EFFECT OF DIETARY PROTEIN ON THE CHEMICAL AND FUNCTIONAL PROPERTIES OF EGG WHITE

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THIS BOOK CONTAINS NUMEROUS PAGES WITH MULTIPLE PENCIL AND/OR PEN MARKS THROUGHOUT THE TEXT.

THIS IS THE BEST IMAGE AVAILABLE.

INTRODUCTION

Efficient use of protein is an important economic factor in the production of food for human consumption. To reduce high production costs, many poultry producers are lowering the bird's dietary protein level to a minimum. This minimal level is not clearly defined, but is a level which has many variables continually changing with time.

Areas of poultry nutrition and economics of egg production have received much attention by research workers. Comparisons of egg quality factors with production costs has enabled the development of many new rations. Those rations are designed for today's hen and the present management techniques allow the hen to produce the largest volume of eggs at least cost. Dietary protein supplementation with crystalline amino acids allows the ration to meet the requirements for essential amino acids, but not to exceed the total nitrogen or protein requirement. Through the addition of high energy sources such as fats, a minimal amount of feed consumed per day can be rigidly controlled. With the aid of linear programming and large flocks of birds, practical feeding has become scientific.

With the cost of protein for human consumption continually rising, quantitative studies of egg protein along with the economics of production merits study. The purpose of this investigation was to determine the effects of different levels of dietary protein upon egg white protein.

REVIEW OF LITERATURE

Egg White Composition

Egg white proteins are synthesized in the oviduct, in contrast to most egg-yolk proteins which are formed in the liver and transported in the blood. Synthesis of egg white proteins conforms to the general theory of protein synthesis; polypeptide chains are assembled in a linear sequence, starting at the N- terminal end. However, the sub-cellular particles 'microsome fraction' associated with albumen synthesis are sedimented by unusually low centrifugal forces (Baker, 1969).

Parkinson (1966) reported that liquid whole egg consists, on average, of 64% white and 36% yolk. The white contains approximately 12% solid matter, which is predominately protein with small amounts of minerals and sugars and only a trace of fat.

Egg white is essentially an aqueous solution of proteins. Four distinct layers can be recognized: (1) a fluid 'outer thin' layer, (2) a firm 'thick' layer, (3) another fluid layer -- 'inner thin', and (4) a shallow dense layer encircling the vitelline membrane of the yolk and continuous with the fibrous 'chalazae' that hold the yolk in position. Those layers differ in chemical and physical properties; in particular, the concentration of ovomucin is much greater in the 'thick' layer than in the 'thin' ones. The proportions of the different layers vary widely, but it has been established that the percentage of thick white is characteristic of the individual hen.

Theory holds that the jelly-like condition of egg white is made possible by ovomucin, an insoluble, fibrous mucoprotein. Ovomucin is

present in a much higher concentration in the 'thick' layer than in either of the 'thin' layers.

The present knowledge of egg white proteins is not vast. Most research has been centered on the biological properties of certain albumen proteins. The following list gives some of the chemical and physical properties of the known egg white proteins.

Protein	Relative amount in egg white (%)	Isoelectric point	Molecular weight
Lysozyme	3.5	10.7	14,600
G_2 globulin	4.0?	5.5	30,000 to 45,000
G_3 globulin	4.0?	5.8	?
Ovomacroglobulin	0.5	4.5 to 4.7	760,000 to 900,000
Ovomucin	1.5	?	?
Conalbumin	13.0	6.6	80,000
Ovoinhibitor	0.1	5.2	44,000 to 49,000
Ovomucoid	11.0	3.9 to 4.3	28,000
Avidin	0.05	9.5	53,000
Flavoprotein	0.8	4.1	35,000
Ovalbumin	54.0	4.6	45,000
Ovoglycoprotein	0.5	3.9	24,400

Electrophoresis

Longsworth et al. (1940), in their classical electrophoretic studies using a standard Tiselius apparatus and covering the pH range 3.9-7.8, identified eight protein fractions. Included were two ovalbumins (A_1 and A_2), three globulins (G_1, G_2, G_3), as well as conalbumin, ovomucoid and ovomucin; also, there was evidence that conal-

bumin could also exist in two forms (C_1 and C_2). They found that crystalline ovalbumin is not homogeneous, but can be resolved partially into two components (A_1 and A_2) which migrate in an electrical field at slightly different rates. Similar migration has been confirmed by later workers by using starch-gel electrophoresis (Baker and Manwell, 1962; Lush, 1964) and evidence has been obtained for the presence of a third component (A_3).

Lush (1961) consistently found two ovalbumin peaks with three closely adjacent components which varied genetically. Feeney et al. (1963) identified three components (3, 4, and 6) as ovalbumin by comparative experiments with crystallized and chromatographed ovalbumin. Their preparations of ovalbumin showed three components like those of 3, 4, and 6 in the approximate proportions of 75, 20, and 5%. Perlmann (1952) found 85%, 14%, and 'traces' for ovalbumins A_1, A_2, and A_3, respectively, by free boundary electrophoresis. Cann (1949) found the ovalbumin fractions differed in phosphorus content. A_1 had two phosphates per mole, A_2 has one, and A_3 had none.

Feeney et al. (1963) crystallized and carefully rechromatographed preparations of conalbumin prepared in their laboratory. Identical patterns to constituents 16 and 17 on their starch-gel electrophorogram were obtained. Fe^{59} radioautographs showed that those were conalbumins.

Chang et al. (1970) separated and identified two conalbumins by polyacrylamide disc gel electrophoresis. Clark et al. (1963) observed that the two chicken conalbumin components observed upon starch-gel electrophoresis could not be distinguished by ion exchange chromatography, moving boundary electrophoresis, electrodialysis, thermal dena-

turation, or chemical modification. Baker and Manwell (1962) separated two conalbumin fractions and observed three preconalbumin fractions migrating just beyond the second conalbumin fraction (C_2).

Rhodes et al. (1958) designated five of their chromatographic fractions from the carboxymethylcellulose column as globulins, but in four of these cases the peaks were very small. Feeney et al. (1963) achieved a partial purification of two globulins. Chang et al. (1970) identified globulins (A_1 and A_2) and tentatively identified four other constituents as globulins. Baker and Manwell (1962) found G_2 and G_3 in whole white, the filtrate from the globin fraction and, to a lesser extent, in the washings from the precipitate (mainly mucin) from the globin fraction. In addition, the two latter solutions contained the proteins (preconalbumins) which migrated between the conalbumin and the bands labeled G_2 during the electrophoresis of whole white. This suggests that the preconalbumins might be globins.

Rhodes et al. (1959) isolated and identified a flavoprotein and its corresponding apoprotein directly from chicken egg white. The flavin moiety was riboflavin and all the riboflavin of egg white was apparently bound as the flavoprotein. Electrophoretically, Baker and Manwell (1962), using starch gel found the flavoprotein-apoprotein complex to migrate just ahead of the ovalbumin component A_1. No electrophoretic heterogenity of apoprotein has been reported. Rhodes et al. (1959) did, however, elute two apoprotein components from cellulose colums; the fractions differed by one phosphate group.

Production

Suboptimal protein dietary levels or intake levels have been shown

by Deaton and Quisenberry (1964), Novacek and Carlson (1969), Harms and Damron (1969), Dewan and Gleaves (1969), Carlson and Guenthner (1969), Harms and Waldroup (1963) to decrease egg production. Body weight was shown to decrease with lower dietary protein levels by Quisenberry et al. (1964), Smith (1967), and Harms and Damron (1969). Egg size and feed conversion was shown to decrease by Quisenberry et al. (1964), Deaton and Quisenberry (1964), Novacek and Carlson (1969), Carlson and Guenthner (1969), and Nivas and Sunde (1969).

Evidence has been found by Smith (1967), and Novacek and Carlson (1969) that hens will exceed their energy requirements (overconsume) to meet protein or amino acid requirements, thus attempting to maintain an equillibrium in their nitrogen balance.

The relative efficiency of conversion of retained nitrogen to egg nitrogen suggest that a large portion (approximately 1/3) of retained nitrogen is utilized for purposes other than egg synthesis (egg protein storage). The nitrogen retention level was found to decrease with time and/or egg production.

The protein requirement for peak nitrogen retention for hens with full protein stores is less than 16-17 gm per day and probably approaches 13 gm. This decreased requirement suggests that once the needs for growth and protein stores are filled the function of the dietary protein is primarily for egg biosynthesis. This work is in agreement with the early findings of Halnan (1925) and of Willcox (1934) who concluded that the nitrogen required for egg production is drawn largely, if not entirely, from the food supplied during the laying period. The results suggest, therefore, that the maximum protein requirement of the hen is governed by her optimum capacity for retaining nitrogen. Csonka et al.

(1947) found when the dietary protein is incomplete (20 percent gelatin in the diet), or when the protein in the diet is low, egg production stops. Apparently, the hen relies on the constituents of the feed for the synthesis of egg protein and connot furnish methionine and cystine from her own reserve protein stores.

Smith (1967) supplemented laying diets of 11, 15, and 19% protein with methionine and lysine singly and in combination, but failed to improve any of the performance criteria, indicating that neither of these amino acids was limiting. It is concluded that the required amino acid pattern of the laying hen is not altered as a result of changing the dietary protein level. The influence of protein level on responses to amino acid supplementation obtained by other investigators must therefore be attributed to the change in amino acid pattern which occurs when the grain and protein supplement portions of the diets are manipulated.

Harms et al. (1967) indicated that the laying hen requires approximately .31% methionine and .53% sulfur amino acids in a diet containing 2024 kilocalories per kilogram in order to maintain a maximum rate of egg production. Increasing methionine levels beyond 0.31% resulted in increasing egg size and body weight gain. It would appear from this data that the methionine requirement of the laying hen is higher in the earlier and latter portion of the production year than during the middle portion.

Deaton and Quisenberry (1964) observed a highly significant strain X protein interaction for average body weight, Haugh unit score and shell thickness in individual cages. When only protein levels were considered, birds housed in individual cages receiving the increasing

protein diet laid significantly more eggs with a significantly heavier egg weight and better feed efficiency. Birds housed in colony cages laid more eggs with better feed efficiency and significantly heavier egg weight when receiving a constant 17% protein diet.

Quisenberry et al. (1964) fed an isocaloric diet varying only in protein content to five duplicate groups of birds. By lowering protein as the laying period advanced, the egg size and body weight were reduced significantly, but egg production and feed efficiency tended to improve. Egg production dropped when the protein level was first lowered to 14%, but recovered. Production was highest at 19% and body weight and egg size at 17% protein. Haugh units were highest at 15% protein. There was no consistent relation of protein level, age, or season to blood spots. Further study is needed to determine the best schedule of protein changes for optimum performance.

It has not been possible to show significant responses for methionine or lysine additions to the layer diets unless dietary protein level was below 13.4% (Stangeland and Carlson, 1961; Britzman and Carlson 1965). Carlson and Guenthner (1969) demonstrated that when methionine did not show a response, either in egg number, egg weight or feed conversion, that protein intake was generally in excess of 16 gm per hen per day. Combs (1960) has reported a method for calculating the methionine requirements of laying hens and it is interesting to note from his data that a 2 kg hen producing 40 gm of egg per day required 302 mg of methionine per day. They found that hens fed 14% (corn-soybean meal) diets plus methionine demonstrated that maximum performance could be obtained with about 15 gm of protein intake per

day when supplemental methionine was provided. No consistent effects of diet on shell thickness or Haugh units were observed. Diets of 16% protein without methionine supplements were quite adequate. About 17 gm of protein per hen was required without methionine supplementation, whereas 15 gm of methionine supplemented protein was adequate for maximum egg numbers, egg size, and feed efficiency.

Ingram et al. (1951) gives the requirement for methionine to be not over 0.38% of the ration and that the combined methionine and cystine requirement is not more than 0.63%.

Deaton and Quisenberry (1964) in studying variable protein levels found that birds housed in individual cages while receiving an increasing protein diet laid significantly more eggs with a significantly heavier egg weight and had a better feed efficiency. Birds housed in colony cages laid more eggs with a better feed efficiency and significantly heavier egg weight when receiving a constant 17% protein diet.

Waldroup and Harms (1962) have demonstrated that low dietary protein levels during the growing period delayed sexual maturity of egg production type pullets, but had no effect on subsequent rate of lay when the pullets were placed on a normal layer diet. They also found that feeding low protein levels during the early part of the laying period did not adversely affect production when the pullets were placed on a good quality feed.

Csonka (1950) found the age of the pullet as a factor controlling the change of nitrogen quantities in eggs. Increased nitrogen, a result of feeding a high-protein diet, occurred mainly in the yolk (25%)

for young pullets. At the same time, egg white nitrogen increased less than 10%. Older pullets showed just the reverse of the conditions stated above.

Novacek and Carlson (1960) found dietary supplementation of methionine and lysine of two strains of layers being fed 9.4% protein corn-soybean meal basal diet improved egg production, feed conversion, and egg weights. No significant response was obtained by further cumulative additions of tryptophan, isoleucine, arginine, valine, or diammonium citrate. In another experiment, their results showed a low protein diet, supplemented with methionine, lysine, and tryptophan, to contain 9.85% protein equivalents which were equal to the basal diet supplemented with 3 or 6% protein equivalents from corn, soybean meal or soybean protein. They found the protein requirement for a 2 kg hen at 60% egg production was not more than 11.3 gm per day. For the total sulfur amino acids, 460 mg per day or 1.23 gm/100gm of egg was adequate for this rate of egg production with 320 mg per day supplied as methionine. An average requirement for feed per dozen eggs was 1.88 kg for the larger layers and 1.83 for the smaller hybrids. Computing the data in terms of feed per 100 gm of egg; the larger layers were less efficient in utilization of total feed or protein by approximately 30%.

Harms and Damron (1969) found that the amino acid requirement for maximum egg weights did not change greatly throughout the production year. Their data also indicated that the sulfur amino acid requirement for maximum egg weight was slightly higher than for maximum rate of egg production. Feed intake was slightly reduced with lower (70 and

80%) levels of amino acid, and this was considered to be due to the lowered rate of egg production. This data indicates that the hen eats to meet energy requirements and cannot overconsume in order to meet amino acid requirements.

Nivas and Sunde (1969) found that 16 gm protein per day per bird was needed for optimum egg production. The diet had 2,156 cal per kg and was supplemented with D-L methionine.

Genetic or strain differences have been observed for Haugh units by Moreng et al. (1964) and Kidwell et al. (1964). Moreng et al. (1964), in their studies with four commercial strains and three protein (13, 15, 17) levels, noticed a strain interaction with protein level for egg production in the lower level of dietary protein. On the 13% protein ration, a test of mean differences between the strains revealed no significant differences with regard to Haugh units. However, at the 15% and 17% levels significant strain differences were shown in Haugh units. Egg weights exhibited significant differences with the 17% rations being greater than the 13 and 15% rations. Strain interactions to egg weight were present at each protein level. As egg production rose, feed utilization became more efficient, and as egg production dropped within a line, utilization of feed became less efficient.

Wilcox and Cole (1957) found that lysozyme concentrations in egg albumen was amenable to selection both up and down, so quantitative differences were to be expected. Age of the hen and position of the egg in the clutch seem to be without effect on albumen phenotype.

Egg Quality

Egg quality parameters such as Haugh units, albumen protein con-

tent, yolk and albumen volume, and solids and ash content of yolk and albumen traditionally have been compared to various production factors. Interaction between production factors and egg quality factors in various experiments have greatly improved efficiency of egg production and quality of eggs.

Almquist and Lorenz (1933), *in a* study of the solids contents of the various layers of egg albumen, found that 'watery' whites did not contain more water than other kinds of whites. The difference in the firm and liquid whites was one of structure and not of water content. They found the percent ash of the layers of white in any one egg to be nearly constant; although, the percent ash of the total solids varied widely. They concluded that the distribution of salts throughout the whites of a single egg was largely independent of the distribution of proteins.

Knox and Godfrey (1934, 1938) indicated that the proportion of thick white was not related to the number of eggs laid. Also, Pope (1960) noted no relationship between intensity of production and Haugh unit scores of eggs.

Mannel (1966) reported that higher internal quality was not related to egg production. Noles and Tindell (1967) indicated that Haugh scores were not related to current production rates. On the other hand, negative relationships of various magnitudes between production rates and interior quality have been noted by Goodman and Godfrey (1955), Johnson and Merritt (1955), King and Hall (1955), Yao (1958), Harms and Douglas (1960), King et al. (1961), and Moiseeva and Tolokonini Kova (1966).

Cunningham (1959) found that on the basis of actual volume, yolk tended to increase throughout the year while albumen volume tended to vary with season, being higher in the winter months and declining in the summer. Haugh units also were found to decline gradually throughout the year. Percent solids in albumen declined steadily throughout the year decreasing somewhat more rapidly during the months of June, July, and August. The actual grams of solids per egg, however, increased from August to April, then gradually decreased through May, June, and July.

Pope et al. (1960) found that the interior quality of eggs slowly decreased throughout the laying period with the greatest decline occurring after the first two months of production. Intensity of production was not found to affect the interior quality of eggs.

Baker and Vadehra (1969) studied albumen quality factors of strains vs. Haugh units and USDA scores. They found a highly significant difference among strains in percent thick albumen, Haugh units, and USDA scores. The data led to the conclusion that placement of the thick albumen is more important than the actual amount since in most strains considerably under 50% of the determination of the interior quality scores (USDA and Haugh units) could be accounted for by the amount of thick albumen.

Newell and Odell (1960) utilizing paper electrophoresis for albumen protein separation did not obtain high correlation between the protein fractions and Haugh units in their studies on age of birds and quality of eggs.

Hudspeth et al. (1965) observed that pH of albumen increased with storage time. Haugh units, as expected, declined with storage. The

relative percentage changes of the various protein fractions were found significant as the storage time increased: lysozyme and ovalbumin percentages increased with storage, Ovalbumin A_3 remained high in refrigerator storage, whereas, Ovalbumin A_2 and A_1 generally decreased with storage time.

Charkey et al. (1947) found that yolks from hens' eggs of high albumen quality did not differ from those of low quality eggs in amount of total solids, total nitrogen, or l(-) tryptophan. Both the fluid and the firm albumen fractions from high quality eggs had greater contents of total solids than did the corresponding low quality fractions. In high quality eggs, fluid albumen contains more solid matter per gram than did the firm albumen. No such differences were found in low quality eggs. Nitrogen content of fresh egg white fractions corresponded roughly to the content to total solids, but there is more nitrogen per gram of high quality firm albumen solids than per gram of low quality firm albumen solids. In addition, an indication has been obtained that fluid and firm albumen fractions from high quality eggs contain higher levels of l(-) tryptophan than do the corresponding fraction from low quality eggs. It appeared likely that the type or composition of the protein complex varies between eggs of high and low albumen quality, especially in the firm albumen fraction, resulting in a reduced water holding capacity in high quality eggs. The solids from such eggs are higher in nitrogen content than are the solids from relatively watery, low quality eggs.

In a life time study on production hens, Skala (1969), found no significant differences in body weight prior to sexual maturity al-

though the tendency for means to differ (higher quality weighing more) was apparent at 8 weeks of age. Body weight changes during laying were found non-significant and did not affect the feed efficiency. Conclusion: Higher quality producing hens are at least as efficient in overall feed utilization as the lower quality producing hens.

Samples of egg white taken at various times in the different studies indicated that the total solids concentration of the higher quality group egg whites generally differed by 0.6%-0.7% higher than that of the lower quality as these groups were constituted. Higher quality eggs may tend to have a slightly lower proportion yolk of total egg weight (Skala and Swanson, 1962a) as inferred from the data of that study which reported significantly more white in higher quality eggs with no difference in yolk weights.

Inspite of the tendency of the higher quality producing hens to be larger in adult body weight, their feed utilization was as efficient when calculated on the basis of equivalent weight of eggs produced.

EXPERIMENTAL PROCEDURE

Birds used in this study were a commercial strain (H & N) hatched June 10, 1969. They were debeaked at seven days of age and again at housing. All were initially fed the KSU starter ration, then switched to the KSU grower ration until 21 weeks of age. No artificial lighting was provided during the growing period. The birds were placed two per cage on a 15 hour stepwise lighting program when housed for laying. The treatments consisted of four isocaloric laying rations with the approximate protein content of 12, 14, 16 and 18% (Table I). The feed was pelleted to insure uniformity of intake.

Sample Collection

The birds were placed on experimental diets at 33 weeks of age. Monthly collection of eggs started 17 weeks later (60 weeks of age) and continued for six months. Egg weights were determined and Haugh units calculated as part of another experiment and will be reported separately. One liter each of egg white and whole egg per treatment was blended in a Waring Blendor for short intervals by turning the switch on and off (Forsythe et al. 1951). After thorough mixing, the samples were placed in screw cap polyethylene bottles and stored in a freezer at -20° C. Before analysis, the frozen samples were defrosted overnight at room temperature.

Solids and Ash

Total solids were determined by methods recommended in NCM publication No. 205 (1970). Five gm were weighed into a tared crucible

and then placed in a convection type oven for 13 hours. Dried whole egg samples were ashed in a muffle at 600° C for 13 hours. Ashing of egg white was done by the procedure recommended by Skala and Swanson (1962b).

Total Nitrogen

Protein (Total nitrogen X 6.25) was determined by the macro Kjeldahl AOAC, 1970. Duplicate samples were run on undiluted whole egg and albumen. Kel-Pak catalyst (10 gm K_2SO_4 + 0.30 gm $CaSO_4$; from Matheson Scientific Co., East Rutherford, N.J.) aided digestion. Two percent boric acid with methylene blue-methyl red mixed indicator, Harrow et al. (1955; 0.24 gm methylene blue + 0.375 gm methyl red in 300 ml ethanol) was used to collect the NH_3 distillate. Titration was carried out with 0.1N HCL.

Sponge Cakes

Whole egg sponge cakes were prepared by the procedure of Hanson et al. (1947). Vanilla extract was omitted as it only adds to the cake flavor. Salt has been shown to be deleterious to functional properties and was therefore excluded. Whipping time required for a soft-medium peak with the sponge cake batter was determined with a Kitchen-Aid (K4B) laboratory mixer on speed 10. Sponge cake batter was placed in rectangular (18.7 X 9.2 X 5.7 cm) aluminum cake pans. The cakes were baked for 21 minutes in a rotary gas oven preheated to 177° C (350° F). Cake volume was determined by the rapeseed displacement method (King et al. 1946) while the cakes were still in the baking pan.

Penetrometer measurements were made with a penetrometer graduated

into 1/10 mm divisions by Precision Scientific, Chicago, Illinois. Six measurements, one at each corner and two in the middle, per cake were made for protein level and a control (16% dietary protein fresh egg).

Disc Electrophoresis

Sample and Buffer preparation. Defrosted, blended egg white was thoroughly mixed, then 0.1 ml was diluted and mixed with 9.9 ml of 40% sucrose. Upper and lower reservoir buffers were prepared from Canalco reagent by their specifications. Tris buffer prepared with 36.6 gm Tris (Hydroxymethyl) Aminomethane, 48 ml 1N HCL and 0.23 ml TEMED ($N_1N_1N_1N_1$ tetramethylenediamine) and distilled HOH to bring volume to 100 ml; the pH was then adjusted to 8.9.

Gel preparation. Polyacrylamide separating gel (14%) was prepared from 1 ml Tris buffer pH 8.9, 2 ml acrylamide solution (28 gm acrylamide plus 0.735 gm Bisacrylamide to 50 ml with distilled HOH), 1 ml distilled HOH and 4 ml ammonium persulfate solution (1 gm in 50 ml HOH). Stacking gel (One part Tris buffer, one part acrylamide gel, one part riboflavin, one part distilled water, four parts sucrose solution) of the Canalco chemical formulation was used according to their specification (0.5 ml RDS-B, 0.5 ml FDS-D, 0.5 ml RDS-E, 0.5 ml HOH and 2 ml RDS-F). Six tubes were prepared for each run. A total of six runs were made.

Clean glass tubes (11.5 X 0.5 (ID/cm)), pre-soaked in non-ionic wetting solution, were inserted into a hollow rubber cap and set in a vertical position. Stacking gel solution was pipetted into the tubes and a thin layer (3-4 mm) of water placed on top of each gel. The gel was allowed to polymerize overnight before electrophoresis. After re-

moval of the water layer, stacking gel was added. A water layer was then placed on top of the stacking gel and allowed to photopolymerize in front of the Canalco light source for 30 minutes.

Electrophoresis. The gel tubes were attached to the upper buffer reservoir with the lower end submerged in the buffer solution of the lower reservoir. The upper buffer was placed in the reservoir and all air bubbles removed from the top of the gel tubes with polyethylene tubing connected to a syringe. Two tenths ml of the sucrose-egg white solution was layered on top of the stacking gel in each tube with a small stringe and polyethylene tubing. Voltage was applied at a rate of one milliampere per sample for one hour, then raised to 2.5 milliamperes per sample until the end of electrophoresis.

The sample ions form a compact ring and migrate downward. The electrophoresis was continued until the samples had migrated to the surface of the lower buffer. At the completion of electrophoresis the power supply was turned off and the gel tubes were removed.

Gel removal and staining. For gel removal, a long 20 gauge needle attached to a syringe was used to rim the surface between the gel and inner tube wall. The bottom part of the gel tubes were rimmed first and the gels were lubricated and loosened by injecting water while rimming the surfaces. Once the gel protrouded beyond the end of the tube, the needle tip was inserted from the opposite end of the tube and the top of the separating gel was rimmed and water injected under pressure. After freeing the gel from the surface of the glass tube, a rubber tube and 50 cc syringe was connected to the top of the glass tube. The gel tube was immersed under water and the gel removed by applying

pressure with the syringe. The electrophoresed gels were fixed in 20% TCA for one hour, rinsed, and then stained with coomassie blue for one hour. After staining, the gels were placed in 7% acetic acid for 12 hours, then stored in 1% acetic acid.

RESULTS AND DISCUSSION

The amount of feed consumed by the birds (Table II) on the 12% ration as compared with other rations supports the theory that hens will overconsume their caloric needs to obtain their protein requirements, this is in agreement with Smith (1967) and Novacek and Carlson (1960).

Daily protein intake has been shown by stangeland and Carlson (1961), Britzman and Carlson (1965), and Combs (1960) to be a function of methionine intake. Approximately 300-320 mg of methionine are required per day for a bird in maximum production, Combs (1960), and Novacek and Carlson (1969). Ingram et al. (1951) gave the combined requirement for methionine and cystine to be not more than 0.63% of the total ration. The 12% ration had a combined methionine and cystine value of 0.415%.

Eggs produced from the hens receiving the 12% ration were 41% mediums, whereas, higher protein diets enabled a greater percentage of large eggs. Egg production also was lower for the 12% dietary protein ration. Since the protein and amino acid consumption was below optimal values, egg size small, and percent production lower; the 12% ration appears to be deficient in essential amino acids.

Ash

The amount of ash (Table III, IV) was not affected by protein level. This is in agreement with Skala and Swanson (1962b) that the total ash content does not vary between high and low quality eggs.

Total Solids

Total solids content of albumen and whole egg was not affected by the dietary protein level (Table III, IV). High quality egg whites have a greater total solids content than lower quality egg whites (Skala, 1969; Charkey et al. 1947). Skala and Swanson (1962) found a greater proportion of albumen to yolk in high quality eggs as compared to low quality eggs.

The high and low quality eggs in the experiments of Charkey et al. (1947), Skala and Swanson (1962a,b), and Skala (1969) were characteristically chosen through hen selection and grouping prior to the actual experimental sample collection and analysis. Thus, their low quality eggs were selected by hen difference and was not an induced dietary factor. In this study, hens of the same genetic background and prior history were grouped with no preference given to previous egg quality. Hen difference factors are compensated for in the computation of the error mean square in the analysis of variance.

Total Nitrogen

Percent albumen and whole egg protein increased as the dietary protein level increased (Table III, IV). High quality firm albumen solids were found by Charkey et al. (1947) to have a greater nitrogen content per gram than low quality firm albumen solids. They also indicated that fluid and firm albumen fractions from high quality eggs contained higher levels of l(-) tryptophan than did the corresponding fraction from low quality eggs.

Skala and Swanson (1962b) observed a greater concentration of total nitrogen in the albumen and whole white solids from high quality

eggs vs. low quality eggs. Results concluded that the difference could be in the composition of the total protein pattern or the pattern of total solids. The latter possibly was favored strongly by subsequent analytical data. Total nitrogen concentration was the same in the outer thin layers of white of eggs of both quality levels. The nitrogen and solids concentration increased toward the inner thin white layer of both quality levels, but at a higher rate in the higher quality eggs. A higher concentration of ovomucin was present in the middle thick layer of higher quality whites. Calculation of the concentration of ovomucin in relation to the total protein of the middle thick white layer again revealed a higher value favoring higher quality eggs. Those results suggested that, while the composition of the protein pattern of different quality whole whites appeared the same, there was a difference between quality levels in distribution of ovomucin in the layers of white. Paper electrophoretic analysis found definite differences in the major proteins between egg white layers within quality levels; none were found between quality levels.

The increasing amount of nitrogen from lower quality to higher quality eggs agreed well with previous research; however, the interaction of total solids and egg quality with dietary factors has not received as much detailed study.

Skala (1969) postulated that the increase of solids content with higher quality eggs may arise from a dilution effect by the uterine secreation, which in turn, might be reflected in water intake. This was evaluated and found not to differ significantly between quality levels, being 2.3 gm per gram of feed for the higher quality groups

and 2.4 gm program of feed for the lower quality groups.

The reason for a greater amount of protein in eggs from hens receiving more dietary protein may be answered by the fact that the lower level protein diets were deficient in essential amino acids. With these amino acids being limited by the diet and the hen unable to utilize them from her bodily stores, the amount of protein formed into egg white was thus limited.

Sponge Cakes

Volume of sponge cakes (Table V) appeared to be related to the amount of protein in the egg. Spong cake whiptime and penetrometer measurements were not affected by dietary protein (Table III, IV).

Electrophoresis of Egg White

Densitometer tracings and gel photographs are shown in Figs. 1-5. The numbering system is that used by Lush (1961) in starch-gel electrophoresis and by Feeney et al. (1963).

The densitometer base line was established through clear, unstained portion of the polyacrylamide gel. Initial gel distortion needle marks and frayed edges of stained stacking gel registered a peak on the strip chart recording. The distortion and peak presence was present at both ends of the gel. The initial peak was indistinguishable from the peak representing non-motile protein (N) observed at the start of each gel.

Feeney et al. (1963) found constituent 18, as yet to be identified. Theoretically, it is in an area where avidin might be expected judging from comparisons of the separations on carboxymethycellulose chromatographic columns (Rhodes et al. 1958) and the mobility of constituent

18 in the gel. The amounts were considered insufficient at this stage, however, to permit extraction and assay. In membrane filtration experiments, constituent 18 was retained by membranes which passed conalbumin. This indicated a molecular weight of less than 80,000.

Melamed and Green (1963) fractionated egg white on ion-exchange cellulose. Three fractions of avidin (A, B, and C) were present. The last fraction was present only in small amount and seemed to be combined with another substance or to have a lower intrinsic biotin-binding capacity. Avidins A and B each had the same capacity to bind three molecules of biotin, but differed slightly in their amino acid composition. Miller and Feeney (1966) purified and identified component 18 on starch-gel as ovomacroglobulin.

In the disc gel scans (Figs. 1-5), there appeared to be more than a single component present before conalbumin. Purified fractions must be electrophoresed before the components of fraction 18 in this experiment can be identified.

Constituents 16 and 17 have been identified by Feeney et al. (1963) as conalbumin. Williams (1962) reported constituent 15 as a third conalbumin.

Intermediate bands 14 through 7 should contain the preconalbumins, globulins and post albumin fractions, as compared to various starch-gel electrophoretic patterns.

Bands 3, 4, and 6 corresponded to ovalbumins in starch gel by Feeney et al. (1963). Chang et al. (1970) identified three ovalbumin in centrifuged egg white fractions by comparison to crystallized ovalbumin.

Bands 1 and 2 are in the preconalbumin range. Tomimatsu et al. (1966) identified purified ovoinhibitor in the conalbumin-preconalbumin region of starch gel. Under some conditions, migration was consortive; in others ovoinhibitor migrated more rapidly than conalbumin and separated into three bands.

Baker and Manwell (1962) found two electrophoretically separate components migrating in front of ovalbumin. They suggested that the slower component was the flavoprotein-apoprotein complex and that the faster component was an unidentified preconalbumin.

Ovoglycoprotein was isolated and purified by Ketterer (1965). The isoelectric point and molecular weight suggested that it might be the faster of the two prealbumins in the chicken (Baker 1969).

Polyacrylamide disc gel electrophoretic separation of egg white protein is comparable to starch gel separation. Starch gel has the advantage of lysozyme separation, a factor not attainable using present methods of sample placement and buffer pH. Disc electrophoresis, does, however, separate fractions slower than conalbumin and faster than ovalbumin. Evidence of starch gel separation and identification of these fractions is limited. Further work is necessary to find the right combination of gel concentration, separating gel length, buffer, and pH necessary to separate and resolve the intermediate area containing the globulins and other unidentified protein fractions.

The application of polyacrylamide gel electrophoresis to egg white protein along with purified and crystallized protein fractions may help identify presently unknown egg protein fractions.

SUMMARY AND CONCLUSION

The response of laying hens fed four isocaloric rations with protein contents of 12, 14, 16, and 18% was studied. The 12% ration was calculated to be deficient in the essential amino acids, methionine, and cystine. Production responses (decrease in egg production and egg size, and overconsumption of energy) verified the deficiency.

The ash and total solids content of whites and whole eggs did not vary with the level of dietary protein. Total nitrogen in whole egg and albumen was a function of the dietary protein intake and increased as the level of protein increased in the diet. Sponge cake volume appeared to improve with the higher levels of protein in the egg.

The amount of egg white formed per egg by hens receiving a suboptimal protein diet is less than a hen receiving an adequate amount of dietary protein.

A method utilizing polyacrylamide disc gel electrophoresis was developed for the separation of egg white proteins. This method using 14% polyacrylamide separating gel yields separation comparable to starch gel patterns of egg white. Fractions faster than ovalbumin and slower than conalbumin were observed, their identification awaits the use of purified protein fractions. With the ease of preparation and high resolution, disc electrophoresis may be an applicable tool to further identify and measure egg proteins.

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APPENDIX

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.

TABLE I

Composition of the Layer Rations used in this Experiment

Ingredients	Amount in Pounds			
	12%	14%	16%	18%
Soybean meal (44%)	152.0	269.6	381.6	496.0
Alfalfa meal (17%)	92.0	92.0	92.0	92.0
Sorghum grain (ground)	1445.0	1301.0	1165.0	1025.0
Fat	24.0	50.4	74.4	100.0
Distillers dried grains & solubles	40.0	40.0	40.0	40.0
Corn gluten meal (60%)	48.0	48.0	48.0	48.0
Dicalcium phosphate	61.0	61.0	61.0	61.0
Ground limestone	108.0	108.0	108.0	108.0
Salt	10.0	10.0	10.0	10.0
Premix[1]	20.0	20.0	20.0	20.0
Per cent protein	12.28	14.21	16.07	17.95
Metabolizable Energy Cal/gm	2.83	2.83	2.82	2.82

Ingredients	PREMIX[1] Amount in grams
Vitamin D_3 (15,000 ICU/gm)	68
B-complex 1233	454
Choline chloride (25%)	1816
Vitamin A (10,000 IU/gm)	400
Vitamin B_{12} (Preferm-20)	454
Methionine	454
Trace minerals "Z 5"	454
Ground sorghum grain	to make 20 pounds

TABLE II

A Summary of the Average Feed Consumption, Protein, and Amino Acid Intake Per Day

	12%	14%	16%
Feed consumed per day (gm)	113	105	103
Protein per day (gm)	14.0	15.0	16.5
Calories per day	320	297	291
Methionine (mg)	266	271	292
Cystine (mg)	203	217	242
Lysine (mg)	522	647	786

TABLE III

Analysis of Variance of the Ash, Solids, Protein Content of Whole Egg and Albumen and Sponge Cake Whiptime, Volume, and Penetrometer Resistance

Source of variation	Degrees of Freedom	Mean Square	F
% Ash Albumen	3	0.0007	0.552
Error	4	0.0013	
Total	7		
% Ash Whole Egg	3	0.012	2.28
Error	4	0.005	
Total	7		
Albumen Solids	3	0.21	0.44
Error	4	0.47	
Total	7		
Whole Egg Solids	3	1.44	1.36
Error	12	1.06	
Total	15		
Albumen % Protein	3	1.88	16.14
Error	12	0.12	
Total	15		
Whole Egg % Protein	3	1.02	7.21
Error	16	0.14	
Total	19		
Sponge Cake Whiptime	4	2.12	11.63
Error	10	0.18	
Total	14		
Cake Volume	4	3578.12	1.88
Error	25	1904.85	
Total	29		
Penetrometer Readings	4	438.96	0.78
Error	20	563.38	
Total	24		

TABLE IV

Duncans Multiple Range Test[1] of Ash, Solids, and Protein Content of Whole Egg and Albumen

% Ash Albumen	18% 0.817	12% 0.805	14% 0.794	16% 0.722
% Ash Whole Egg	12% 10.7	16% 0.94	14% 0.94	18% 0.84
Albumen Solids	18% 11.89	16% 11.56	12% 11.42	14% 11.11
Whole Egg Solids	12% 26.21	18% 25.41	16% 25.12	14% 24.82
Albumen % Protein	18% 10.73	16% 9.88	14% 9.59	12% 9.10
Whole Egg % Protein	18% 12.43	16% 12.16	12% 11.59	14% 11.49

[1] Means underscored with same line are not significantly different at the 0.05 level of probability.

TABLE V

Duncans Multiple Range Test[1] of Sponge Cake Whiptime, Volume, and Penetrometer Resistance

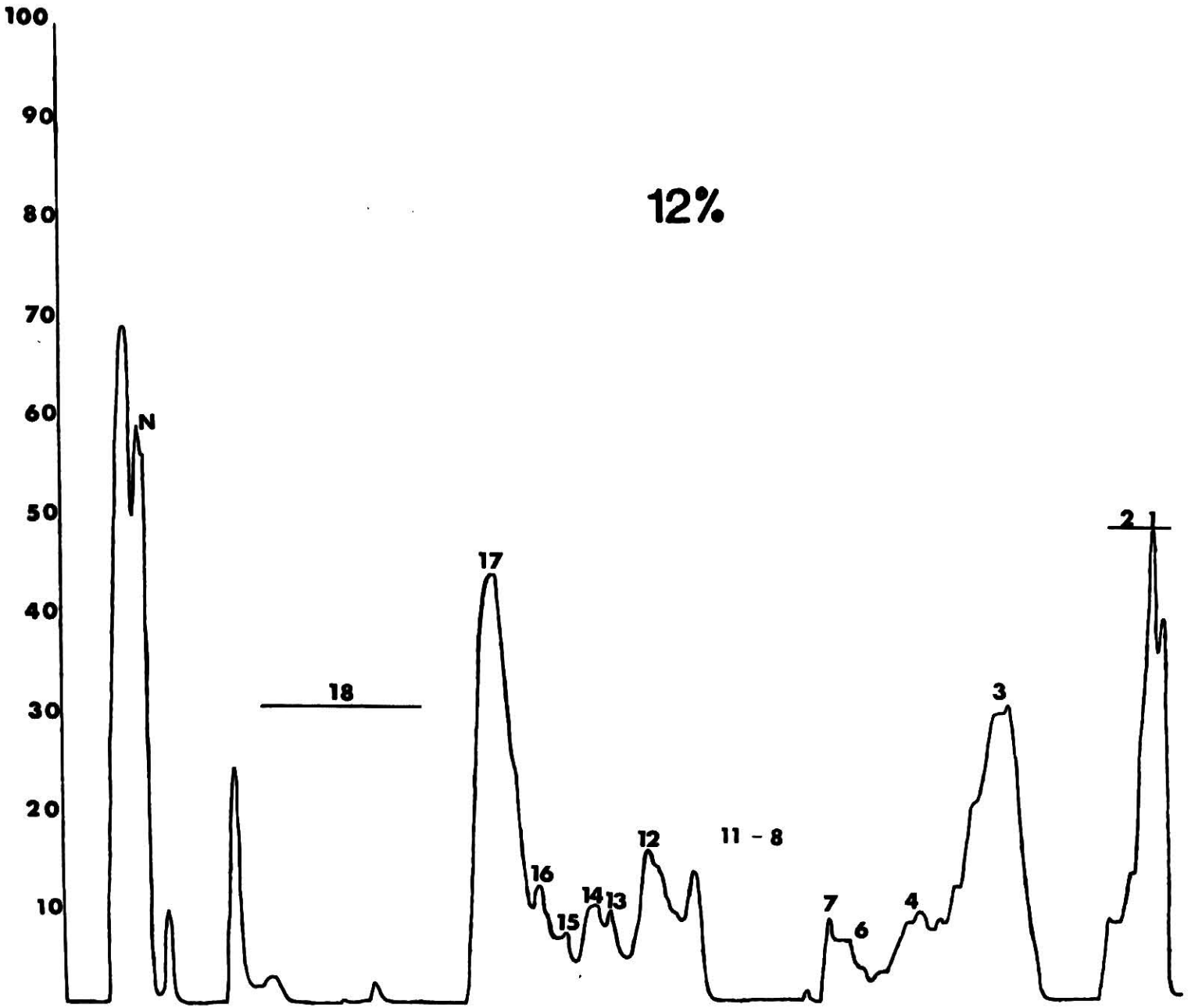
	12%	18%	16%	14%	Control
Sponge Cake Whiptime	6.27	5.67	5.60	5.33	4.00
	Control	16%	18%	14%	12%
Cake Volume	544.2	526.3	522.2	502.2	480.5
	Control	12%	16%	18%	14%
Penetrometer	161.60	150.00	148.80	139.20	138.80

[1] Means underscored with same line are not significantly different at the 0.05 level of probability.

EXPLANATION OF FIGURE I

Densitometer tracings of the disc gel electrophoretic patterns of egg albumen from the 12% ration. The gel speed was 60 seconds per inch and chart speed was 20 seconds per inch. The wavelength was 550 mm; gels were stained with coomassie blue.

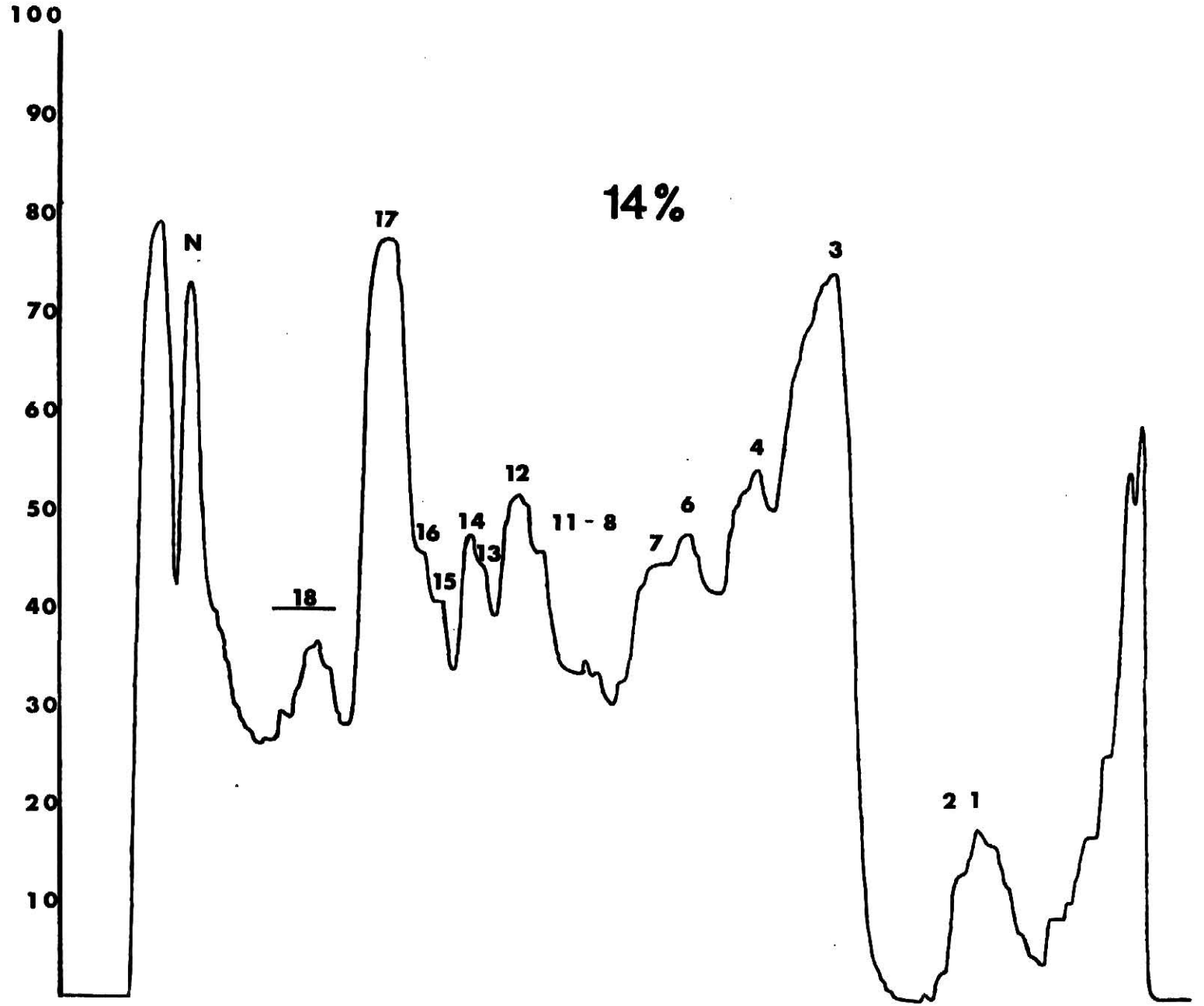
FIGURE I



EXPLANATION OF FIGURE II

Densitometer tracings of the disc gel electrophoretic patterns of egg albumen from the 14% ration. The gel speed was 60 seconds per inch and chart speed was 20 seconds per inch. The wavelength was 550 mm; gels were stained with coomassie blue.

FIGURE II



EXPLANATION OF FIGURE III

Densitometer tracings of the disc gel electrophoretic patterns of egg albumen from the 16% ration. The gel speed was 60 seconds per inch and chart speed was 20 seconds per inch. The wavelength was 550 mm; gels were stained with coomassie blue.

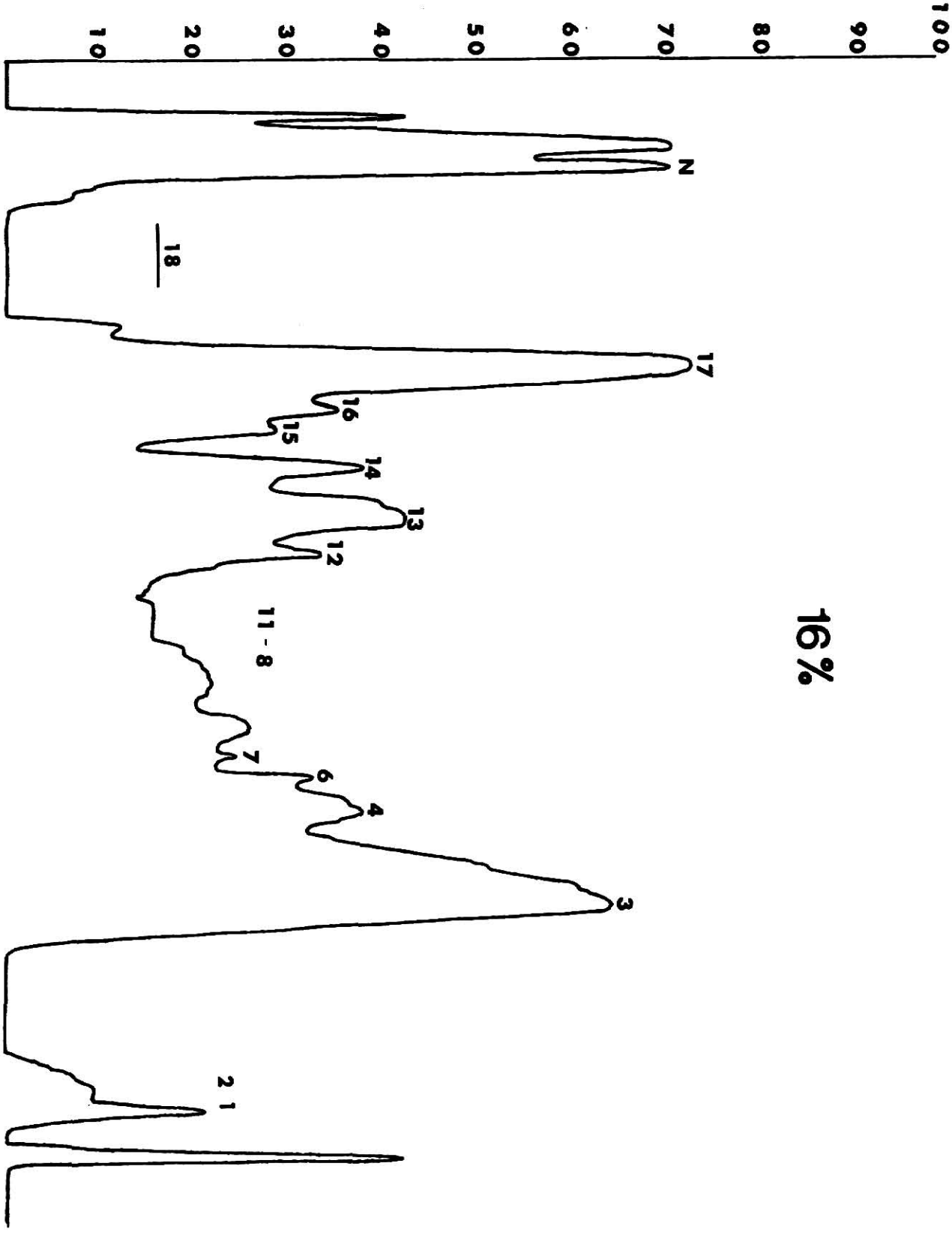


FIGURE III

EXPLANATION OF FIGURE IV

Densitometer tracings of the disc gel electrophoretic patterns of egg albumen from the 18% ration. The gel speed was 60 seconds per inch and chart speed was 20 seconds per inch. The wavelength was 550 mm; gels were stained with coomassie blue.

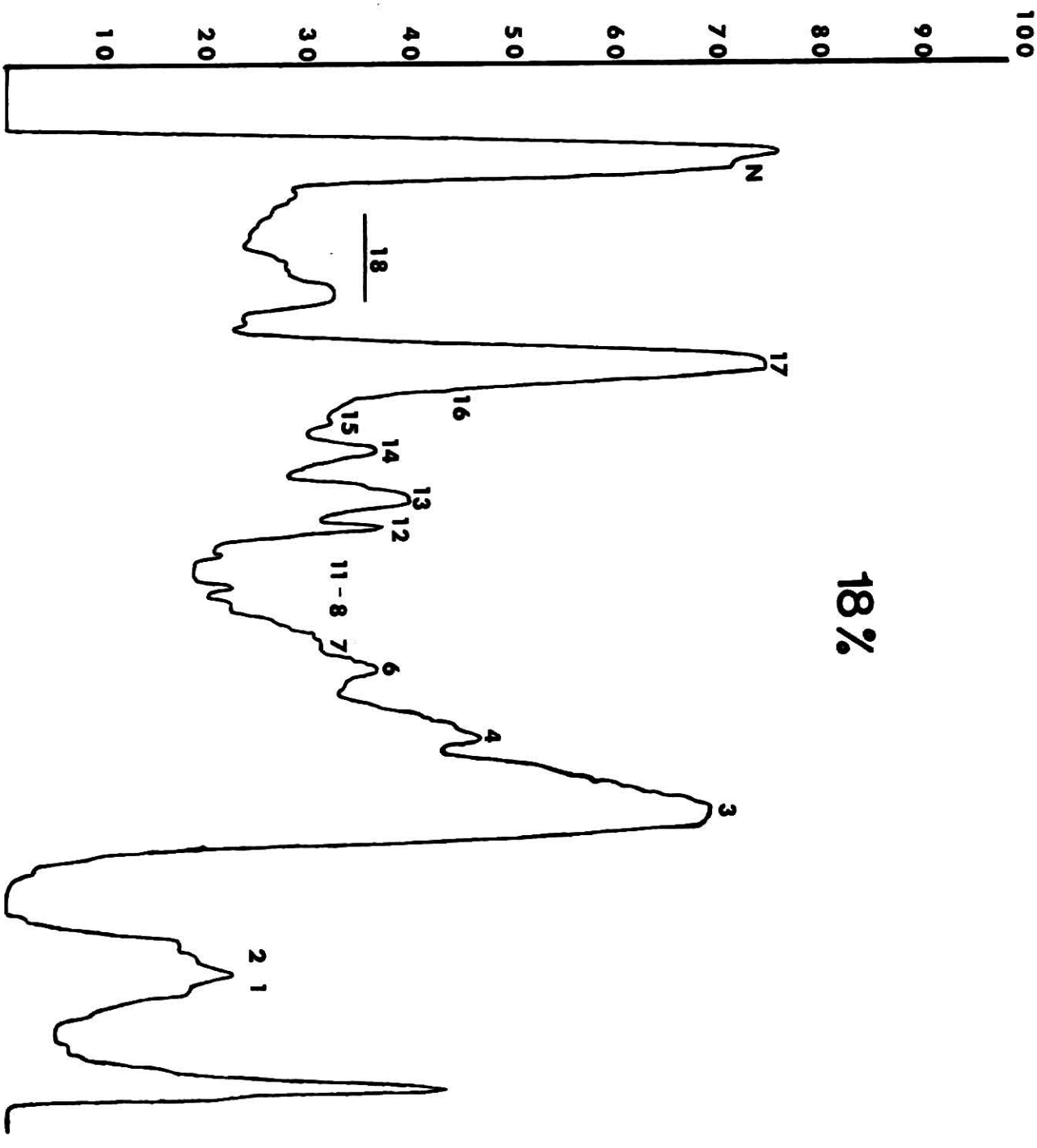
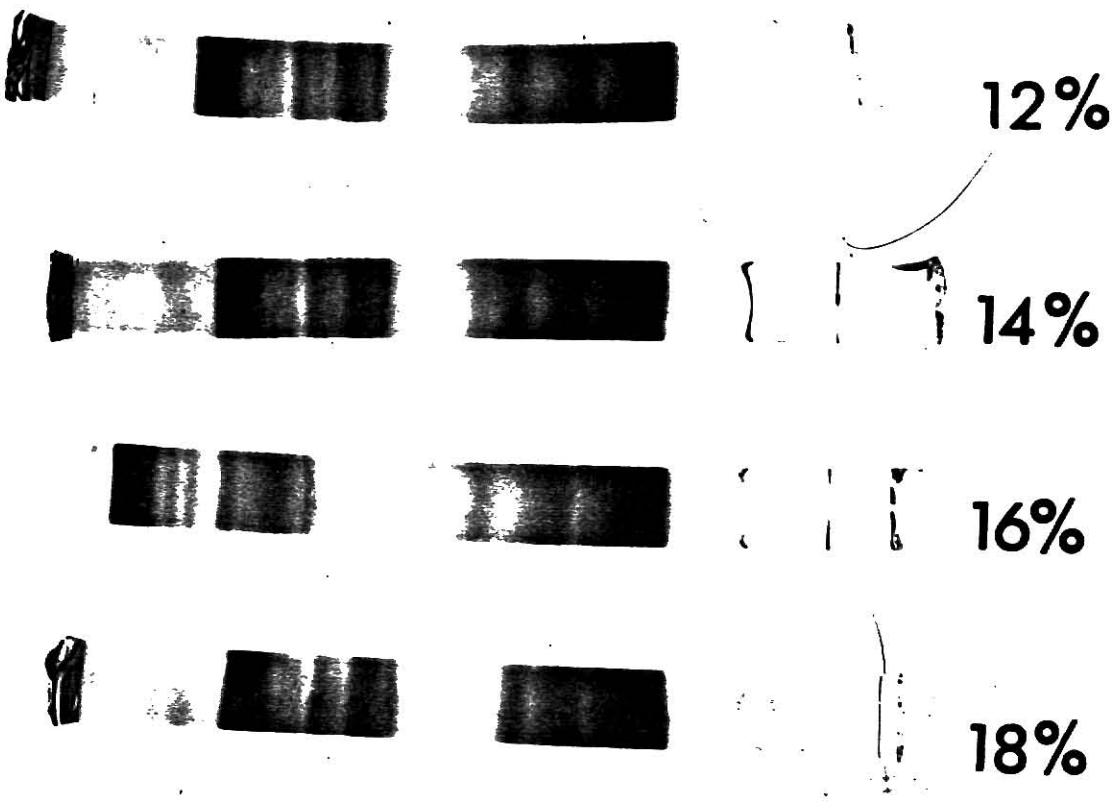


FIGURE IV

EXPLANATION OF PLATE I

Disc gel electrophoretic patterns of egg albumen from the four fractions. Migration of the protein fractions is from left to right. Gels were fixed with 20% TCA and stained with coomassie blue.

PLATE I



EFFECT OF DIETARY PROTEIN ON THE CHEMICAL AND FUNCTIONAL PROPERTIES OF EGG WHITE

by

JOHN NELSON BUTTS

B.S., Kansas State University, 1970

AN ABSTRACT OF A MASTER'S THESIS

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requirements for the degree

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Commercial strain (H & N) chicks were hatched June 10, 1969. The birds were reared until laying at the KSU Poultry Farm. At 21 weeks of age, the birds were housed, two per cage, on a 15 hour stepwise lighting program. At 33 weeks of age, the caged hens were divided without preference into four groups and fed the experimental isocaloric rations of 12, 14, 16, and 18% protein. Sample collections began at 60 weeks of age and continued for six months. Whole egg and albumen samples were taken at monthly intervals. One liter of each egg white and whole egg per treatment was mixed by blending, placed in polyethylene bottles and stored in a freezer until analysis.

Total solids, ash, and total nitrogen content was determined for each dietary treatment on both whole egg and albumen. Whole egg sponge cakes were made and analyzed for batter whiptime, sponge cake volume, and penetrometer resistance. Egg-white proteins were separated by polyacrylamide disc gel electrophoresis.

The results indicated that the 12% ration was protein deficient as measured by production responses. Birds receiving the 12% ration laid fewer and smaller eggs and overconsumed their energy requirement in order to meet their protein requirement. As the level of dietary protein increased, total nitrogen in albumen and whole egg increased, ash and total solids remained unaffected. Sponge cake volume appeared to improve with the higher levels of protein in the egg.

A 14% polyacrylamide separating gel was used as the medium for separation in the disc gel electrophoresis. This method of electrophoresis yields separation and resolution comparable to starch gel

patterns of egg white, but is much easier and less time consuming than the latter method. Several unidentified fractions faster than ovalbumin and slower than conalbumin were observed.