially if *ad libitum* feeding practices were used. Much of the variation in suggested protein levels for hens can be attributed to the level of feed intake as well as the amino acid pattern and availabity of the protein.

In view of the long-term consideration of requirements expressed in terms of amino acids, it is difficult to understand why continued emphasis is placed on protein. As late as 1967, a report discussed the protein and calorie-protein ratio requirements of coturnix quail, without consideration for amino acid levels. These classifications can have limited scientific merit because they poorly define a specific dietary need. Both are expressions of total nitrogen without consideration of the compounds which comprise the total nitrogen value. Neither expression can be reasonably used with diets containing different ratios of different feedstuffs or even different ratios of the same feedstuff when the amino acid patterns of the protein are unequal. The only condition under which protein per se may be important is when protein is associated with a so-called "unidentified growth factor". Even considering that condition, a classification of protein requirement would be meaningless unless the specific source of protein is specified. If a specific diet was used and this diet compared as a feed for various strains or conditions, then it would be possible to make valid interpretations. If a specific diet was used with different feed additives, then valid interpretations could be made. If the diets supply simple differences in protein levels from various mixtures of ingredients, then a response may be more closely related to the ratio of feedstuffs than to the amount of protein, and valid interpretations become impossible. It would not be possible to separate the effect due to protein from the effect due to several other factors associated with a change in ingredients. On this basis, attempts will be made to restrict the literature review to those studies in which single protein sources or amino acids were used as variables.

Influence of Genotype

Perhaps one of the least understood factors affecting the response to proteins or the requirement for amino acids has been the difference between birds with similar but not identical parentage. The effect of strain and of differences within a strain has been reported in several papers. One of the first observations about the effects of genotype on amino acid or protein requirements has been published by McDonald (1957). A difference in response to methionine supplements to layer diets by Black Australorp and Single Comb White Leghorn (SCWL) hens was noted. These data were supported by those of Griminger and Fisher (1962) who reported that the growth potential of chicks reared on diets deficient in lysine and arginine was inherited.

Nesheim and Hutt (1962) reported a 25 percent difference within two SCWL strains in the requirement for arginine. Hess et al. (1962) observed that lines differing in requirement for methionine could be obtained by selecting breeding stock according to response on a methionine-deficient diet. Lepore (1965) also observed that lines differing in the requirement for methionine could be selected from random-bred SCWL stock. Enos and Moreng (1965) showed that the lysine requirement of chicks from New Hampshire stock was under genetic influence. The chicks, which were selected for weight after being fed a deficient diet, transmitted different potentials for growth when lysine-deficient diets were fed to their offspring.

Moreng et al. (1964) showed that a response from different protein levels expressed as maximum egg production during any given four-week period was affected by strain. Of four strains tested, maximum production was obtained with 15 and/or 17 percent protein. Maximum production varied from 78 percent for strain A to 75 percent for B, C and D. Peak production varied from 65 percent for strain A fed the 13 percent protein diet to 70 percent for the same diet fed to strain C. Strain B peaked at 75 percent for the 13 percent protein diet, which indicated that their requirement for the limiting amino acid in the diet was lower than the remaining three strains.

Deaton and Quisenberry (1965a) also showed that when amino acid levels were calculated to be equal, a strain difference was observed in response to total protein level. The difference was detected by a factorial analysis of variance as a strain X protein interaction; that is, the response to changes in protein level was different between the four strains. Protein levels were varied by changing the ratio of milo and soybean meal in the diet. It was of interest to observe that the amount of defluorinated rock phosphate increased with increased amount of milo. Egg weight was also affected by the change in protein level but did not appear to be related to strain.

Brown and Hale (1965) compared corn, oats and barley as the major ingredients in diets for caged layers. Their results with a large crossbred strain and a small commercial hybrid showed that the smaller strain performed better than the crossbred strain when the diets contained no supplemental amino acids. No evidence was found to indicate that the small bird's requirement for energy differed from that of the larger bird.

Deaton and Quisenberry (1965b) showed that egg production (65-67 percent) was not improved when a 14 percent protein diet was supplemented with methionine and tryptophan to equal National Research Council standard levels. The addition of amino acids was made to 14 percent protein corn or milo based diets. Egg weights for the birds fed milo were smaller (57 vs. 60 gm.). The smaller eggs from the birds fed the milo diet were believed to be due to a deficiency of linoleic acid.

Lillie and Denton (1967) presented data on the effect of protein level of the grower and layer diets on subsequent performance of the laying stock. Literature published prior to 1966 on the effect of grower and layer protein levels was reviewed in a selective manner. It was concluded that significantly improved production was observed for 21 and 16 percent protein diets over lower protein levels. The 14 percent protein diet was adequate for egg production but not for maintenance of body tissue. A difference in the rate of egg production and in weight of eggs produced was observed between strains. The hens fed the 12 percent protein diets also consumed greater amounts of feed, but feed intake was not affected by strain.

Factors Contributing to the Strain Effect

Jaffe (1964), in a study on the factors affecting egg size and yolk size, found that yolk size was correlated to genotype (r=0.82) and to environment (r=0.33) as well as phenotype (r=0.55). The ability of the sire to transmit egg size was greater than the dam (r=0.73 vs. r=0.29). Yolk size was transmitted equally by sire and dam. It was apparent that larger sized eggs would require more nitrogen per egg. The relationship of these data to the strain difference in response to protein level was not discussed.

Fisher (1969) reported on the effect of protein on the relative contribution of shell, yolk and albumen to the whole egg. The studies were made with two hybrid strains 67 weeks old and fed a 9.3 percent protein diet. Egg weights were reduced from 63 to 56 gm. by the low protein diet. The percentage of yolk increased from 31.6 to 32.4 for a brown egg-laying strain and 30.1 to 30.6 for the

Leghorn type hybrid fed the higher protein diet. The relationship between protein level and yolk size was highly significant. A relationship of protein to shell thickness was also observed, but it was ignored because shell thickness is frequently affected by the rate of lay.

Effect of Chemical Form of Nitrogen

Featherston et al. (1962) in a trial with chicks suggested that the L and D amino acids were metabolized equally well. Criteria for that study were growth and feed utilization. Free amino acids in plasma were also considered. The addition of excess D or L indispensible amino acids promoted equal growth rates and feed consumption. Plasma free amino acids were increased for the indispensible as well as the dispensible forms.

The source of nitrogen was also investigated by Akintunde et al. (1968). The diets contained 12 and 14 percent protein with supplements of two and four percentage equivalents of protein from diammonium citrate, diammonium phosphate and soybean meal. The diets were fed for fourteen weeks to hens forty-two weeks of age which had previously been selected for similarity in egg production. Significant increases were obtained by supplementing the 12 percent protein diet with either level of soybean protein. No effect was observed for the supplements to the 14 percent protein diet. The non-protein sources did not affect production at either level of protein. This report is in aggreement with Moran et al. (1968) and is in contrast to the work of Chavez et al. (1966) and of Young et al. (1965). The diets used by the latter two groups were also between 12 and 14 percent in protein and of a similar composition. No explanation was given for the discrepancies in results.

Absorption and Retention

Adibi et al. (1967 a-b) were concerned that the relative absorption rates of the amino acids were different. Their tests on young men involved absorption rates from the small intestine after perfusing the jejunum with free amino acids and simultaneously withdrawing samples. Although absorption may be different in the avian species, it was shown that methionine, isoleucine and leucine were absorbed most rapidly. The rates of absorption of proline, arginine, alanine and phenylalanine were slightly slower. The dicarboxylic acids, glutamic and aspartic, were either absorbed more slowly, or perhaps they were replaced by transamination of the alpha Keto acid as they were absorbed. Changes in the plasma amino acid levels may have been slightly related to absorption pattern, but the relationship was not consistent. The absorption rates of amino acids in the hen were studied by Tasaki and Takahashi (1966). They found that the relative absorption rates of arginine, tyrosine, aspartic acid, glutamic acid and glycine were all slow compared to other amino acids.

Williams (1969) tested the absorption rates of amino acids in sheep. The sheep were fed standard low roughage diets for six weeks and starved for 48 hours before the test. The amino acids were infused into 60 centimeters of exteriorized small intestine. His data given in comparison to that of Delhumeau et al. (1962) for the rat, Orten (1963) for man, and Tasaki and Takahashi (1966) for the fowl, indicated that the rates of absorption of most amino acids were similar for all species. However, marked differences in absorption rates were shown between Mammalia and Aves for arginine, lysine and proline. Rates of absorption of glutamic acid, glycine and proline were similar for the two classes. Histidine was absorbed more rapidly by Aves than by Mammalia.

Boorman and Fisher (1966) studied interactions of several amino acids in chicks. It was reported that methionine at higher levels (0.8 percent) resulted in decreased growth rates. A leucine, isoleucine and valine interaction was also observed. Leucine depressed growth rates of chicks when added to diets containing added lysine whereas arginine did not. It was suggested that arginine acted as a detoxifying agent in the presence of excess lysine.

Boorman et al. (1968), after first reporting on an arginine-lysine interaction (1966), tested the effect of lysine on reabsorption of arginine from the kidney of cockerels. Twenty cockerels weighing between 1.1 and 2.0 kg. were infused for 100-minute periods while under anesthesia. The results indicated that as the rate of lysine infusion increased from 0.0 to 4.0 micromoles per minute per kilogram, efficiency of reabsorption of lysine dropped from 98 to 64 percent. Efficiency of reabsorption of arginine was almost identical and dropped from 97 to 66 percent. Although considerable variation was observed between individuals infused with the higher levels of lysine, the reduced efficiency of absorption of both amino acids was highly significant. It was suggested that an inhibitory effect on reabsorption at the tubular level was involved. The reabsorption of ornithine and histidine also appeared to be affected at the higher rates of lysine infusion.

Waterlow and Stephen (1968) in an extensive review of the literature about the effect of low dietary protein levels on amino acid incorporation suggested that an adaptation to low protein levels occurred in rats. The specific research reported on concerned the incorporation of lysine into protein when the rats were fed low protein diets. In normal rats the rate of turnover of nitrogen was approximately 350 mg per 100 gm per day. When the low protein diet was imposed the rate was reduced to approximately 260 mg. The reduction was primarily due to a reduced turnover of amino acids in the essential organs or tissues. Turnover in the less essential organs or tissues, such as skin and muscle, was not affected. With normal rats, the rate of turnover (350 mg) plus dietary nitrogen (250 mg) was estimated to be 600 mg. of nitrogen per 100 grams of body weight per day. The dietary contribution of amino acids to the amino acid pool or plasma was estimated to be 40 percent of the total and turnover of amino acids was 60 percent of the total. Thus, the endogenous source of amino acids contributed 150 percent of that contributed by dietary sources.

Kaufman et al. (1966) investigated the effect of a deficiency in methionine on values for chemical and histological properties and growth in the rat. It was observed that iron was either poorly retained or poorly absorbed in the absence of methionine, and endogenous stores of body iron were transported to the liver. In spite of this compensation or adaptation, anemia was evident after only two weeks of feeding the deficient diet. Hematocrit values, hemoglobin levels, body and kidney weight were all suppressed in the methionine deficient rats. It was surprising that in spite of a lower body weight, the livers of the deficient rats were larger (4.70 vs. 4.51 gm.). It was suggested that the greater weight may have been due to "citrilobular fatty metamorphosis" which probably means intracellular fat infiltration.

Sidransky and Verney (1968) reported that when rats were force-fed a diet deficient in threonine, several morphological changes occurred. The most striking change was the marked atrophy of the pancreas and submaxillary glands. Atrophy of the parotid gland, stomach and thymus also occurred. It was also suggested that threonine was preferentially used for the synthesis of plasma and hepatic protein over skeletal muscle when the rats were fed diets deficient in threonine. Threonine is a four-carbon hydroxy amino acid, and reamination of alpha ketoglutarate produced by deamination does not occur. It is possible to form the L-amino acid in some organisms by combining acetaldehyde with glycine and by carboxylating amino acetone followed by reduction with NADH. No reports were observed which indicate that this pathway or a similar pathway of regeneration was present in the avian Class.

Amino Acid Requirement Standards

The amino acid requirements of poultry are also affected by several other factors and it is sometimes impossible to separate the confounded effects of secondary factors from the true effect of the amino acids.

Nelson et al. (1960) suggested that the amino acid requirements of chicks increased with increased level of protein. Methionine and cystine were fed at levels from 0.55 to 1.35 percent in 0.10 percent increments. The diets contained from 20.5 to 26.4 percent crude protein.

Naber and Touchburn (1963) used three different standards in an attempt to improve performance of hens fed low protein diets supplemented with amino acids. A 12 percent protein corn-soybean meal layer diet was supplemented with amino acids according to National Research Council standard levels. A response was obtained from methionine but not from lysine supplements even though both were calculated to be deficient. The 12 percent protein diet was also supplemented with amino acids to equal a standard calculated from the 16 percent protein diet. A response was obtained from lysine, but the combination of lysine and arginine depressed egg production. In addition to the two previous standards, the diet was also supplemented to provide amounts based on the ratio of amino acids in whole egg. In this case a response was obtained from lysine, threonine, tryptophan and valine. From these data it appeared that lysine, methionine, threonine, valine and tryptophan may all be limiting amino acids in this diet.

Sherman (1959) in a selected review of literature concerning amino acid supplements for livestock stated that the amino acid requirements of poultry were affected by energy level as well as protein level. It was suggested that a correction of 0.07 percent of the amino acid per 125 cal ME, and an 0.10 percent correction for each three percent change in protein should be made. It was also suggested that the requirements were affected by age, temperature and sex. He stated that the factors are interrelated with amino acids and may have different effects on the requirements depending upon the extent that each factor deviates from a normal standard. The situation was said to provide one of the most complicated areas of research because neither minerals, vitamins nor energy have such varied relationships with themselves and other factors. The statement receives support from the data of Naber and Touchburn (1963) previously cited.

Davidson and Matheison (1965) reported that the methionine levels recommended by the National Research Council were generous.

They suggested a 20 percent decrease in diets for cockerels grown under conditions in Scotland. The birds used to establish NRC recommended levels probably were from fast-growing broiler strains and it is possible that the rate of increase in the nitrogen requirement was greater than the rate of increase for energy. Thus, the amino acid requirement per day may increase at a faster rate than the increase in feed consumption with birds selected for rapid growth potential.

Gleaves et al. (1968) investigated the interrelationships of four physiological food intake regulators in the laying hen. Neither dietary protein, volume, energy or weight had significant effects upon food consumption. Feed consumption increased in a linear manner with level of protein and density and decreased with increased levels of energy. Significant interactions of protein and energy and of volume and density were found. These data indicate that feed intake and the resulting intake of nutrients was governed not only by the amounts of each single factor but were affected by the relative amounts of all factors as well.

Morris (1968) in a review of evidence of the hen's ability to regulate food intake according to diet given, suggested that birds fed high energy diets tend to overconsume energy. Increased feed intake generally resulted in an increase in body weight, which in the mature bird would be essentially laid down in the form of fat deposits. The correlation of extra energy consumed and body weight gain was highly significant ($r=0.675$; $P < 0.001$). Thus, 46 percent of the variation in body weight was associated with variation in caloric intake. It was suggested that strains which normally consume large quantities of energy tend to compensate less efficiently for changes in dietary energy. These data are in agreement with the work of Payne and Lewis (1966) with broiler chicks.

Balloun et al. (1960) fed 24 percent protein starter diets to turkey poults. The diets were supplemented with 0.5 percent methionine, 0.2 percent lysine and 0.2 percent arginine. Their results indicated that the 24 percent protein and supplemented diet supported growth equivalent to a 28 percent protein diet. These data indicate that under certain conditions protein can be replaced by amino acids, without affecting the rate of growth. Other literature concerning amino acid supplements for low protein turkey starter diets has been reviewed by Carlson (1967).

Nitrogen Losses in Urine and Feces

Losses of nitrogenous compounds in the urine and feces of hens have also received considerable attention. Thomas et al. (1969) used a new technique presented by Davidson and Thomas (1969) to estimate several nitrogenous compounds in hen excreta. They used a basal diet which was predominantly barley, oats, wheat and either ground nut or white-fish meal to supply 10.4 percent protein. A greater percentage of nitrogen was lost as uric acid from ground nut meal (37.4 percent of the dietary N) than from the diet containing fish meal (27.0 percent). When ground nut meal was supplemented with 0.1 percent of methionine and lysine, urate N was reduced to 30.5 percent of the dietary N. A difference in digestibility, which approached significance, was attributed to an effect of methionine which aided absorption of other amino acids.

O'Dell et al. (1960) showed that the predominant nitrogenous losses in urine from chicks were uric acid, glutamic acid, glycine, ornithine, and lysine. Uric acid accounted for 81 percent and the total amino acid content accounted for 2.01 percent of the nitrogen from the urine of five-week old chicks. Lysine and glycine were among the predominant amino acids found. The dicarboxylic amino acids and ornithine were also present.

Waring (1969) studied digestibility of amino acids from protein supplements in the colocutaneous fistulated hen. The protein content of fish meal was 64 percent compared to 46 percent for meat and bone scraps and 24 percent for field beans. Each test protein was mixed to supply a highly digestible diet. The digestibility of the three proteins was approximately 89, 69 and 83 percent respectively. In this study, in contrast to the works of Tasaki and Takahashi (1966) and Williams (1969) absorption coefficients for most of the amino acids were within five percent of the mean value. Thus, amino acid availability would be affected equally for all amino acids proportional to the digestibility of the protein. Approximately 0.4 grams of endogenous urinary nitrogen and 0.6 grams of metabolic fecal nitrogen were lost per day. Urinary nitrogen was calculated to be 0.80 mg x gram of body weight to the 0.75 power. For a two kg. hen the loss of nitrogen in the urine should be nearly 240 mg/day.

Source of Nitrogen

Bornstein et al. (1968) compared performance of hens fed diets containing 15 percent protein from either corn-soybean meal, milo-soybean meal or these diets supplemented with fish meal, tallow, or acidulated soap stock. The performance of the birds fed either basal was improved by each supplement. It was suggested that methionine was a limiting amino acid in the 15 percent protein layer diets and that linoleic acid was a limiting factor in the milo diet for the production of normal sized eggs.

Lewis (1966) presented an approach which compared the relative economics of supplying amino acids as protein or as a synthetic source. It was clear from the data presented that a 12.5 percent protein layer diet containing a mixture of fish meal, soybean meal, corn, wheat and milo should supply adequate amounts of most of the essential amino acids. Lysine, methionine and tryptophan respectively were calculated to be 83, 96 and 96 percent adequate. The diet was 107, 109, and 114 percent adequate respectively, when the protein level was increased to 14.5 percent. The data showed that a response to methionine and lysine could be expected at the 12.5 percent level but not at the 14.5 percent protein level.

Britzman and Carlson (1963) (1965) found that performance of hens fed a high-energy 11 percent protein corn-soybean meal layer diet was improved by supplements of lysine and methionine. Production improved as a result of tryptophan supplements, but it was not equivalent to that expected of a 16 percent protein diet. Production rates were not further improved even when lysine, methionine, tryptophan, isoleucine, glycine, valine and arginine were added in a cumulative manner.

Jackson et al. (1967) also tested the effect of lysine and methionine supplements to corn-soybean meal and to barley-fish meal diets. Supplements were made to each of the diets such that each contained an additional 0.1 and 0.2 percent of each amino acid. Their results indicate that a 14.3 percent corn-soybean meal layer diet was adequate in both amino acids. Addition of the amino acids to an 11.1 percent protein barley-fish meal layer diet improved both feed conversion and egg production.

Bray (1960) has indicated that the ratio of protein from corn to that of soybean meal should be 60:40. Working with various corn-soybean meal layer diets, he found (1965) that when corn was the principal constituent of the layer diet, lysine was more limiting than methionine, and when soybean meal was increased methionine became more limiting than lysine. In 1964 he observed that isoleucine and lysine were most limiting in a layer diet containing 8.5 percent protein and a 60:40 ratio of corn to soybean meal protein.

Kirkpatrick and Foulton (1967) compared choline and methionine in 15 percent protein layer diets. The effects of choline were evident only on egg production. The addition of methionine to the diet had no effect on the criteria studied. Levels of methionine were calculated to be 0.53, 0.61 and 0.66 percent. Choline was calculated to be at levels of 0.54, 0.73 and 0.92 gm. per pound of diet. The 0.92 gm. level of choline appeared to have the greatest influence on egg production. The 0.53% level of methionine appeared to support egg production as well as the 0.92 gm. level of choline.

The factors that influence a response to amino acid supplements and the problems involved in measuring a response have been reviewed only in part. Unfortunately, no thorough review of the data has been published in recent years that would be suitable for reference. This review serves only as an introduction to studies reported herein.

GENERAL EXPERIMENTAL PROCEDURE

Experiments with Laying Hens

The experiments with laying hens were made in wire cages. All trials were made with one bird per eight-inch cage except the last trial which had two birds per eight-inch cage or three birds per twelve-inch cage. The trials were generally ten months in duration. Feed and water were given *ad libitum* in all cases. The basal diet, shown in Table I, was essentially the diet of Britzman and Carlson (1965), but further diluted to 9.4 percent protein with glucose monohydrate. As a result of the dilution, the metabolizable energy was increased to 3.40 Kcal/gm. The ratio of protein from corn to that from soybean meal was maintained at 60:40 as recommended by Bray (1960). Amino acid supplements were made to 125 percent of the standard of Johnson and Fisher (1958), and the values are given in Table 2 along with levels recommended by the National Research Council (NRC) (1966). Amino acid levels were calculated from the table of average values according to Merck (1965).

Criteria for response to treatment for the laying trials included egg production and weight, egg shell thickness, quality as measured by Haugh units, body weight, feed conversion and mortality. Free amino acids in blood serum and specific proteins as reflected by electrophoretic patterns on polyacrylamide gel were also considered in limited trials. In the last test, in addition to the basal diet with methionine, lysine and tryptophan, supplements of inositol, methionine, lysine and valine were tested in a factorial arrangement of treatments. Analyses were also made for liver and plasma lipid.

For the egg production data, records were calculated on a hen-day basis. Egg weights were obtained by group-weighing the entire egg production for two days of lay at least twice a month. Shell thickness and egg quality were determined on at least ten average eggs for each treatment group at least once each month. Body weights were taken initially and at the termination of the trials and at other irregular intervals. Mortality was calculated at the end of each experiment.

When considered, serum and egg protein electrophoretic patterns were determined on polyacrylamide gels according to the method of Ornstein (1964) and Davis (1964). Serum and eggs for chemical studies were obtained from hens which had laid eggs on five consecutive days after a rest of at least one day.

For the last trial, livers and plasma were obtained from hens that had been fed the basal diet for a six-month period and then either continued on the basal for a four-month period to provide a control or fed one of the 16 diets from the factorial set of treatments. Hens were also selected from a group which was fed a typical cage layer diet for ten months. Hens were selected at random from each group fed a common diet. The birds were killed electrically and blood was drawn from the jugular vein and carotid artery while the hen was hanging from a track. The livers were removed immediately and both the blood and livers were cooled on ice.

Analysis for lipid in fresh plasma was made by extracting 2 ml. aliquots of plasma with 10 ml. of a 1:2 ratio of ethanol and diethyl ether. Five mls. were transferred to a previously weighed aluminum pan and the samples were first air dried, then dried for two hours at 100° C in a drying oven.

For liver lipid, the livers were sectioned at the center of the right lobe. The tip of the lobe and external fatty tissue were discarded. Sections of the remaining tissue were randomly selected to provide approximately 3.5 grams of wet liver. The samples were dried for 16 hours at 100° C and the fat was extracted by refluxing with diethyl ether for 16 hours.

Experiments with Turkey Poults

For the turkey growth studies, day-old turkey poults were placed in Petersime batteries for either four or five weeks. Feed and water were given *ad libitum* and heat was maintained near 37.5° C for the first week. Temperatures thereafter were adjusted to the bird's apparent comfort, which varied with room temperature. Body weights and feed consumption were obtained either at seven-day or at 14-day intervals. Several diets were used and the compositions of each low-protein diet for each trial are given separately in Tables 3 and 4. The amino acid standards used are given in Table 5.

Analyses of serum for free amino acids were made on an average of five poults and on an individual basis. The blood was collected by heart puncture and allowed to clot. The plasma was deproteinized with picric acid, centrifuged and the excess picrate removed on Bio-Rad Ag 2 x 10 resin. Analysis was made on a Beckman 120 amino acid analyzer. Several tissues and ages of poults were analyzed but are not reported. In one case, cockerels were fed the turkey diets for a 4.5-day period, the birds were killed by cervical dislocation without loss of blood and immediately frozen. The entire frozen chicks were individually ground while frozen in a #1 Universal hand-operated grinder. The resulting material was well mixed and two grams were added to twenty ml. of saturated picric acid solution. The mixture was homogenized in an omni-mixer for two minutes, centrifuged and the excess picrate removed from the supernatant liquid on Bio-Rad Ag 2 x 10 resin. The free amino acids were analyzed as previously described for serum. A completely random design was used in each growth study except for trial 3, which involved the use of a factorial application of treatments in a randomized complete block. Complete and specific procedures pertinent for each trial are given in the appendix.

TABLE 1. COMPOSITION OF THE CAGE-LAYER TEST DIETS USED DURING EXPERIMENT 3.

	Protein Treatment					
	Basal	Soybean Meal	Soybean Protein	#2 Yellow Corn	Soybean Meal	Soybean Protein
	%	%	%	%	%	%
Ground Yellow Corn	41.8	41.8	41.8	75.8	41.8	41.8
Soybean Meal (50%)	11.2	17.1	11.2	11.2	23.0	11.2
Soybean Protein (90%)	...	...	3.3	...	...	6.6
Cerelose	34.0	28.1	30.7	...	22.2	27.4
Yellow Grease	5.0	5.0	5.0	5.0	5.0	5.0
Limestone	5.0	5.0	5.0	5.0	5.0	5.0
Dicalcium Phosphate	2.0	2.0	2.0	2.0	2.0	2.0
Salt Mix[1]	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin Mix[2]	0.5	0.5	0.5	0.5	0.5	0.5
DL-Methionine	0.25	0.17	0.20	0.16	0.09	0.15
L-Lysine	0.19	...	...	0.14	...	...
DL-Tryptophan	0.04	...	0.01	0.01	...	...
ME kcal./gm.	3.34	3.28	3.34	3.28	3.22	3.34
Protein Equivalents	9.85	12.53	12.54	12.73	15.35	15.45
Cystine & Methionine[3]	.500	.500	.500	.500	.500	.500
Lysine	.625	.625	.643	.625	.812	.836
Tryptophan	.150	.153	.150	.150	.192	.173

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 IU Vit. A; 820 ICU Vit. D_3; 11 IU. Vit. E., 2.2 mg. Vit. K; 29 mg. Niacin; 4.8 mg. Pantothenate; 375 mg. Choline; 4.8 mg. Riboflavin; 9.7 mcg. Cyanocobalamine and 110 mg. Ethoxyquin.

3 Not more than 0.200% as cystine or less than 0.300% as methionine.

TABLE 2: AMINO ACID REQUIREMENTS OF LAYING HENS AND THE CALCULATED CONTENT OF THE LOW PROTEIN BASAL DIET.

	National Research Council[1]	Johnson & Fisher (1958)		Basal Diet[2]
		100 Percent	125 Percent	
	%	%	%	%
Arginine	0.8	0.50	0.63	0.60
Cystine	0.25	0.16	0.20	0.15
Glycine	...	0.50	0.63	0.48
Histidine	...	0.18	0.23	0.21
Isoleucine	0.50	0.50	0.63	0.49
Leucine	1.20	0.68	0.85	0.82
Lysine	0.50	0.50	0.63	0.47
Methionine	0.28	0.24	0.30	0.17
Phenylalanine	...	0.42	0.53	0.45
Threonine	0.40	0.30	0.45	0.25
Tryptophan	0.15	0.12	0.15	0.12
Tyrosine	...	0.30	0.38	0.36
Valine	...	0.54	0.68	0.48

[1] National Research Council (1966).

[2] Calculated on the basis of the amino acid content of the feedstuffs as given by Merck (1961).

TABLE 3. COMPOSITION OF THE TEST DIETS FOR STARTING TURKEY POULTS.

	Protein Treatment					
Ingredient	Soybean Meal	Fish Meal	Safflower Meal	Hydrolyzed Blood Meal	Corn Gluten Meal	Meat & Bone Scraps
	%	%	%	%	%	%
Yellow Corn	57.0	57.0	57.0	57.0	57.0	57.0
Fish Meal	2.0	2.0	2.0	2.0	2.0	2.0
Dried Buttermilk	2.0	2.0	2.0	2.0	2.0	2.0
Alfalfa Meal	2.0	2.0	2.0	2.0	2.0	2.0
Protein Treatment	25.2	21.0	30.0	17.5	21.0	22.9
Glucose Monohydrate	2.3	12.5	...	13.0	9.5	11.6
Yellow Grease	3.5	0.5	1.0	0.5	0.5	1.5
Sand	...	1.0	...	...	...	...
Limestone	3.0	1.0	3.0	3.0	3.0	...
Dicalcium Phosphate	2.0	...	2.0	2.0	2.0	...
Vitamin Mix[1]	0.5	0.5	0.5	0.5	0.5	0.5
Trace Mineral Mix[2]	0.5	0.5	0.5	0.5	0.5	0.5
Protein (%)	19.93	19.93	19.93	19.93	19.93	19.93
ME (kcal./gm.)	3.11	3.14	3.17	3.09	3.18	3.11
Calcium (%)	1.86	1.94	1.85	1.87	1.83	2.50
Phosphorus (%)	0.76	0.93	0.67	0.75	0.69	1.15

[1] To supply per kg of feed, vitamin A 10,572 IU; D_3 2,753 ICU; E 44 IU; K 2.2 mg; pantothenate 8.8 mg; riboflavin 17.6 mg; choline 880 mg; niacin 88 mg; folic acid 2.2 mg; B_{12} 17.6 mcg; biotin 0.2 mg and ProStrep 1 mg.

[2] To supply per kg of feed NaCl 4.85 gm; Mn 22.5 mg; Zn 25 mg; I 0.5 mg; Fe 8.5 mg; Co 0.5 mg; Cu 2.5 mg; and S 15.0 mg.

TABLE 4: COMPOSITIONS OF THE LOW PROTEIN TURKEY STARTER DIET AND A TYPICAL STARTER DIET.

	Low Protein %	Typical Diet %
Ground Yellow Corn	43.8	40.0
Soybean Meal	27.2	43.8
Fish Meal	2.0	2.0
Dried Whey	2.0	2.0
Alfalfa Meal	2.0	2.0
Corn Oil	3.5	4.2
Yellow Grease	3.0	...
Dicalcium Phosphate	2.0	2.0
Limestone	3.0	3.0
Vitamins[2]	0.5	0.5
Trace Mineral Salt[1]	0.5	0.5
Glucose Monohydrate	10.5	...
Protein Level	20.0	28.0

[1] To supply per kg of feed, vitamin A, 10,572, IU; D_3 2,753 ICU; E 44IU; K 2.2 mg.; pantothenate 8.8 mg., riboflavin 17.6 mg., choline 880 mg., niacin 88 mg., folic acid 2.2 mg., cyanocobalamin 17.6 micrograms, biotin 0.2 mg., and ProStrep 1 gm.

[2] To supply per kg of feed, NaCl 4.85 gm., manganese 22.5 mg., zinc 25 mg., iodine 0.5 mg., iron 8.5 mg., cobalt 0.5 mg., copper 2.5 mg., and sulfur 15 mg.

TABLE 5. AMINO ACID STANDARDS FOR TURKEY STARTER DIETS.

	Standard[1]	Standard[2]
	%	%
Arginine	2.36	1.81
Glycine	2.49	1.43
Histidine	0.90	0.65
Isoleucine	1.82	0.65
Leucine	3.14	2.20
Lysine	2.60	1.65
Methionine (minimum)	0.96	0.50
Cystine (maximum)	0.54	0.35
Methionine plus Cystine	1.50	0.85
Phenylalanine	2.28	1.10
Threonine	1.50	0.84
Tryptophan	0.42	0.30
Tyrosine	1.05	0.73
Valine	2.14	1.17

[1] Calculated from the values used by Dunkelgod et. al. (1962), adjusted to a diet containing 3.52 kcal. ME/gm.

[2] Calculated values based upon the 28% protein control starter diet.

RESULTS OF HEN STUDIES

Experiment 1

The hens used to initiate the first study were two replicate groups of 15 Single Comb White Leghorns (SCWL) of the Cornell control strain which were randomly assigned to each treatment. The hens were in the fourth month of production at the start of the experiment. Two replicate groups of commercial hybrids (Dekalb 131) at 50% production were also randomly assigned to the same treatments. All birds had been placed in individual wire cages in groups of 15 when approximately 22 weeks of age. The SCWL had been fed the 10 percent protein high energy diet of Britzman and Carlson (1965) for a period of four months prior to the start of the experiment. They were then assigned the 9.4 percent protein test diets along with the hybrids which had not received prior treatment. Diets 5-8 contained only supplements of methionine, lysine and tryptophan as in diet 4 for the next three months. After the hybrids had been in production for three months, diets 5-8 were further supplemented cumulatively with isoleucine, arginine, valine and diammonium citrate, in that respective order. Each nitrogen source was added in addition to the supplements preceding it, such that the diet containing valine contained all of the added amino acids and the next diet contained diammonium citrate in addition to the amino acids.

The results given in summary in Table 6 indicate that the SCWL did not respond to methionine during either three-month period as measured by hen-day production and egg size. The hybrids showed a marked improvement with the methionine supplements. This may indicate that the older hen has a lower requirement or that the methionine requirement for the SCWL strain is lower than for the hybrids.

The response to lysine was apparent for the SCWL during both periods but for the hybrids the response was more marked in the first than the second period. Perhaps the lower production in the second period was due to the stress imposed by higher production during the first period.

The effect of tryptophan was variable but was believed to show a trend toward improvement. Isoleucine and arginine either depressed or did not affect production.

The response to valine was also variable but not greater than for the combination of methionine and lysine. Thus, it was not clear whether or not the response would have occurred in the absence of methionine and lysine supplementation.

Supplementation of diammonium citrate in addition to the amino acids was without effect. The absence of response is in contrast to the report of Chavez et al. (1966) and of Young et al. (1968) and Moran et al. (1968). The discrepancy could be surmised to relate to the higher (12.7-13.7%) protein diets used in the former studies but this was discounted by Akintunde et al. (1968) and Moran et al. (1967) who also used 12-14 percent protein diets.

TABLE 6. SUMMARY OF PRODUCTION DATA OF HENS FED LOW-PROTEIN DIETS WITH AMINO ACID SUPPLEMENTS, EXPERIMENT 1.

	Hen-Day Production				Feed/Doz. Eggs		Ave./Hen/Day				Egg Weight		Haugh units	
	Period 1[2]		Period 2[2]				Protein		Methionine					
Treatment[1]	SCWL	Hyb.	SCWL	Hyb.	SCWL	Hyb.	SCWL	Hyb.	SCWL	Hyb.	SCWL	Hyb.	SCWL	Hyb.
	%	%	%	%	kg	kg	gm	gm	mg	mg	gm	gm	..	..
(1) Basal	50.5a[4]	43.2a	48.3a	37.8a	2.9	2.8	9.5	8.3	153	133	57	55	78	83
(2) 1+0.15% M	49.1a	48.4ab	59.7b	62.9c	2.4	2.2	8.9	10.8	282	342	59	61	81	77
(3) 2+0.11% L	59.5b	55.4b	68.0c	58.1bc	2.4	2.1	10.3	10.0	321	312	57	60	79	80
(4) 3+0.02% T	63.7bc	48.5ab	64.3bc	62.7c	2.6	2.0	9.9	10.3	309	321	59	59	79	80
(5) 4+0.08% I[3]	62.8bc	53.1b	60.7b	50.2b	2.4	2.3	10.5	9.3	324	297	58	58	78	78
(6) 5+0.25% A[3]	62.5bc	56.4b	62.0b	62.4c	2.3	2.1	10.7	11.0	321	330	58	60	77	76
(7) 6+0.12% V[3]	61.1bc	52.7b	69.9c	65.5c	2.3	2.0	10.0	11.2	297	333	57	61	72	76
(8) 7+3.5% DAC	66.3c	52.6b	63.6bc	64.2c	2.4	2.1	14.1	15.2	315	339	58	58	78	78

[1] The amino acid supplements were: M, DL-Methionine; L, L-lysine; T, DL-tryptophan; I, DL-isoleucine; A, L-arginine; V, DL-valine; DAC, diammonium citrate.

[2] Period 1 included the first 3 and period 2 included the last 3 months of the trial. The SCWL had been fed Diet 1 for 4 months prior to initiation of the experiment. The hybrids had just attained 50% production. Two groups of 15 hens of each strain received each treatment.

[3] The diets were fed during period 2 only. Diet 4 was fed during period 1.

[4] Data followed by similar letters were not statistically different at the 1% level of significance.

It was observed that egg production was approximately 10 percentage points less for the hybrids fed the basal diet supplemented with methionine, lysine and tryptophan than was obtained from similar hens fed a typical 16% protein layer diet. It was also evident that the 9.4 percent protein diet was inadequate for all criteria except egg quality. When methionine was added at 0.15 percent of the diet, hen-day egg production, egg weight and feed utilization were improved. Haugh unit measurements were not affected.

Further supplementation with lysine, tryptophan and isoleucine resulted in an apparent increase in production although egg weights and feed utilization were unchanged. Isoleucine was believed to be without effect or perhaps detrimental during period 2. Indications of a detrimental effect could be explained on the basis that an imbalance occurred with the addition of isoleucine. Failure to obtain a consistent response to amino acid supplements and indications that the response was affected by the age and genetic strain of the hen suggested that it would be necessary to determine whether or not protein or amino acids was deficient in the basal diet.

Experiment 2

The basal diet was supplemented with methionine, lysine and tryptophan and compared to an equivalent amount of these amino acids from protein. The substitution was made on a weight for weight basis with glucose monohydrate so that the concentration of as many dietary components as possible remained the same. Also, when a dietary effect was obtained, it could be attributed to either protein, factors associated with protein, amino acids or the absence of glucose, *per se*. The assumption was made that any response would be due only to the amino acids supplied by the supplemental protein.

DL-methionine, L-lysine and DL-tryptophan were added to provide 125 percent of the standard of Johnson and Fisher (1958). They were added to supply 0.500, 0.625 and 0.150 percent, respectively, for any diet which contained less than the standard values. Methionine was calculated as the sum of methionine plus cystine for all diets and for the standard. Each was calculated separately to insure a proper level of methionine as well as total sulfur amino acids.

The protein sources tested included dehulled soybean meal, isolated soybean protein and #2 ground yellow corn. The soybean materials were fed at levels to supply three and six percent protein equivalents. As each test material was added at the expense of glucose monohydrate, the amount of material added could not exceed the level to supply three percent equivalents of protein only. The substitutions were made on an air dry basis.

The results of the second experiment as shown by hen-day egg production are given in summary in Table 7. It is evident that the additional protein substituted for glucose monohydrate did not promote a consistent improvement in egg production. Although hen-day egg production from replicate groups varied from 54 to 65 percent for the hybrid strain during the ten-month trial, the variation was not attributed to diets. Average production for the SCWL strain varied from 64 to 72 percent.

It was evident for most of the criteria that performance was not affected by diet but was affected by strain. The difference in performance between the strains was apparent when production was expressed in terms of replicate pens, dietary treatment or an average of all birds of a common strain. The effect of strain was large and consistent when expressed in terms of hen-day production. For the commercial hybrids, egg production was consistently low, although most replicate groups exceeded 70 percent for at least one month. Production was improved for the SCWL strain for all test diets. All groups exceeded 70 percent for at least one month and the group fed three percentage units of isolated soybean protein and the group fed the basal diet maintained production at 72 and 70 percent respectively.

The results as they relate to weight of eggs produced are also given. These data have been adjusted for egg weight differences and are expressed as grams of egg per bird-day. The adjusted data were less consistent than the percentage data. No difference was observed between diets for either strain. Production ranged from 30.9 to 40.5 gm/bird-day for the hybrids and 36.6 to 40.5 for the SCWL strain. The data indicate a trend toward lower production at lower levels of protein for the hybrids only. This effect was not evident for the SCWL strain.

The egg weight data are summarized in Table 7 and are given in Table 8 as an average by periods of four replicate groups for each diet and strain. The data are intended to show comparative values at specific times as well as trend characteristics for the two strains and six diets. An analysis of variance of the data and the subsequent use of Dunnett's test (Steel and Torrie, 1960) showed that for the June-July data, the hybrid hens fed the basal diet produced smaller eggs than any other group. The effect was significant at the 0.01 level. The effect was not observed for the SCWL strain; however, average egg size was six grams less for this strain than for the hybrids. The smaller eggs were evident for all replicate groups and for all treatments.

Average values for Haugh units are given in summary in Table 7. The average values for the hybrids ranged from 70.1 to 81.3 and 75.2 to 80.3 for the SCWL strain. Considerable variation was evident, but the variation was not attributed to strain or diet. Average body weights obtained at the end of the trial are also given in Table 7. Average body weights were greater for the SCWL but were not affected by diet.

Average hen-day feed consumption data were as shown in Table 7 for each strain and diet. A greater feed intake and consequent

TABLE 7. SUMMARY OF PRODUCTION, BODY WEIGHT, MORTALITY AND NUTRIENT INTAKE FOR STRAINS AND DIETS-EXP.2.

	Egg				Body			Average per hen per day						Per doz.	
	H.D. Production		Wt.	Haugh unit	Wt.	Gain	Death Loss	Feed	Prot	ME	Meth	M+C	Lysine	Feed	Prot
	%	gm.	gm.	..	kg.	gm.	%	gm.	gm.	kcal.	gm.	gm.	gm.	kg.	gm.
Hybrid Strain (4 groups of 13 hens each, per treatment)															
Basal[1]	58.2	33.8	58.2*	76	1.73	100	23	93	9.2	311	.36	.47	.58	1.9	182
Soybean meal[2]	59.8	36.9	61.8	73	1.82	248	19	91	11.3	298	.32	.46	.56	1.8	248
Soybean protein[2]	58.6	36.3	61.4	73	1.74	261	27	92	11.5	306	.34	.46	.58	1.9	241
Yellow Corn[2]	58.3	36.0	61.9	73	1.88	250	31	89	11.3	290	.31	.44	.56	1.8	235
Soybean meal[3]	59.1	39.0	62.5	75	1.89	235	31	92	14.2	297	.29	.47	.75	1.9	278
Soybean protein[3]	61.3	38.2	62.0	73	2.03	415	27	94	14.5	314	.32	.45	.76	1.8	281
Average	59.2	36.4	61.3	74	1.85	250	26	92	12.0	303	.32	.46	.63	1.9	244
SCWL Strain (2 groups of 15 hens each per treatment)															
Basal[1]	70.6	38.5	55.1	77	2.11	554	33	121	11.9	404	.47	.61	.76	2.1	197
Soybean meal[2]	65.2	36.4	55.2	77	2.14	569	33	108	13.5	354	.38	.55	.67	1.9	237
Soybean protein[2]	72.5	39.6	54.9	76	1.97	427	10	104	13.1	248	.38	.52	.66	1.6	198
Yellow Corn[2]	65.6	36.5	55.9	77	2.09	518	23	105	13.4	346	.38	.53	.66	1.8	229
Soybean meal[3]	67.2	36.4	54.7	76	1.97	457	30	109	15.9	349	.34	.55	.88	1.8	281
Soybean protein[3]	66.5	36.8	55.5	76	2.06	522	43	100	14.8	334	.35	.50	.84	1.8	277
Average	66.4	37.4	55.2	77	2.09	508	29	108	13.9	356	.38	.54	.74	1.8	237

* Significantly different from the other dietary groups at the 0.01 level of significance. The difference between strains was also highly significant.

[1] Basal contained 9.4% protein.

[2] Test protein included at 3% protein equivalents to supply 12.4% protein, C-1 Assay protein, dehulled soybean meal and No. 2 yellow corn, respectively.

[3] Test protein included at 6% protein equivalents to supply 15.4% protein.

[4] M + C = methionine plus cystine.

TABLE 8. THE EFFECT OF STRAIN, SOURCE OF PROTEIN AND LEVEL OF PROTEIN ON EGG WEIGHT FOR THREE PERIODS OF EXPERIMENT 2.

	Month of Trial							
	3-4-5		6-7-8		9-10		Ten Month Average	
Diet	SCWL	Hybrid	SCWL	Hybrid	SCWL	Hybrid	SCWL	Hybrid
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Basal[1]	50.3a	58.2a	56.2a	59.4a	58.8a	59.3a	55.1a	58.2a
Soybean Meal[2]	51.2a	62.4b	56.8a	63.6b	57.5a	63.3b	55.2a	61.8b
Soybean Protein[2]	51.3a	61.8b	56.8a	63.4b	57.2a	63.3b	54.9a	61.4b
Yellow Corn[2]	52.3a	61.6b	57.7a	63.2b	58.1a	63.8b	55.9a	61.9b
Soybean Meal[3]	50.9a	62.6b	56.3a	64.8b	56.6a	64.6b	54.7a	62.5b
Soybean Protein[3]	52.4a	62.1b	56.8a	63.9b	57.4a	64.0b	55.5a	62.0b

[1] Basal contained 9.4% protein.

[2] Test protein included at 3% protein equivalents to supply 12.4% protein.

[3] Test protein included at 6% protein equivalents to supply 15.4% protein.

[4] Data with the same subscripts did not differ at the 1% level of significance.

TABLE 9. A COMPARISON OF UTILIZATION EFFICIENCY FOR STRAINS AND DIETS USED IN EXPERIMENT 2.

	Basal[1]		Soybean Meal[2]		Soybean Protein[2]		Yellow Corn[2]		Soybean Meal[3]		Soybean Protein[3]		Average	
	SCWL	Hyb.	SCWL	Hyb.	SCWL	Hyb.	SCWL	Hyb.	SCWL	Hyb.	SCWL	Hyb.	SCWL	Hyb.
Amount Consumed per 100 gm. of Egg														
Feed (gm)	314	275	263	253	289	246	297	246	298	246	298	246	293	253
ME (kcal)[4]	1050	920	878	844	946	807	973	807	960	817	996	821	967	836
Protein (gm)	30	26	33	31	36	31	37	31	46	39	46	38	38	33
Calculated Percent Utilized[5]														
Met plus cys.	42	48	50	52	46	54	45	54	44	52	44	54	45	52
Lysine	35	50	41	43	38	45	37	45	38	33	28	34	35	40
Tryptophan	28	32	33	34	30	35	28	27	25	31	28	32	28	32
Isoleucine	45	50	39	41	38	45	36	44	29	34	27	33	36	41
Leucine	39	45	37	39	31	36	33	40	27	32	27	33	32	37
Arginine	39	44	32	34	33	39	30	36	24	28	20	24	30	34
Histidine	37	42	32	33	31	37	29	35	23	27	22	27	29	33
Threonine	70	80	58	60	53	62	53	62	39	46	39	47	52	60
Phenylalanine	45	51	41	42	45	45	53	36	44	29	34	29	35	38
Valine	53	60	47	49	44	51	42	50	33	39	33	40	42	48

1 9.4% protein.

2 9.4% protein from basal plus 3 percentage equivalents from the test protein.

3 9.4% protein from basal plus 6 percentage equivalents from the test protein.

4 kcal. of Metabolizable Energy.

5 Calculated value for the percentage of ingested protein used for synthesis of egg protein.

amino acid intake was evident for the SCWL but was not affected by diet. The hybrid strain consumed an average of 40 gm less feed per 100 gm of egg produced than the SCWL strain.

"Feed consumed per egg" ratios are given in Table 9 along with calculated values for percent of amino acid intake used for synthesis of egg protein. The percent of amino acids calculated for synthesis of egg protein indicate that either methionine plus cystine or threonine could have been first limiting in the basal diet. Valine and threonine were also considered border line. The remaining amino acids should have been present in the diet in adequate amounts for tissue synthesis as well as the production of egg protein. Production was not improved by any of the protein supplements and consequent increase in threonine and valine, therefore these amino acids were probably adequate in the basal diet.

Experiment 3

The results for Experiment 3 are given in separate tables and summarized in Table 13. Hen-day egg production for each treatment group of four replicates with fourteen hens per replicate is given in Table 10 for each period of the trial. Analysis of variance data are given at the bottom of each table for all pertinent data.

It was evident from the data that considerable variation existed between hens treated alike. The average rate of egg production varied approximately ten percentage units between replicate groups of birds for the 10-month data. According to an analysis of variance and subsequent Dunnett test (Steel and Torrie, 1960) a difference of 11 to 12 percentage units between the control and treatment groups for the May-June data would be required to show a difference at the 1% level of significance. Highly significant differences were observed between the control and diets containing 3 percentage equivalents of protein from corn, wheat or barley. Despite the numerical size difference required, differences were consistently shown for these diets for the first two periods and for the 10-month average. Variations appeared to be greater during the last five months, although this was not shown by the error mean square. No significant differences were observed for the egg production data during the last five months of the trial. Hens consuming the least cost diets performed as well as the hens fed the basal for all periods. A trend toward increased production by the hens fed the least-cost corn diet was observed, but this trend was small and not consistent.

Egg weight data are given in Table 11 for each replicate group. Except for the first month and the 10-month average where egg size may have been reduced for the hens fed the least-cost milo diet, no significant differences or important trends were observed.

Feed consumption data are given in Table 12. It is apparent that feed intake by the hens fed the diet containing oats was less than for hens fed the other diets. Except for the first period, feed intake by hens fed the diet containing oats was less ($P < 0.01$) than for the control basal diet. These birds consumed less feed than any other treatment group with the possible exception of the group fed the barley diet.

Eggshell thickness, Haugh unit values, protein and amino acid intake, mortality and body weights are given in summary in Table 13. The effects of diet on egg quality, Haugh unit and shell thickness, were inconsistent and not significant. No trends or other important indications of a dietary effect were observed for any of the criteria studied except as related to feed intake as was expected. Differences in protein content were reflected in protein intake data. Mortality and body weight at the end of the ten-month trial were not affected by the diet. Trends toward increased body weight were observed for the hens fed the diets containing either corn, wheat, milo or soybean meal. Average body weight at the end of the experiment had increased for all groups by at least 200 grams during the preceeding ten months.

Average amino acid intake for hens fed each diet is given in Table 14. The data were calculated from feed intake records and average composition tables of Merck (1961).

TABLE 10. AVERAGE HEN-DAY EGG PRODUCTION DURING FOUR PERIODS OF EXPERIMENT 3.

Treatment	September-November	December-February	March-June	May-June	Ten-Month Average
	%	%	%	%	%
Basal (9.4% protein)	63.7	57.6	54.4	49.0	58.1
Corn[1]	74.0**	67.6**	55.1	48.7	65.0**
Milo[1]	71.4*	64.3	55.7	54.4	62.9*
Spring Wheat[1]	75.4**	70.9**	59.8	52.9	67.8**
Barley[1]	74.4**	66.8**	58.0	55.2	65.5**
Oats[1]	69.7	63.1	48.4	46.4	59.2
Soybean Meal[1]	71.3*	67.3**	58.9	52.6	65.1**
Least-cost Corn[2]	61.0	65.2*	58.7	53.7	61.4
Least-cost Milo[2]	59.2	58.7	57.0	53.7	58.1

1 Added to basal diet by substitution for glucose monohydrate to supply 3% equivalents of protein.
2 Calculated least-cost diets with maximum corn or milo and 12% protein.
* Significantly different than basal ($P < 0.05$) ** Significantly different than basal ($P < 0.01$)

Analysis of variance

September-November

source	df	SS	MS	"F"
treatment	8	1180.99	147.62	6.455**
error	27	617.46	22.87	
Dunnett's Test		(0.05 = 6.74)	(0.01 = 8.00)	

December-February

source	df	SS	MS	"F"
treatment	8	599.48	74.935	3.116*
error	27	649.24	24.05	
Dunnett's Test		(0.05 = 6.9)	(0.01 = 8.2)	

March-June

source	df	SS	MS	"F"
treatment	8	382.30	47.79	1.944
error	27	663.50	24.57	
Dunnett's Test		(0.05 = 7.0)	(0.01 = 8.3)	

Ten-month average

source	df	SS	MS	"F"
treatment	8	402.35	50.29	3.516**
error	27	386.22	14.30	
Dunnett's Test		(0.05 = 5.3)	(0.01 = 6.3)	

TABLE 11. AVERAGE WEIGHT OF EGGS PRODUCED DURING FOUR PERIODS OF EXPERIMENT 3.

Treatment	September-November	December-February	March-June	May-June	Ten-Month Average
	gm	gm	gm	gm	gm
Basal (9.4% protein)	53.2	60.5	62.5	62.6	59.1
Corn[1]	53.8	60.7	63.0	63.2	59.6
Milo[1]	53.0	59.7	61.2	61.4	58.3
Spring Wheat[1]	53.6	59.6	61.5	61.3	58.6
Barley[1]	52.7	59.3	61.6	61.6	58.3
Oats[1]	53.9	61.0	62.8	62.5	59.6
Soybean Meal[1]	54.1	60.7	62.7	62.5	59.5
Least-cist Corn[2]	53.4	61.1	62.3	62.3	59.3
Least-cost Milo[2]	51.9	59.6	60.9	61.3	57.8

1 Added to basal diet by substitution for glucose monohydrate to supply 3% equivalents of protein.
2 Calculated least-cost diets with maximum corn or milo and 12% protein.
* Significantly different than basal ($P < 0.05$) ** Significantly different than basal ($P < 0.01$)

Analysis of variance

September-November

source	df	SS	MS	"F"
treatment	8	20.839	2.61	1.47
error	27	47.778	1.77	
Dunnett's test		(0.05 = 1.88)	(0.01 = 2.23)	

December-February

source	df	SS	MS	"F"
treatment	8	18.94	2.37	1.24
error	27	51.36	1.90	
Dunnett's test		(0.05 = 1.94)	(0.01 = 2.31)	

March-June

source	df	SS	MS	"F"
treatment	8	18.05	2.26	1.08
error	27	56.26	2.08	
Dunnett's test		(0.05 = 2.03)	(0.01 = 2.42)	

Ten-month Average

source	df	SS	MS	"F"
treatment	8	16.18	2.02	1.29
error	27	42.13	1.56	
Dunnett's test		(0.05 = 1.74)	(0.01 = 2.06)	

TABLE 12. AVERAGE HEN-DAY FEED CONSUMPTION DURING FOUR PERIODS OF EXPERIMENT 3.

Treatment	September-November	December-February	March-June	May-June	Ten-Month Average
	gm	gm	gm	gm	gm
Basal (9.4% protein)	97.4	112.0	110.6	98.7	106.7
Corn[1]	98.4	109.1	102.9	97.0	103.4
Milo[1]	97.2	110.8	105.1	96.2	104.4
Wheat[1]	97.2	107.7	100.3	94.7	101.7
Barley[1]	94.4	102.4*	101.7	99.4	99.5*
Oats[1]	91.6	95.8**	87.4**	79.5**	91.6**
Soybean Meal[1]	96.7	113.9	109.6	97.5	104.1
Least-cost Corn[2]	97.5	115.7	101.6	101.8	107.8
Least-cost Milo[2]	93.4	115.2	111.9	107.6	106.8

1 Added to basal diet by substitution for glucose monohydrate to supply 3% equivalents of protein.

2 Calculated least-cost diets with maximum corn or milo and 12% in protein.

* Significantly different than basal (P < 0.05) ** Significantly different than basal (P < 0.01)

Analysis of variance

September-November

source	df	SS	MS	"F"
treatment	8	168.8	21.10	0.688
error	27	828.1	30.67	
Dunnett's test		(0.05 = 7.8)	(0.01 = 9.3)	

December-February

source	df	SS	MS	"F"
treatment	8	1363.5	170.4	3.70*
error	27	1242.3	46.01	
Dunnett's test		(0.05 = 9.6)	(0.01 = 11.4)	

March-June

source	df	SS	MS	"F"
treatment	8	3615.2	451.9	3.281*
error	27	3718.4	137.7	
Dunnett's test		(0.05 = 19.7)	(0.01 = 16.5)	

Ten-Month Average

source	df	SS	MS	"F"
treatment	8	794.0	99.2	4.747*
error	27	564.0	20.0	
Dunnett's test		(0.05 = 6.4)	(0.01 = 7.6)	

TABLE 13. SUMMARY OF PRODUCTION, NUTRIENT INTAKE AND OTHER DATA, EXPERIMENT 3.

Treatment	Hen-Day Egg Production	Egg Wt.	Shell Thickness	Haugh Units	Amount/Hen-Day Feed	Prot.	Lys.	Meth.	M+C	Amount per 100 gm. egg Feed	Prot.	Body Wt.	Death Loss
	%	gm.	mm.		gm.	gm.	mg.	mg.	mg.	gm.	mg.	kg.	%
Basal[1]	58.1	59.1	3.3	79	107	10.0	667	373	534	311	29.2	1.73	10
Yellow Corn	65.0**	59.6	3.3	78	103	12.8	647	287	517	265	32.9	1.84	13
Milo	62.9*	58.3	3.2	77	104	13.0	652	310	522	283	35.1	1.89	11
Spring Wheat	67.8**	58.6	3.2	77	102	12.6	636	305	508	256	31.7	1.88	8
Barley	65.5**	58.3	3.2	78	100*	12.3	622	272	496	261	32.4	1.80	13
Oats	59.2	59.6	3.2	78	92**	11.3	573	270	458	260	32.2	1.79	9
Soybean Meal	65.1**	59.5	3.3	76	104	12.9	651	317	520	183	22.7	1.88	9
Least-Cost Corn[2]	61.4	59.5	3.4	79	108	12.9	674	323	539	295	36.6	1.73	8
Least-Cost Milo[2]	58.1	57.8**	3.3	79	107	12.8	668	288	535	318	39.4	1.68	13

* Significantly different than the basal ($P < 0.05$).

** Significantly different than the basal ($P < 0.01$).

[1] Basal diet was 9.4% protein with methionine, lysine, tryptophan and isoleucine added to supply standard levels. Feedstuffs were added to supply 3 percentage equivalents of protein to replace an equal amount of glucose monohydrate and amino acids were recalculated and made to standard levels.

[2] Least-Cost diets were formulated by computer by reducing the price of either corn or milo.

TABLE 14: AVERAGE DAILY INTAKE OF AMINO ACID DURING TEN MONTHS OF EXPERIMENT 3.

Treatment	Arg. mg.	Lys. mg.	Met. mg.	M + C[3] mg.	Trp. mg.	Ile. mg.	Leu. mg.	Val. mg.	Thr. mg.	Gly. mg.
Basal (9.4% prot.)	680	670	374	534	160	667	958	509	509	560
Corn[1]	834	646	288	517	155	646	1312	659	532	528
Milo[1]	790	652	310	522	157	653	1376	664	517	553
Spring Wheat[1]	789	636	305	509	153	636	1101	609	478	495
Barley[1]	741	622	272	586	149	622	1035	584	460	490
Oats[1]	767	573	270	458	137	573	1029	593	455	501
Soybean Meal[1]	902	651	318	521	156	651	1171	546	528	606
Least-Cost Corn[2]	675	674	323	539	162	674	1259	652	527	515
Least-Cost Milo[2]	528	668	280	535	160	668	1403	664	464	537

[1] Basal diet was 9.4% protein with methionine, lysine, tryptophan and isoleucine added to supply standard levels. Feedstuffs were added to supply 3 percentage equivalents of protein to replace an equal amount of glucose monohydrate and amino acids were recalculated and made to standard levels.

[2] Least-cost diets were formulated by computer by reducing the price of either corn or milo.

[3] M + C refers to methionine plus cystine.

Experiment 4

The results of the fourth experiment are given in a manner similar to that used for experiment three. Modifications in the basal diet were the addition of 0.05 percent methionine (+meth.), 0.3 percent nf-180, 0.1 percent 3-Nitro-4-hydroxyphenylarsenic acid (3-Nitro), 3 and 6 percentage equivalents of protein from a 60:40 ratio of corn and soybean meal (3% corn-soy) (6% corn-soy), 3 percentage equivalents of protein from either hydrolyzed feather meal (feather meal), fish meal, or meat and bone scraps. The basal diet was also fed at 0.575 percent lysine (-Lysine) instead of 0.625 percent. All diets except the +meth. and -Lysine were calculated to be equal in methionine, lysine and tryptophan content. DeKalb 131 pullets were distributed at random in groups of twenty four to supply two replicate groups per treatment. Three hens were placed in each 12-inch cage. A complete procedure is given in Appendix 3.

The results of the trial as related to egg production are given in Table 15. Consistent differences which were highly significant were observed for the -Lysine, 3 Nitro, fish meal and 6% corn-soy diets. Decreases were observed for the former two and increases were observed for the latter two diets. For the 10-month average the data were essentially the same except that a significant increase was also observed for 3% corn-soy. Differences during the last period, May-July, due to supplements of nf-180, +meth., feather meal and meat and bone scraps may have been due to a reduced production in groups fed the basal diet. Egg production was affected during the trial as early as the fourth month of production for the groups fed nf-180, -Lysine, 3 Nitro and feather meal diets. The trend for reduced production continued for the duration of the trial although the effect of the feather meal diet decreased with time as compared to groups fed the basal diet. The apparent detrimental effect of nf-180 disappeared after the first period.

Egg weight data are shown in Table 16. It was evident that egg weights increased as the hens became older; however, no effect of diet on egg weight was observed. Eggs produced by one group of hens fed the 3% corn-soybean diet were approximately one gram heavier than for any other group during any selected period; however, only one of the two replicate groups produced larger eggs.

Feed intake data are shown in Table 17. It was apparent that feed intake was similar for all groups except those fed the fish meal diet. Feed intake was greater ($P < 0.01$) for this group than for those fed the basal diet for all periods except the first one. Feed intake was reduced for the hens fed the 3-Nitro diet ($P < 0.01$) only during the February-April period.

The remainder of the data presented are given in summary in Table 18. Egg quality as measured by Haugh units and egg weight were not affected by any of the diets. A trend towards larger egg size was observed for the hens fed the 9.4 percent protein +3-nitro diet as well as the 12.4 and 15.4 percent protein fish meal and 6%

TABLE 15. AVERAGE HEN-DAY EGG PRODUCTION DURING THREE PERIODS OF EXPERIMENT 4.

Treatment	November-January	February-April	May-July	Ten-Month Average
	%	%	%	%
Basal (9.4% protein)[1]	69.2	57.9	43.2	56.8
+ .05% Methionine	66.3	60.7	51.8*	59.6
- .05% Lysine	52.8**	42.7**	29.6**	41.7**
nf-180	56.0**	57.4	53.2**	55.5
3 Nitro	59.5**	39.6**	28.0**	42.4**
Corn-Soy (3%)[2]	72.6	61.9	55.3**	63.3*
Corn-Soy (6%)[2]	70.0	66.6	58.8**	65.1**
Feather Meal (3%)[2]	59.6**	59.9	52.0*	57.2
Fish Meal (3%)[2]	66.7	70.5**	61.7**	66.3**
Meat and Bone Scraps (3%)[2]	70.4	62.3	50.4*	61.0

1 Basal contained added methionine, lysine and tryptophan to 125% of the standard (Johnson and Fisher 1958).

2 Protein supplements were made by adding the feedstuff to supply 3 or 6% equivalents of protein, removing equal amount of cerelose and adjusting levels of methionine, lysine and tryptophan to the standard level.

* Significantly different than basal ($P < 0.05$) ** Significantly different than basal ($P < 0.01$)

Analysis of Variance

November-January

source	df	SS	MS	"F"
treatment	9	781.61	86.846	16.648**
error	10	52.165	5.3165	
Dunnett's test		(0.05 = 3.48)	(0.01 = 4.61)	

February-April

source	df	SS	MS	"F"
treatment	9	1716.61	190.73	20.42**
error	10	204.24	9.34	
Dunnett's test		(0.05 = 6.9)	(0.01 = 9.1)	

May-July

source	df	SS	MS	"F"
treatment	9	2005.90	222.877	15.664**
error	10	142.29	14.229	
Dunnett's test		(0.05 = 5.8)	(0.01 = 7.6)	

Ten-month Average

source	df	SS	MS	"F"
treatment	9	1267.94	140.882	18.178**
error	10	90.89	9.089	
Dunnett's test		(0.05 = 4.6)	(0.01 = 6.1)	

TABLE 16. AVERAGE WEIGHT OF EGGS PRODUCED DURING THREE PERIODS OF EXPERIMENT 4.

Treatment	November-January	February-April	May-July	Ten-Month Average
	gm	gm	gm	gm
Basal (9.4% protein)[1]	50.2	57.9	59.7	55.9
+ .05% Methionine	49.9	59.3	59.6	55.6
- .05% Lysine	50.5	58.9	61.2*	56.9
nf-180	51.0	58.4	60.7	56.7
3 Nitro	51.2	57.4	61.7**	56.7
Corn-Soy (3%)[2]	50.4	58.4	62.0**	57.5**
Corn-Soy (6%)[2]	50.4	60.1**	62.5**	57.9**
Feather Meal (3%)[2]	51.2	59.3	62.0**	56.6
Fish Meal (3%)[2]	50.6	59.6*	61.8**	57.4**
Meat and Bone Scraps (3%)[2]	50.6	58.6	61.0*	56.7

1 Basal contained added methionine, lysine and tryptophan to 125% of the standard (Johnson and Fisher 1958).

2 Protein supplements were made by adding the feedstuff to supply 3 or 6% equivalents of protein, removing equal amount of cerelose and adjusting levels of methionine, lysine and tryptophan to the standard level.

* Significantly different than basal (P < 0.05) ** Significantly different than basal (P < 0.01)

Analysis of Variance

November-January

source	df	SS	MS	"F"
treatment	9	3.685	0.4093	0.9972
error	10	4.105	0.4105	
Dunnett's Test		(0.05 = 0.98)		(0.01 = 1.29)

February-April

source	df	SS	MS	"F"
treatment	9	15.720	1.747	2.208**
error	10	7.910	0.791	
Dunnett's Test		(0.05 = 1.36)		(0.01 = 1.79)

May-July

source	df	SS	MS	"F"
treatment	9	15.862	1.762	2.719**
error	10	6.48	0.648	
Dunnett's Test		(0.05 = 1.23)		(0.01 = 1.62)

Ten-month Average

source	df	SS	MS	"F"
treatment	9	8.462	0.940	1.951
error	10	4.820	0.482	
Dunnett's Test		(0.05 = 1.06)		(0.01 = 1.40)

TABLE 17. AVERAGE DAILY FEED INTAKE DURING THREE PERIODS OF EXPERIMENT 4.

Treatment	November-January	February-April	May-July	Ten-Month Average
	gm	gm	gm	gm
Basal (9.4% protein)[1]	87.6	100.8	98.4	95.6
+ .05% Methionine	85.9	102.1	101.3	96.4
- .05% Lysine	90.2	102.8	99.5	97.5
nf-180	90.3	92.8	104.3	98.6
3 Nitro	89.1	87.5**	99.3	91.9
Corn-Soy (3%)[2]	96.7**	101.0	101.6	99.8
Corn-Soy (6%)[2]	96.5**	102.6	105.1	101.4
Feather Meal (3%)[2]	87.5	98.4	103.4	96.4
Fish Meal (3%)[2]	96.2**	106.8	111.6	104.9**
Meat and Bone Scraps (3%)[2]	98.7**	99.3	101.3	99.8

1 Basal contained added methionine, lysine and tryptophan to 125% of the standard (Johnson and Fisher 1958)

2 Protein supplements were made by adding the feedstuff to supply 3 or 6% equivalents of protein, removing equal amount of cerelose and adjusting levels of methionine, lysine and tryptophan to the standard level.

* Significantly different than basal ($P < 0.05$) ** significantly different than basal ($P < 0.01$)

Analysis of Variance

November-January

source	df	SS	MS	"F"
treatment	9	392.19	43.58	5.15**
error	10	84.61	8.46	

Dunnett's Test (0.05 = 4.71) (0.01 = 6.22)

February-April

source	df	SS	MS	"F"
treatment	9	554.43	61.60	2.16**
error	10	285.25	28.53	

Dunnett's Test (0.05 = 8.2) (0.01 = 10.*)

May-July

source	df	SS	MS	"F"
treatment	9	357.85	39.71	1.29
error	10	309.20	30.92	

Dunnett's Test (0.05 = 8.5) (0.01 = 11.2)

Ten-month Average

source	df	SS	MS	"F"
treatment	9	236.99	26.33	1.48
error	10	178.40	17.84	

Dunnett's Test (0.05 = 6.45) (0.01 = 8.52)

TABLE 18. SUMMARY OF PRODUCTION, NUTRIENT INTAKE, AND OTHER DATA COLLECTED DURING EXPERIMENT 4.

Treatment	Hen-Day Egg Production	Egg Weight	Shell Thickness	Haugh units	Amount/Hen/Day Feed	Amount/Hen/Day Prot.	Amount/Hen/Day Lys.	Amount/Hen/Day Meth.	Meth.Cys.	Amount per 100 gm Egg Feed	Amount per 100 gm Egg Prot.	Body Weight	Death Loss
	%	gm	mm	..	gm	gm	mg	mg	mg	gm	gm	kg	%
Basal	56.8	55.9	3.4	80	96	8.9	598	335	478	302	28.4	1.92	22
+.05% Meth.[1]	59.6	55.6	3.3	80	96	9.0	603	386	530	289	27.2	1.01	20
-.05% Lys.[1]	41.7**	56.9	3.4	79	98	9.1	560	341	488	413	38.8	1.98	31
nf-180[2]	55.5	56.9	3.4	79	96	9.0	600	336	480	303	28.5	1.85	20
3-Nitro.[3]	42.4**	56.7	3.3	81	92	8.5	574	322	460	382	35.9	1.86	31
Corn-Soy.(3%)[4]	63.3*	57.5**	3.4	80	100	12.3	624	299	499	274	25.8	1.93	8
Corn-Soy.(6%)[4]	65.1**	57.9**	3.4	81	101	15.6	740	304	527	267	25.1	2.01	16
Feather Meal	57.2	56.6	3.3	80	96	11.9	603	296	482	296	27.8	1.91	16
Fish Meal	66.3**	57.4**	3.3	79	105	13.0	682	336	525	275	25.9	2.02	20
Meat & Bone Scraps	61.0	56.7	3.3	78	100	12.3	625	319	499	289	27.2	1.84	13

* Significantly different than the basal ($P < 0.05$).

** Significantly different than the basal ($P < 0.01$).

1 Plus or minus refers to change from the basal diet.

2 Furazolidone

3 3-Nitro-4-hydroxyphenylarsenic acid.

4 Contains 9.4% protein equivalents from the basal and 3.0 or 6% protein equivalents from a 60:40 mixture of corn and Soybean Meal.

TABLE 19: AVERAGE DAILY INTAKE OF AMINO ACIDS DURING THE SECOND PERIOD OF EXPERIMENT 4.

Supplement to Basal Diet[1]	Arg.	Lys.	Met.	M + C	Trp.	Leu.	Ile.	Phe.	Thr.	Val.	Gly.
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Basal	642	630	353	504	150	905	450	256	390	481	425
+.05% Meth.[2]	650	638	408	562	151	917	455	255	395	487	431
-.05% Lysine[3]	655	591	360	514	152	923	458	257	398	490	434
nf-180	591	575	322	461	140	833	414	232	359	443	392
3-Nitro	557	546	306	438	131	786	390	219	339	417	369
Corn-Soy 3%[4]	849	631	303	505	150	1192	606	525	515	636	566
Corn-Soy 6%[5]	1067	749	308	523	205	1508	770	800	656	800	708
Feather 3%[4]	767	615	295	492	148	1043	531	344	482	630	581
Fish Meal	861	694	342	534	160	1185	630	395	545	705	673
Meat & Bone Scraps 3%[4]	804	621	318	497	150	1053	526	338	467	596	784

[1] Except as indicated, all diets were made equal in methionine, lysine and tryptophan.
[2] Contains 0.550 percent of total sulfur amino acids instead of 0.050.
[3] Contains 0.575 percent of lysine instead of 0.625.
[4] Contains 9.4 percent protein equivalents from the basal and 3.0 from the test feedstuff.
[5] Contains 9.4 percent protein equivalents from the basal and 6.0 from the test feedstuff.

TABLE 20. AVERAGE DAILY INTAKE OF PROTEIN AND AMINO ACIDS DURING EXPERIMENT 4.

	Protein	Arg	Lys	Meth	Meth+ Cys	Tryp	His	Leu	Isol	Phen	Thre	Val	Gly
	gm	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg
Basal	8.94	606	595	333	476	114	211	854	424	238	368	454	401
+ 0.05% Meth	9.06	614	603	386	530	117	217	866	430	241	373	460	407
- 0.05% Lys	9.17	621	561	341	488	118	220	876	435	244	378	466	412
nf-180	9.02	611	600	335	480	116	216	862	428	240	372	458	405
3 Nitro	8.64	586	574	322	460	111	207	825	410	230	356	439	388
Corn-Soy (3%)	12.28	832	619	297	495	159	297	1168	594	515	505	624	555
Corn-Soy (6%)	15.64	1056	742	315	528	203	376	1493	762	793	650	792	701
Feather Meal (3%)	11.98	751	603	288	482	145	231	1022	521	338	473	617	569
Fish Meal (3%)	13.06	849	685	316	527	157	316	1169	622	390	538	696	664
Meat and Bone Scraps (3%)	12.37	808	623	309	499	140	269	1057	529	339	469	598	788

DUNNETT'S TEST

5% level	2.26	50.9	41.4	24.3	32.4	9.6	17.9	71.2	35.9	279	29.8	38.1	35.6
1% level	2.99	67.2	54.7	32.1	42.8	12.6	23.7	94.0	47.5	369	39.4	50.4	47.1

corn-soy diets. The differences were small and variable between replicates and periods. If a difference was present, these variations may have masked the true effect.

Protein and amino acid intake per day for period 2 and for the 10-month average are given in Tables 19 and 20. Intake per day was related to feed intake and amount of protein in the diet. Feed intake was not the same for all diets and different protein levels were used. Thus, the amount of protein consumed per day by any group would have been affected by these factors. Mortality and body weight were not affected by diet.

Experiment 5

The fifth trial of the series involving low protein diets for laying hens employed a completely randomized design with factorial arrangement of treatments. The treatments employed included supplements to the basal diet containing 125 percent of the standard for methionine, lysine and tryptophan, methionine (0.05%), lysine (0.05%), valine (0.1%), and inositol (0.1%). The hens were selected from the same group as Experiment 4 but were placed in pairs into eight-inch cages. Each of the 16 dietary treatments was given to six pairs of hens.

Average hen-day egg production is given in Table 21. Included in the table are the factorial effect totals for each of the sixteen diets. A numerical response in percent hen-day production was shown for methionine, ML, VI, LVI, and MVI. These data indicate that diets containing either methionine alone, methionine or lysine in combination with valine and inositol or valine with inositol would be equally satisfactory supplements to the 9.4% protein layer diet for the production of eggs.

When the data were considered as factorial effects, it was apparent that only valine and the combination of valine and inositol resulted in a numerical improvement in production. Combinations of other amino acids either produced negative effects or very small positive effects.

Egg weights were not affected by the various dietary treatments nor was egg quality or body weight. Mortality from these hens was less than from similar hens fed during Experiment 4. This may have been due to conditions imposed with two birds per eight-inch cage which may have contributed less stress to the birds.

Plasma lipid varied from 15 to 55 mg/ml for the hens fed the low-protein diets compared to 11 to 46 mg/ml for hens fed diets containing 16 percent protein. Liver lipid also varied extensively. Values of from 30 to 67% of dry matter were obtained for the hens fed low-protein diets compared to 28 to 68% for hens fed diets containing 16% protein.

TABLE 21. HEN-DAY EGG PRODUCTION BY HENS FED FACTORIALLY ARRANGED TREATMENTS DURING THE LAST THREE MONTHS OF EXPERIMENT 5.

Treatment	Hen-Day Production	Factorial Effect Total	Agreement[2]
	%		
Basal[1]	51.8	..	..
Methionine	56.7	1.7	yes
Lysine	37.8	-25.7	yes
ML	54.5	- 7.7	no
Valine	47.0	40.7	no
MV	49.4	-44.5	no
LV	51.6	- 4.5	no
MLV	41.2	-35.5	yes
Inositol	41.4	- 5.5	yes
MI	41.1	-25.5	yes
LI	40.9	13.9	no
MLI	42.5	- 5.9	yes
VI	57.5	63.9	yes
MVI	55.2	14.7	yes
LVI	58.4	-20.7	no
MLVI	47.3	13.7	yes

[1] Basal diet contained added methionine, lysine and tryptophan.

[2] Comparison of response calculated as percent production and as a factorial effect, given for agreement between the two measures of response to dietary treatment.

RESULTS OF STUDIES WITH TURKEY POULTS

Experiment T-1

The composition of the 20 percent protein starter diets is given in Table 3. The test diets were formulated by substituting an equivalent amount of protein from a test protein for the soybean meal portion. Glucose monohydrate and corn oil were varied to maintain nearly iso-caloric and iso-nitrogenous diets.

Amino acid supplements were made to provide calculated minimum amounts of 40, 60 and 80 percent of the values given for standard 1 in Table 5. Body weights and pooled blood samples were taken for each replicate group at four weeks of age.

Average weights are given in Table 22 for each replicate group and each treatment. It was apparent that growth rates were affected by treatment. Highly significant differences were present between sources of protein and between levels of amino acid supplements. An interaction of amino acid and protein source suggested that not all sources of protein were affected equally by the supplement. Improvement was obtained for most sources only at the 60 percent level. Further improvement was not obtained at the 80 percent level except for a trend towards improvement with two replicates of poults fed the diet containing fish meal. The greatest growth response to amino acids was obtained from the poults fed either soybean or safflower meals.

Best growth rates were evident for the poults fed fish meal at all levels of amino acid supplementation. Growth rates of poults fed soybean or safflower meal were also satisfactory. The remaining test proteins were unsatisfactory for poults under these conditions because the growth rates were 50% less than for diets containing fish, soybean or safflower meal as the test protein.

Pooled samples of blood were prepared for analysis by the method of Moore *et al.* (1958) and Spackman *et al.* (1958). It was evident from the results shown in Table 23, that random variation for most of the amino acids was so great that a meaningful interpretation could not be made.

It was apparent even from a casual observation that the three analytical values given in the table for similar protein treatments were extremely variable. Only histidine, cystine, methionine, phenylalanine and tyrosine showed consistent trends with increased levels of amino acid supplements. No consistent association could be made for serum free amino acids and growth. Free amino acid values for different groups of poults fed different protein sources were relatively characteristic of the protein.

Experiment T-2

As the previous trial showed evidence of a major source of variation besides that due to amino acid and protein supplements, this trial was made to test for a source of variation. Turkey poults were fed a low-protein corn-soy starter diet to four weeks of age.

TABLE 22. AVERAGE WEIGHT OF EACH REPLICATE GROUP OF SIX TURKEY POULTS FED DIETS WITH DIFFERENT SOURCES OF PROTEIN AND AMOUNTS OF AMINO ACIDS UNTIL FOUR WEEKS OF AGE.

		Percent of Standard[1]		
Treatment	Replicate	40	60	80
		gm	gm	gm
Soybean Meal	1	314	440	477
	2	313	489	455
	3	293	455	424
	Average	306	450	452
Fish Meal	1	473	487	458
	2	471	474	502
	3	489	475	512
	Average	478	478	491
Hydrolyzed Blood Meal	1	152	176	169
	2	157	163	193
	3	167	187	198
	Average	158	186	201
Meat and Bone Scraps	1	130	176	169
	2	145	177	176
	3	128	178	185
	Average	134	177	177
Corn Gluten Meal	1	118	163	170
	2	117	166	199
	3	124	200	171
	Average	119	176	180

[1] Essential amino acids added to supply a percentage of the standard used by Dunkelgod *et. al.* (1962).

TABLE 23. AVERAGE FREE AMINO ACID LEVELS IN SERUM FROM THREE REPLICATE GROUPS OF FIVE TURKEY POULTS FED DIETS WITH DIFFERENT SOURCES OF PROTEIN AND AMOUNTS OF AMINO ACIDS UNTIL FOUR WEEKS OF AGE.
(micromoles/100 ml.)

	Percent of Standard																
	Soybean Meal			Fish Meal			Safflower Meal			Blood Meal			Corn Gluten Meal		Meat and Bone Scraps	
	40	60	80	40	60	80	40	60	80	40	60	80	40	60	40	60
Taurine	103	66	64	88	64	89	60	47	68	30	51	102	44	56	41	83
Aspartate	34	18	36	43	30	25	27	..	36	21	13	22	26	16	12	13
Threonine	49	24	43	68	60	57	44	..	58	25	26	27	60	33	34	13
Serine	157	110	111	184	126	174	110	..	119	134	178	206	60	33	34	13
Proline	46	47	45	55	48	54	44	39	53	31	37	50	89	60	59	81
Glutamine	165	41	32	182	103	74	62	..	63	86	18	81	68	30	60	26
Glutamate	77	88	75	90	90	75	87	178	88	47	68	61	78	71	73	36
Glycine	122	105	133	128	120	54	127	120	146	139	193	187	126	161	238	267
Alanine	157	162	152	259	189	183	144	142	166	148	155	210	176	188	162	144
Valine	30	24	28	32	25	32	45	37	55	23	24	36	29	27	22	23
½ Cystine	18	48	38	28	24	32	34	29	44	11	24	26	33	41	19	27
Methionine	5	13	32	9	8	19	10	15	29	1	19	30	9	7	4	14
Isoleucine	12	12	12	15	11	11	12	11	15	6	8	11	12	9	7	7
Leucine	23	28	26	30	21	33	25	29	37	24	42	46	44	34	19	20
Tyrosine	35	62	64	50	60	75	44	43	53	50	37	68	..	67	40	42
Phenylalanine	17	39	63	18	33	86	33	35	65	23	28	43	73	57	22	71
Lysine	28	32	41	49	32	98	30	117	43	20	18	40	12	18	..	16
Histidine	10	11	12	8	11	19	17	14	22	15	12	13	13	11	..	11
Tryptophan	4	4	4	3	3	6	4	5	5	..	..	5	7	2	..	3
Arginine	48	48	41	29	20	25	75	69	81	10	21	92	24	23	..	39

TABLE 24. MICROMOLES OF FREE AMINO ACID PER 100 MILLILITERS OF SERUM FROM INDIVIDUAL TURKEY POULTS FED SOYBEAN MEAL AFTER FASTING FOR 34 HOURS COMPARED TO FASTING FOR 36 HOURS.

	FED SOYBEAN MEAL BODY WEIGHT					FASTED 36 HOURS BODY WEIGHT						
	460	480	510	560	580	420	500	550	610	610	640	715
Aspartic Acid	30	25	41	40	35	16	22	27	22	68	17	24
Proline	56	41	53	59	48	39	32	55	35	24	32	48
Glutamic Acid	45	36	45	177	69	52	41	68	39	135	67	47
Glycine	145	106	125	115	133	163	122	176	115	..	91	115
Alanine	169	121	140	151	117	103	94	148	99	..	80	117
Valine	35	15	32	27	30	17	19	24	11	13	17	23
Cystine	8	17	20	21	23	18	16	25	52	5	18	20
Methionine	5	5	6	8	6	5	6	8	8	2	4	7
Isoleucine	22	12	24	21	21	13	14	19	16	8	12	14
Leucine	27	16	30	28	29	19	20	29	24	14	17	23
Tyrosine	33	10	34	47	24	10	18	25	14	19	11	20
Phenylalanine	18	9	20	20	15	11	11	15	10	9	10	23
Lysine	55	32	42	45	47	46	38	49	49	11	28	38
Arginine	52	18	50	40	36	13	24	30	37	17	20	34

They were then fasted for 34 hours. After fasting they were either fed a single source of protein or fasted for an additional 2 hours before blood was taken by cardiac puncture. Samples were taken from individual poults and prepared as in trial 1 for amino acid analysis.

The results given in Table 24 indicate that the source of variation was neither associated with variation in body weight nor was it associated with time interval since the last feeding. Variations appeared to be in a random pattern. Only proline and perhaps methionine and leucine levels were consistent for each trial. Exceptions were evident even for these amino acids.

Experiment 3

The results are given for body weight at 1, 2, 3, 4 and 5 weeks of age for both series in Table 25. Table 26 shows the factorial effect means for the first series and Table 27 the factorial effect means for the second series for each of the five weeks of the trial. Table 27 also shows the data collected on feather development.

For the methionine series, average weight was greatest for the birds fed the combination of five amino acids for each of the five observations. The largest birds were found in the treatment groups which were fed methionine alone or in combination with another amino acid. The pens with the largest birds at any weighing were those containing the M, ML, MLT, MLTI, MLC, MTC and MLTC groups. It appeared from these data that at three weeks of age maximum growth was obtained from the MLT diet. At four weeks of age, the MLTIC group was 70 grams heavier than the nearest weight group, MLC. From these data it appeared that over the five-week period, isoleucine and cystine were not at critical levels until after the poults had reached three weeks of age. This interpretation is, in part, supported by the results of the single degree of freedom analysis of variance of the effect totals.

The data shown in Table 26 indicated that for the main effects, methionine, lysine and tryptophan, in that order, were the most important amino acid supplements. The two factor interactions ML, TI, and MC and the three factor interactions MLI, MLC, and MIC indicate that combinations of amino acids were also important. All of the main effects and interactions were positive except the MLC interaction which was consistent and negative for each period. This may indicate that the utilization of lysine is impaired at higher levels of methionine or when methionine is spared by cystine. The corresponding F value for MLC was significant at two weeks of age and highly significant thereafter. In light of this negative effect, the contradictory positive interaction of MC is not understood. In the absence of lysine supplements, growth was improved by the MC combination. This may indicate that the amount of methionine supplied in the diet was too low. The size of the three factor interactions suggests that the response from additional cystine is applicable only in the absence of lysine supplements.

The results of the second series for average weight are given in Table 25. These data indicate that valine and threonine were the important amino acid supplements in this series. The average weights of poults from the groups not receiving valine and threonine supplements were nearly 30 grams less than that of those receiving the sulfur amino acids. The results of the effect means given in Table 27 support this interpretation.

Of the single factor effect means, isoleucine, leucine and valine were consistently significant for each of the four seven-day periods. However, only valine showed a positive effect. Those effects are supported by the feather data. The effect of amino acid supplements to a 20 percent protein starter diet containing supplements of methionine, lysine and tryptophan suggest that valine was unable to overcome the detrimental effects of isoleucine or leucine. In fact, all two factor interactions with isoleucine or leucine were negative except the IA interaction at three weeks of age. It is remarkable that the only consistent positive three factor interactions involved valine and threonine together, however, this effect was not always significant at even the 5 percent level. The IVTh interaction was highly significant at three and at four weeks of age.

TABLE 25. AVERAGE WEIGHT OF TURKEY POULTS FED A LOW-PROTEIN DIET SUPPLEMENTED WITH AMINO ACIDS IN A FACTORIAL MANNER IN TWO SERIES OF TWO SIMILAR DESIGNS.

	Series 1					Series 2				
	Weeks of Age						Weeks of Age			
Treatment	1	2	3	4	5	Treatment	1	2	3	4	5
	gm	gm	gm	gm	gm		gm	gm	gm	gm	gm
Basal	79	139	211	304	418	MLT	89	178	275	451	646
Methion	93	137	297	446	663	Isoleuc	92	186	307	509	701
Lysine	85	142	231	340	438	Leucine	97	195	311	496	721
ML	77	174	312	468	644	ILe	96	176	300	520	714
Tryptop	89	145	225	318	425	Valine	99	204	337	551	759
MT	80	143	227	354	506	IV	88	189	302	469	655
LT	86	144	203	294	401	LeV	101	201	327	519	740
MLT	84	186	326	492	653	ILeV	91	177	284	467	609
Isoleuc	85	158	229	378	568	Arginine	87	199	318	532	754
MI	78	158	202	308	438	IA	86	189	310	512	676
LI	90	133	236	350	502	LeA	88	177	299	470	673
MLI	95	152	278	454	644	ILeA	86	186	299	472	656
TI	92	160	254	378	509	VA	91	202	335	511	711
MTI	89	150	259	404	576	IVA	99	204	339	543	741
LTI	82	142	200	317	450	LeVA	92	185	311	533	724
MLTI	89	182	331	509	722	ILeVA	86	175	281	469	664
Cystine	81	140	209	286	425	Threoni	90	198	321	532	744
MC	94	159	262	410	570	ITh	88	179	293	460	637
LC	89	149	235	362	506	LeTh	90	179	291	483	681
MLC	79	187	322	512	646	ILeTh	86	170	276	440	686
TC	83	128	188	258	397	VTh	99	213	344	559	791
MTC	91	168	282	452	660	IVTh	96	196	331	553	739
LTC	82	155	268	434	600	LeVTh	96	206	337	531	742
MLTC	99	184	300	466	605	ILeVTh	97	189	327	514	701
IC	80	131	182	272	369	ATh	97	199	338	543	737
MIC	91	169	254	462	637	IATh	86	194	307	507	728
LIC	84	135	202	295	452	LeATh	94	179	331	534	712
MLIC	91	160	284	450	624	ILeATh	96	169	303	461	676
TIC	82	140	208	318	479	VATh	99	187	319	503	710
MTIC	85	186	293	444	589	IVATh	90	222	362	574	741
LTIC	87	140	228	360	510	LeVATh	105	196	343	536	762
MLTIC	98	194	333	582	788	ILeVATh	92	184	304	514	703

[1] Names of the amino acids have been shortened to 7 letters.

[2] amino acid supplements were made to equal the amounts in standard 2 (Table 5) derived from calculated values for the 28% protein control diet and for series 2 MLT was added to all diets.

TABLE 26. FACTORIAL EFFECT MEANS OF BODY WEIGHT OF TURKEY POULTS FED LOW PROTEIN AMINO ACID SUPPLEMENTED DIETS.

	Effect Mean				
	1 wk	2 wk	3 wk	4 wk	5 wk
	gm	gm	gm	gm	gm
Methionine	3.46*	25.50**	62.06**	121.81**	157.25**
Lysine	1.43	4.75	35.44**	55.81**	59.75**
ML	-0.26	9.38*	15.81**	25.81**	26.12
Tryptophan	1.61	7.75	14.94	17.69	36.39
MT	0.41	4.38	2.56	6.44	8.88
LT	0.33	4.12	3.69	10.19	13.75
MLT	4.49	2.00	2.31	6.94	9.63
Isoleucine	1.81	0.62	-4.06	5.31	18.75
MI	0.18	1.00	-7.69	-3.69	-9.88
LI	2.79	-6.50	-1.56	-11.69	6.13
MLI	-0.44	4.50	22.44	25.19	18.23
MTI	0.01	1.63	9.56	16.94	25.50
LTI	-2.96	3.13	-3.06	1.69	-0.38
MLTI	-3.06	4.50	-1.44	8.44	15.00
Cystine	1.39	5.00	5.56	15.56	18.75
MC	3.99	10.63	6.69	27.31	15.38
LC	1.11	-3.37	8.81	14.06	15.88
MLC	-1.14	-9.00	-23.06	-35.19	-50.00
TC	0.59	0.38	11.31	15.44	19.49
MTC	1.74	1.75	-7.31	-12.06	-17.50
LTC	3.01	-1.75	6.56	12.44	5.13
MLTC	1.01	-3.13	-20.56	-14.06	-8.25
IC	1.61	-2.50	1.31	-4.94	-13.86
MIC	0.26	3.63	9.94	27.81	44.25
LIC	0.06	5.88	-0.19	-10.44	-6.75
MLIC	-1.14	-0.25	-5.06	0.31	-0.63
TIC	-0.36	3.36	1.31	-2.06	2.89
MTIC	-3.16	1.50	-10.81	-10.56	29.88
LTIC	2.61	-2.25	10.31	17.94	21.50
MLTIC	0.56	1.88	7.19	31.36	49.63

* Significantly different than zero ($P < 0.05$).

** Significantly different than zero ($P < 0.01$).

TABLE 27. FACTORIAL EFFECT MEANS FOR BODY WEIGHT AND FEATHER DEVELOPMENT OF TURKEY POULTS FED LOW PROTEIN DIETS SUPPLEMENTED WITH AMINO ACIDS.

	Body Weight Factorial Effect Mean					Feather Development 5 weeks of age	
	1 wk	2 wk	3 wk	4 wk	5 wk		
	gm	gm	gm	gm	gm	Score	"F"
Isoleucine	-2.98*	-7.23**	-13.3**	-18.7**	-34.2**	0.1	0.56
Leucine	1.8	-12.3**	-13.4**	-22.0**	-21.2**	-2.3	212.9**
ILe	-1.14	-4.6*	-8.6*	-11.6	-9.2	0.2	1.76
Valine	3.8**	10.6**	19.1**	26.1**	24.2*	3.0	386.7**
IV	0.8	-0.5	-2.0	1.2	-9.6	1.1	55.34*
LeV	-0.4	-1.0	-5.9	-0.5	0.9	1.6	106.6**
ILeV	-2.1	-4.5*	-6.3	-9.1	19.9	0	0
Arginine	-0.4	0.5	8.5*	10.2	4.2	0	0
IA	-1.1	6.8**	2.1	5.0	9.4	0	0
LeA	-1.5	-6.0**	-6.5	-7.6	-7.1	0.4	8.0**
ILeA	0.7	-1.6	-4.3	-13.8	-8.9	0	0
VA	0.7	-3.2	-7.7*	-1.3	-6.0	0.3	5.6
IVA	-0.4	3.7	7.8*	16.4*	20.0	0	0
LeVA	-1.2	-0.2	-3.4	4.0	15.3	0.1	0.8
ILeVA	-1.8	-4.9*	-9.3**	12.2	-7.0	0.1	1.2
Threonine	1.4	2.5	11.9**	13.8	19.6	-0.3	4.1
ITh	-0.3	0.1	-1.8	-6.1	0.9	-0.2	3.2
LeTh	1.1	-2.3	0.7	-4.9	0.8	-5.3	9.3**
ILeTh	1.1	-0.6	0.9	-2.2	10.0	1.1	53.6**
VTh	0.4	4.6*	6.7	14.1	12.0	0.8	26.3**
IVTh	1.6	4.1	12.5**	30.0**	13.0	1.3	77.9**
LeVTh	2.3	4.9*	7.6	4.6	19.4	0.4	6.7
ILeVTh	-0.9	-2.5	-5.6	-2.8	-0.8	0	0
ATh	4.4**	-0.6	2.5	2.2	1.9	-0.5	10.1**
IATh	-3.4*	1.8	-0.8	4.7	5.9	0.4	6.7**
LeATh	2.1	2.4	8.0	14.5	12.0	-0.4	8.0**
ILeATh	2.2	6.8*	-7.7*	-4.5	-21.0	0	0
VATh	-1.4	-0.2	-5.8	-19.0**	-14.2	-0.2	2.4
IVATh	-3.9	1.3	-2.4	-8.3	-19.1	0.7	21.8**
LeVATh	-0.5	0.2	-4.2	-0.9	-12.9	0.6	16.8**
ILeVATh	0.6	1.2	0.3	10.5	11.7	0	0

* Significantly different than zero ($P < 0.05$).
** Significantly different than zero ($P < 0.1$).

DISCUSSION OF EXPERIMENTS WITH HENS

Experiment 1 and Experiment 2

It has been shown that for Experiments 1 and 2, where protein levels varied from 9.4 to 15.4% (9.8 to 15.5 with amino acid supplements) most criteria for egg production were unaffected by protein level. Egg size for the hybrid hens fed the basal diets was smaller than for the hybrid hens fed any other diet. In Experiment 2, the SCWL were superior to the hybrids in percent hen-day production but the overall egg size was smaller and not related to diet. Thus, when the data were considered as grams of egg/ bird/ day the larger number of smaller eggs were corrected to an approximately equivalent value for both strains. Hen-day egg production for all groups of hybrid hens appeared to be suppressed whereas the effect was not evident for the SCWL. Perhaps feed intake as it is related to body size was inadequate for the smaller hybrid hens to support normal egg production. The larger body size and concomitant greater energy need may have resulted in a greater intake of the dietary factor limiting production. On the other hand, production of smaller eggs by the SCWL would, in effect, reduce the protein requirement for egg production. It appeared from the data that protein *per se* was not a limiting factor even at the 9.4 percent level because production was not improved at either 12 or 15.4 percent when other dietary factors were constant.

The data show a consistent difference between the two strains used in the study. It was noted that the average body weight of the SCWL was nearly 250 gm. greater than for the hybrids. The larger body size was probably reflected in a greater maintenance requirement for energy. As the energy requirement of the larger bird is proportionally greater than its requirements for other dietary factors when compared to the smaller bird, the dietary requirement for factors for egg production could be more easily met as a result of the greater feed intake.

Feed intake when expressed as a function of numbers of eggs produced show that the two strains were approximately equal. However, when feed intake was considered in terms of the weight of eggs produced, the hybrids show an advantage; that is, when egg production data were weighted to consider the size of egg, the two strains became more nearly equal in terms of production and less nearly equal in terms of efficiency of production. A trend towards increased production with increased level of protein was observed for the hybrids. This may reflect a minimum level of intake of an amino acid supplied by the protein or of a dietary factor associated with protein. However, the effect of higher levels of protein was not significant for either the hybrid or the SCWL strain.

It was observed in all groups, strains, and diets that although egg production was not severely affected, the appearance of the birds was markedly inferior to those fed normal diets. This effect was especially evident as regarding feathering condition. Either through feather pulling or through normal loss without replacement, in all experimental groups the birds appeared as though they were almost without feathers for the last six months of the trial. The deficient factor or factors may be required only for feather maintenance and probably was related more to feather picking than to the development or state of health of the feather per se. The color of the comb, wattles or ear lobes was also affected. Instead of a bright flesh color, they were somewhat pale and lacked normal brightness. The discrepancy in general appearance was not noticeably improved with higher levels of protein. The appearance effect may have been partly due to glucose monohydrate adhering to the tissues.

Experiment 3

It was apparent that egg production was improved as a result of supplements of three percentage equivalents of protein from corn, wheat, barley and soybean meal. The response was detectable by an analysis of variance and subsequent use of Dunnett's test for the data from the first six months of the trial. The influence of the diet was great enough to permit differences to be shown for the 10-month average. Differences were greater during the first three months than any subsequent period as indicated by the F test. During the first period rather than later, differences between diets were greater and more consistent than differences between replicate groups fed the same diet. Perhaps some of the diets could support peak production better than others during this period of stress. During later periods when production was reduced it may have become easier to consume adequate amounts of nutrients to maintain the lower rate of production. It would also appear that as nutrient intake becomes a limiting factor, egg production should fluctuate to replace reserve stores for the hen. Production should cease before the health of the hen is endangered. If marginal levels were being fed, then it would be conceivable that during any given month, with small numbers of hens, production between replicate groups would vary greater than during periods when greater amounts of reserve nutrients are available to supplement the dietary intake.

It was evident that production by the hens fed diets containing wheat, corn, and soybean meal were essentially equivalent. This was evident even though intake of glycine varied from 500 to 610 milligrams per day compared to 450 for the hens fed the basal diet. Threonine, valine, and leucine intake also varied from 480 to 530, 550 to 610, and 1100 to 1380 mg per day respectively. A comparison of amino acid intake with production indicated that approximately 740 milligrams of arginine, 610 milligrams of lysine, 290 milligrams of methionine, 200 milligrams of cystine, 550 milligrams of valine and 480 milligrams of threonine per day were adequate for good production. These values were obtained by finding the lowest level of intake at which production was adequate. They may not be applicable when compared to diets containing a different ratio of amino acids. The wide variation in daily intake which did not appreciably affect production might suggest that the ratio of amino acids was of little importance when the maximum level of an amino acid was less than twice the suggested standard for requirements. It was also of interest to note that approximately 100 grams of feed were consumed daily. The use of 100 grams as a standard for feed intake in a practical sense permits the values to be expressed directly as a percent of feed by converting to grams from milligrams. The values obtained are similar to the values given for requirements in Table 2. The similarity between the two sets of data indicates that the requirements are indeed approximately 125 percent of the requirement values suggested by Johnson and Fisher (1958).

Experiment 4

The data for egg production in Table 14 indicate that an effect of diet was evident as early as the third month of production. Perhaps a reduced rate of lay resulted from feeding the low protein diets to the hens when they were only twenty weeks of age and not sexually mature. A deficiency may have been precipitated earlier because the hens were unable to accumulate a reserve of amino acids due to the demands made for the amino acids during the final stages of growth and sexual development. The stress of peak production during the first three months of lay may have resulted in an exaggerated suppression of performance for the hens fed deficient diets compared to those fed more adequately.

Reduced performance was especially apparent for the hens fed 0.575 percent lysine. The standard level (0.625%) which was 125 percent of the standard of Johnson and Fisher (1958) was reduced to 115 percent for this study. The reduced rate of production evident in the third month of lay indicated that the 125 percent level may have been only marginal with regard to the lysine requirement of caged layers. Rate of production was also reduced for the hens fed diets supplemented with 3-Nitro, nf-180 and feather meal. The effects may have been, at least in part, due to lack of acceptance of the diets or for the group fed the 3-Nitro diet, may have been evidence of arsenic toxicity. Feed consumption is affected by rate of production; however, maximum production cannot be maintained when feed intake is reduced. In this case, cause and effect are closely related and it was not possible to determine whether feed intake was reduced because of a low rate of production or if production was reduced due to reduced feed intake. Feed intake was also low for the hens fed the plus methionine diet despite rather satisfactory production. Feed intake was below normal for all groups of hens fed the 9.4 percent protein diet, although even those fed the basal diet maintained satisfactory production through the seventh month of lay. Feed intake of the hens fed the basal diet was no different than for the basal diet plus methionine. Nutrient intake must have been nearly satisfactory for the continuous production of eggs through the tenth month of production at rates greater than 50 percent on a hen-day basis. Certainly if the 9.4 percent protein diet was deficient in amino acids other than methionine, lysine and tryptophan then a marked response over the plus methionine or nf-180 groups should have been obtained from the diets containing additional protein. The additional protein was equivalent to one-third of the amount in the basal. A 33 percent increase in protein from corn-soy, fish, or meat and bone scraps should have promoted more of a response, over the +methionine diet, if further severe deficiencies of other amino acids were present. Egg production was improved over the level of production of hens fed the basal diet during most periods by supplements of 3 percentage protein equivalents from fish meal or supplements of 6 percentage equivalents of protein from the mixture of corn and soybean meal. The trend towards increased production was especially evident in the final two months of the trial. The hens fed the diet containing fish meal continued to produce in excess of 60 percent even after 10 months of lay. The response supposedly reflected a more adequate level of an amino acid or acids; however, the amino acid pattern was not markedly different than for the remaining diets containing 12 percent protein. The response may have been a product of greater feed intake and concomitant increased intake of all amino acids, of a single amino acid, or of a factor associated with the fish meal. Identification of the causative agent was not made. When the data were calculated as milligrams of amino acid per egg, differences were more apparent. Daily intake between 500 and 525 mg of the sulfur amino acids appeared to be sufficient for good egg production. At least 610 mg of lysine per day was required. It was evident from the performance of the group fed the lysine diet that 560 mg of lysine per day was not sufficient to maintain egg production. Although egg production was severely depressed for both replicate groups fed the -lysine diet, it was unlikely that the effect was due to lysine deficiency alone. Certainly as little as 500 mg of lysine per day should support continued production at a rate not less than 50 percent, providing that other dietary nutrients are at satisfactory levels. An examination of the nutrient intake data failed to reveal a causative factor.

It was of further interest to compare amino acid intake for the various diets. Although the hens fed fish meal produced at a rate equal to or greater than the hens fed the corn-soy 6% diet the pattern of amino acid intake was grossly different. The intake of phenylalanine was 100 percent greater for those fed the corn-soy 6% diet than for those fed the diet with fish meal. Tryptophan intake was also greater for the hens fed the corn-soy 6% diet, although in this case only 50 percent more was consumed. The remaining amino acids were consumed in similar amounts.

Intake of amino acids was also compared for the hens fed the diet with the corn-soy 3% protein supplement and those fed the diet containing meat and bone scraps. Those fed the former diet as compared to the latter diet maintained a higher rate of production during the last months of the trial. If this response was due to a different intake of amino acids then it should be possible to show an association between the amount consumed and the rate of production. Intake of crude protein, arginine, lysine, methionine, sulfur amino acids, leucine, tryptophan and glycine were similar. Intake of tryptophan by the hens fed the diet containing fish meal was 10 mg a day more and their rate of production was excellent even during the last months of the trial. The only amino acid intakes which appeared to be consistent with production were threonine and valine. These amino acids were consumed by the hens fed the low protein diets at rates of approximately 370 mg and 460 mg, respectively per day. These rates are compared to 515 and 636 mg per day for the diet with 3% protein from corn-soy and 467 and 596 mg per day for the hens fed the diet containing meat and bone scraps. It is doubtful that an intake increase of 25 percent would result in similar production rates, if indeed these amino acids were the limiting factor for production. The correlation of valine intake to egg production was high (r=0.72) with a standard error of 0.11. A correlation would be expected because increased egg production alone will result in a greater intake of feed and concomitant increase in amino acid intake (r=0.72).

Perhaps this serves as an excellent example of a response to a dietary treatment, but due to an inability to identify the factors involved they remain unidentified. Plasma studies were made for free amino acids for electrophoretic separations of the white of egg and plasma but neither showed indications of a response. This would be expected on the basis of the work of Nasset et al. (1963), Mitchell (1962), Williams (1969), Elwyn (1968) and others who indicate that physiological adaptation by the individual and endogenous sources of amino nitrogen tend to modify the potential effect of diet.

Experiment 5

The data for egg production in Table 21 indicate that a response may have been obtained when the 9.4 percent diet with added methionine, lysine and tryptophan was further supplemented with methionine or with valine and inositol. Valine alone may have improved production as indicated by the factorial effect total or it may have suppressed production as indicated by a lower numerical value for hen-day egg production from the twelve hens fed the diet supplemented with valine alone. Unfortunately, no definite conclusions can be made from the data obtained from this experiment. The data do indicate that valine may be deficient and that when valine is added inositol may become limiting.

EXPERIMENTS WITH TURKEY POULTS

Experiment T-1

The results of Experiment T-1 indicated that the source of protein was important for starter diets for turkey poults. It was apparent from the results that even though the diets were calculated to be equal in amino acids at the 80 pecent level of the standard, growth rates were markedly different. Perhaps the factors involved were related to taste or flavor of the feed but this would be highly unlikely based upon the difference in growth rates. Certainly if flavor alone were affecting intake, adaptation would be expected to take place before growth was reduced to one-fourth of the normal expected. The effect mediated by the diet may have been of a physiological nature characteristic of the poult, because when the diets were fed to day-old cockerels, growth was as good on the corn gluten meal as for the fish meal diets. If the effect was physiological it was not reflected in the free amino acid levels of plasma. The variations between replicates were large and showed no trend towards consistently high or low values for any protein source. Variations in plasma amino acids did not appear to be correlated to growth. It was evident that free amino acid levels generally increased with increasing amounts of amino acid supplements, especially at the 80 percent levels of the standard. This indicated that the free amino acid patterns could be changed by supplementing the diet, but large variations were observed between replicate groups treated alike. As the variations did not appear to reflect dilution of the serum, and the source of variation was not evident, it was not possible to have confidence in the results. The variations may have been random and by chance alone may have affected single amino acid values.

Experiment T-2 and Experiment T-3

The results obtained during Experiment T-2 and Experiment T-3 indicate that the source of variation was neither associated with body weight nor the time interval elapsed since the last feeding. Variation appeared to be associated with the individual poult and did not appear to be a simple dilution of serum during preparation of the sample or relative hydration or dehydration of the poult prior to taking blood. The data give support to and are supported by the reports of Mitchell (1962), Williams (1969), Elwyn (1968), Ganapathy and Nassett (1962) and others that indicate that the variations between animals in levels of free amino acids in plasma are as great within groups as between groups. The results do not support and are not supported by the data of Hill and Olsen (1963) (1965), Longnecker and Hause (1967), and Richardson *et al.* (1953a).

Perhaps one reason for the random variation is that physiological activities are not static but rather they are dynamic. Several factors probably work to readjust physiological levels of nutrients and the nutrients themselves may induce changes in the levels of others. For instance, Richardson *et al.* (1953a) showed that several vitamins affected the level of amino acids in plasma. Most of the amino acids increased but methionine decreased as a result of the addition of Vitamin B_{12}. Charkey et al. (1950) also reported that free amino acids were increased when chicks were fed B_{12} deficient diets. Insulin has been shown to affect plasma levels (Lotspeich 1949) and insulin levels vary in response to blood glucose levels. Galactose has been reported (Alvarado 1968) to inhibit amino acid absorption. Perhaps even more important are the secretions of endogenous nitrogen and sloughing of tissues which are degraded to amino acids (Ganapathy et al., 1962; Mitchell, 1962). The degradation was suggested by Williams et al. (1968) to contribute to the variation found between animals and between collection periods for the same animal.

SUMMARY AND CONCLUSIONS

Studies made with caged laying hens and turkey poults fed low protein diets have indicated that growth rates and egg production were improved by supplementing the low protein diet with DL-Methionine, L-Lysine, and D-L-tryptophan. Further supplements of amino acids resulted in inconsistent responses that may have indicated that the low protein diets supplemented with methionine, lysine and tryptophan were further deficient in valine.

When the low protein layer diet consisted of a basal portion and a portion from several different protein sources, the rate of egg production was either unaffected relative to the low protein basal diet, or the rate of lay was improved. On the other hand, the rate of growth of turkey poults was markedly affected by the replacement of soybean meal by either hydrolyzed blood meal, meat and bone scraps or corn gluten meal. Possibly the effect was mediated by the relative acceptance of the diet by the poult more than by the relative amount of amino acids supplied. The laying hen may not be as fastidious or as easily disturbed by dietary change.

Plasma amino acid patterns of the poults fed the various protein sources were affected by amino acid supplements from 60 to 80 percent level of Dunkelgod's standard (1962). Variations of specific amino acids were so great that meaningful interpretations were not made. Increasing the supplement of amino acids from 40 percent to 60 percent of the standard would not be expected to cause any great change because most proteins were adequate to 60 percent for most of the amino acids. However, most of the essential amino acids were supplemented for all proteins at the 80 percent level; thus, all diets should have been equally adequate within the limits of accuracy for average analytical values.

It was concluded that a 20 percent protein corn-soybean meal turkey starter diet or corn-fish meal starter diet each supplemented with lysine, methionine and tryptophan to supply at 1.65, 0.50, and 0.30 percent respectively would support growth in turkey poults almost equal to a 28 percent protein diet. These standard levels appeared to be applicable to turkey starter diets only when feed intake was not a limiting factor.

The 9.4 percent protein layer-diet was also adequate in most amino acids. However, supplements of L-lysine, D-L Methionine and D-L tryptophan to supply 0.625, 0.500 and 0.150 percent respectively resulted in improved rates of production. Further supplements of isoleucine, arginine, and diammonium citrate did not improve production. Supplements of valine in the presence of additional methionine, lysine and tryptophan may have improved production although the response was small and inconsistent. The 9.4 percent protein supplemented diet was found to be deficient in a factor supplied by fish meal but also present in corn, wheat, barley, soybean meal, meat and bone scraps and milo. More work is necessary to identify the factor. Fish meal should be an excellent source of raw material.

Criteria used in this study to measure response to treatment were not satisfactory for quantitative work. Egg production, feed consumption, Haugh units and egg weight were not precise indicators of nutritional adequacy. Electrophoretic patterns of protein from the white of egg and from samples of blood were also poor indicators of the status of the nutritional adequacy of the diet. Growth rates, feed conversion and plasma free amino acid levels in turkey poults were also inadequate measures. Whereas factorial effects of growth rates appeared to provide adequate information on response to amino acid supplements in highly controlled studies with turkey poults, they were of little value for measuring response in laying hens in this limited study.

Trials should be continued with fish meal to find the minimum amount required to promote adequate production. Fractionation or solubility studies could then continue to provide further information on the substance. It is doubtful that the substance is an amino acid as reflected by calculated amino acid intakes. If indeed it is not an amino acid, identification could result in reducing protein levels by 50 percent for laying hens. The same substance may be required by the poult as reflected by growth rates on the unsupplemented 20 percent protein starter diet. Protein supplies for man's use can be spared by more liberal use of methionine, lysine and tryptophan in poultry diets.

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APPENDIX

APPENDIX A

Procedure - Experiment 2

For the second experiment a total of 312 commercial hybrid pullets (DeKalb 131) at about 50 percent production were placed at random into 24 groups of 13 individual cages to provide four replicates of six dietary treatments. Feed and water were provided *ad libitum* by one drinking cup for two hens in back-to-back cages and one feeder for 13 hens in adjacent cages. The ten-month trial began October 1 and ended July 31. The criteria measured included number, quality and weight of eggs produced, feed consumed, body weight and mortality. One month after the hybrids were distributed, 180 Single Comb White Leghorns (Cornell Control Strain) just coming into lay were also assigned to cages in groups of 15 to provide two replicates of six diets. They were fed the same as the hybrids except that one replicate group assigned to the basal diet was given a milo-based 16 percent protein layer diet.

A composite sample of two days egg production was used for egg quality and egg weight. Egg weights were obtained each week, and Haugh Unit values and shell thickness were obtained at 14-day intervals. Egg production and feed consumption were calculated at the end of each month. Body weights were obtained at intervals during the ten-month trial. Mortality was recorded as it occurred but the cause of death was not determined.

APPENDIX B

Procedure - Experiment 3

As single protein supplements used in the previous study failed to induce a response at levels of either three or six percent protein equivalents, further studies were made to determine whether or not the diet could be improved by the amino acids supplied by several other common feedstuffs. Corn, milo, wheat, barley, oats, and soybean meal were added to the basal diet to supply three percentage equivalents of protein. Methionine plus cystine, tryptophan, lysine, and isoleucine were maintained at constant levels of 125 percent of the 1958 Johnson and Fisher standard as described for trial 2. All substitutions were made by removing an equal amount of glucose monohydrate from the basal diet. Phosphorous, calcium, and metabolizable energy were also maintained at constant calculated levels.

Random diets were obtained by computer in which the level of corn was maximized for one diet and the level of milo was maximized for the other. To obtain the diets corn was priced at 50 cents a hundred pounds for the first diet and milo was priced at 50 cents for the second. The restrictions imposed on the least-cost program were the same as for diets discussed previously except that metabolizable energy was reduced from 3.34 to 2.87 kcal./gm.

The hens used in the study were DeKalb 131 commercial hybrids. They were grown on range until approximately 22 weeks of age and then distributed to the various individual eight inch cages in groups of fourteen to provide four replicate groups for each of nine test diets. The treatments were given after the hens had been in the cages for about one week to permit adaptation to their new environment without the added stress of dietary changes.

Data collected during the trial included egg production, egg weight, Haugh units, shell thickness, feed consumption, mortality and body weight. Egg production and feed consumption were calculated at the end of each month on a hen-day basis. Haugh unit values and egg weights were obtained at intervals of two weeks. Egg weights were obtained by weighing the entire production of two-days. Haugh units and shell thickness were determined with a sample of ten eggs from the two-day production. Body weights were obtained at irregular intervals. Mortality data were calculated at the end of the trial. All birds that died during the trial were sent to the Veterinary Science Diagnostic Laboratory for necropsy. Results of necropsy tests are not reported.

Blood samples were obtained by cardiac puncture from hens that had laid five consecutive eggs after one day of rest. The blood was allowed to clot and the serum was diluted to 1 mg. protein per milliliter by adding distilled water. Protein concentrations were verified by repeating the biuret test. Serum containing 1 mg. protein per milliliter was stored at 2^{o} C overnight and electrophoretic patterns were developed within 16 hours after collection. A sample of

0.2 ml portion of the diluted serum was placed at the top of the tubular polyacrlamide gel and developed according to the procedures of Ornstein (1964) and Davis (1964). The gels were later photographed over a subdued light for reference.

APPENDIX C

Procedure - Experiment 4 and 5

The commercial hybrid hens (DeKalb 131) were purchased from a hatchery as day-old pullets and reared in floor pens until 20 weeks of age. They were then distributed to twenty sets of eight, twelve-inch cages and ten sets of twelve eight-inch cages to provide one pullet per four linear inches of cage width. Two pullets were placed into one eight-inch cage and three were placed into the twelve-inch cages.

The pullets were fed the low protein basal diet for the first six weeks. The ten test diets were each fed to two replicate groups of the twenty-six-week old hens in the twelve-inch cages. The birds in the eight-inch cages were fed the low protein diet for the subsequent six months and then fed a factorial set of treatments. A different diet was fed to each group of six, eight-inch cages.

The diets which were fed to the birds in twelve-inch cages in this trial were all similar to the basal diet shown in Table 1. The (1) basal 9.4 percent protein diet was supplemented with an additional (2) 0.05 percent methionine, (3) 0.05 less lysine, (4) nf-180, (5) 3 Nitro-4-hydroxyphenly-arsenic acid, (6) 3% equivalents of protein from a 60:40 mixture of corn and soybean meal protein, (7) 6% equivalents of the same mixture, (8) 3% equivalents from hydrolyzed feather meal, (9) fish meal and (10) meat and bone scraps. Lysine, methionine, and tryptophan were calculated to be equal in each of the ten different test diets. All substitutions were made by replacing an equivalent amount of glucose monohydrate with the test material.

For the factorial study, sixteen different diets were given to the hens after they had been in production for seven months with the 9.4 percent protein basal diet as the only feed. The factors used in this study were: methionine, lysine, valine and inositol. The various treatments were applied to a pre-mixed basal containing added methionine, lysine and tryptophan.

Egg production and feed consumption were calculated for each replicate group on a hen-day basis at the end of each month. Egg weights were obtained by group weighing two day's production of eggs at fourteen-day intervals. Egg quality and shell thickness data were obtained on ten eggs from each replicate group at 28-day intervals. Body weights were taken at five irregular intervals. All birds that died during the experiment were sent to the animal disease diagnostic center for necropsy. Mortality was calculated only at the end of the 10-month trial. Statistical analysis was as given by Steel and Torrie (1960.

For the factorial study egg production was calculated for each of the twelve pairs of hens on a hen-day basis. The calculation of effect means was made by the automatic method of Yates (1935) and tests for significance were based on the "error" mean square obtained by an analysis of variance of the hen-day production data. Egg weights, body weights and feed consumption were obtained in the same manner as for the birds in the twelve-inch cages. In addition to the routine data, plasma and liver samples were obtained from a random selection of six hens after they had been fed the basal diet seven months and the factorial diets for three months. Liver samples were obtained by slicing the tip off the large right lobe and then removing a portion approximately one centimeter in width from the center for analysis for lipid. A sample of approximately four grams of wet tissue was used for extraction purposes. The sample was dried for sixteen hours at 100° C. and extracted with a continuous flow of recycled diethyl ether for sixteen hours.

Plasma lipids were determined by mixing two milliliters of fresh plasma with eight milliliters of a 3:1 ratio of diethyl ether and 95 percent ethanol. The sample was allowed to remain on ice for approximately four hours and then re-mixed and 5 ml of the supernatant liquid placed in a pre-weighed aluminum dish to dry over steam. After the sample appeared to be free of ether, the residue was dried at 100° C for one hour. The samples were then placed into a dessicator and allowed to cool before weighing.

Liver lipid was calculated on a dry and on a wet basis as percent by weight. Plasma lipid was calculated as milligrams of lipid per milliliter of plasma.

APPENDIX D

Procedure - Experiment T-3

A total of 425 day-old, straight-run turkey poults were maintained on a 20 percent protein diet with added lysine, methionine and tryptophan until five days of age. At five days of age the birds were weighed and placed in common weight groups with graduations in weight of 2.5 grams. Approximately 4 percent of the poults weighed less than 55 grams and 1 percent weighed more than 85 grams and were discarded. The remaining birds were allocated to each diet in such a manner that each group of a series contained an equal number of birds from each weight range. The extra birds were placed in a reserve group and fed the 20 percent diet shown in Table 4. Poults that died within the first four days of the trial were replaced with a bird of equivalent weight from the reserve group.

The trial was divided into two separate series. The first contained one group of five birds for each of 32 diets and consisted of a 2^5 factorial arrangement of methionine, lysine, cystine, tryptophan and isoleucine. The low level diet was the 20 percent protein basal diet. The second series contained one group of seven birds for each of 32 diets. This series was a 2^5 arrangement of isoleucine, valine, arginine and threonine. The low level diet for this series was supplemented with lysine, methionine and tryptophan as given in Table 1. The amino acids which made up either series were selected arbitrarily except that the methionine, lysine and cystine interactions were of interest in the first series and that isoleucine, leucine, valine, and arginine interactions were of interest in the second series. Previous tests had indicated that glycine was not as important in the diet as threonine. Thus, threonine was used as the fifth amino acid in the second series.

The composition of the basal diet is given in Table 4. The amino acid standard (2) is given in Table 5. The basal diet was first mixed without glucose monohydrate. This ingredient was added with each amino acid and combination of amino acids to supply 3.20 percent of the final diet. At three days of age poults were given 0.5 ml. of a 1:10 water dilution of an nf-180 suspension. This was done in an effort to control unusually high mortality. Subsequent microbiological tests were reported negative for salmonella and paracolon.

The birds were weighed at seven day intervals beginning at seven days of age. The weight data were expressed as average weight in grams per bird. The factorial effect means were calculated in terms of grams per bird for each main effect and interaction. The main or single factor effects express the average change due to that single factor. The multi-factor effects express the difference between the main effect and the effect of the combinations and provide information about the additivity of the response.

The analysis of variance was made by first calculating the total variation, then subtracting the variation due to treatments and initial weight blocks (B) from the total. The remainder, the T x B interaction was used as the error term for blocks B and for treatments (Snedecor and Cox 1967). Individual degrees of freedom were broken out with their respective mean square by the automatic method of Yates. Confounding was done with the 5 factor interaction but it was ignored in the analysis of variance because the effect if present appeared to be small. Confounding was done on two levels of the four batteries. If an effect of level was present it was confounded with the 5 factor interaction. Feed records were kept but were not calculated.

Information on feather formation was obtained at five weeks of age. Each bird was classified according to relative feather development. The best development of feathering was classified as 10 and worst was classified as 1. The analysis of variance and calculation of effect means were the same as for body weight except that a completely random design was used instead of the randomized complete block.

ANALYSIS OF VARIANCE
FACTORIAL DESIGN FOR TURKEY POULTS

Source	df	SS	MS	"F"
2 weeks of age: Series 1				
Total	159	137.484		
Treatment	31	55.304	1.784	2.77**
Error	128	82.180	0.642	
Series 2				
Total	223	213,745.9		
Treatment	31	35,545.9	1,146.6	3.23**
Block	6	112,191.3	18,698.5	52.69**
Error	186	66,008.7	354.8	
3 weeks of age: Series 1				
Total	159	619,199.4		
Treatment	31	311,889.4	10,060.9	7.11**
Block	4	131,955.6	32,988.9	23.33**
Error	124	175,354.4	1,414.1	
Series 2				
Total	223	596,525.8		
Treatment	31	103,768.6	3,347.4	3.476**
Block	6	286,649.7	47,774.9	49.614**
Error	186	179,107.4	962.9	
4 weeks of age: Series 1				
Total	159	1,900,873.6		
Treatment	31	1,055,353.6	34,043.7	9.78**
Block	4	414,0166	10,350.1	29.74**
Error	124	431,503.4	3,479.9	
Series 2				
Total	223	1,610,230.2		
Treatment	31	265,565.9	8,566.6	2.40**
Block	6	681,062.3	113,503.9	31.81**
Error	186	663,602.0	3,567.8	
5 weeks of age: Series 1				
Total	159	3,383,844.4		
Treatment	31	1,776,194.0	57,296.6	8.13**
Block	4	734,017.8	183,504.4	26.05**
Error	124	873,632.6	7,045.4	
Series 2				
Total	223	2,805,393.6		
Treatment	31	358,622.2	11,568.4	1.46*
Block	6	971,874.1	161,979.0	20.43**
Error	186	1,474,897.3	7,929.6	