IN-PROCESS CONTROL OF PROTEIN LEVELS IN FORMULA FEEDS

by

KERMIT EDWARD ADELGREN

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Approved by:

C W Deyoe
Major Professor

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INTRODUCTION

The need for a rapid routine method for the determination of protein content of commercial formula feeds has been evident for several years. In many cases the formula feed has left the manufacturer before a Kjeldahl analysis for crude protein content has been determined. Many manufacturers do not have laboratory facilities for such a determination, thus a sample of the formula feed must be sent to a commercial laboratory for an analysis. Since essentially all formula feed is sold on a guaranteed protein basis, a quick in-process method of determining the protein content of the feed ingredients and formula feeds would be of great benefit to feed manufacturers.

The Kjeldahl nitrogen analysis which was developed by Johan T. Kjeldahl in 1883 is applicable to all plant and animal protein sources. This analysis, even though developed 80 years ago, remains today the standard method for crude protein analysis by which all other protein analysis methods are compared. The original procedure has been modified from time to time to include the addition of, or substitution of, various salts and catalysts to accelerate the reaction. Including these modifications and modern laboratory equipment, this method requires approximately two hours to obtain a crude protein determination.

The biuret test was developed in 1873 as a qualitative test for proteins and later in 1915 adapted to the quantitative estimation of proteins in biological fluids. In this test, copper in strongly alkaline solution reacts with protein material and forms a reddish-violet solution. Under proper conditions, the intensity of the color produced is proportional to the protein concentration. Copper has been found to react in a similar way with non-protein materials which contain amino and carboxyl groups characteristic of proteins.

Fraenkel-Conrat and Cooper (1944) developed a convenient method for measuring the acidic and basic groups of proteins using an insoluble protein dye complex. This protein dye complex is formed by a bond between the guanidyl, imidazole and amino groups of basic amino acids and the sulfonic acid group of the dye molecule. The acid dye, orange G was found to combine stoichiometrically with the basic protein groups in a buffered solution of pH 2.2. At pH 2.2. the basic groups on a protein molecule are positively charged and the negatively charged sulfonic acid groups of the dye molecule react to form the dye-protein complex.

This technique has been found to be rapid, simple and applicable to both soluble and insoluble proteins. Since it is based on the photoelectric determination of the uncombined dye, it can be carried out with protein samples as small as the accuracy of weighing permits.

Dye binding has also been adapted to the quantitative determination of proteins of several biological materials. Some of these materials include milk, wheat, flour, corn, milo, and blood serum. A number of workers have reported high correlations between dye binding capacities and crude protein as determined by the Kjeldahl method.

The Kjeldahl determination of crude protein content is based upon the total nitrogen content of the material being tested. Since nitrogen from numerous non-protein sources is detected by this method, the crude protein content determined in this manner may not be an actual measure of the protein present. An additional error may also be introduced by the calculation of the protein content based on the total nitrogen in the sample. The value most commonly used in this calculation, (6.25 X %N), is based on the average nitrogen content (16%) of feedstuffs.

Some proteins have higher levels of nitrogen than this and others have lower levels, thus determination based upon the actual protein or some constituent of the protein molecule could give a more reliable value for the actual protein available. If this were based on certain bonds or amino acids, it could also provide information regarding the quality of the protein.

This research was planned to investigate the feasibility of adapting dye binding techniques to in-process protein analysis of formula feeds.

REVIEW OF LITERATURE

Dye Binding of Milk Proteins

Udy (1956) studied this technique for the determination of total protein in milk products. He substantiated the assumption that the total proteins in milk have a common dye binding capacity by the results of tests on more than 100 samples of milk. Samples of fresh whole milk and spray-dried milk were analyzed. The results indicated that the only constituents binding the dye were the milk proteins, because a regression line drawn through the experimental points passed very near the origin.

The results also indicated a high correlation between dye absorbed and Kjeldahl protein, the standard error of estimate being 0.07%.

The use of amido black and orange G dyes for protein estimation in milk were investigated by Dolby (1961). Orange G and amido black reacted in the same molar ratio with basic groups of proteins, but in this work, amido black, owing to its higher molar optical density, gave a more sensitive indication of the protein content of milk.

Ashworth and Seals (1957) studied various factors affecting dye binding techniques. These workers developed a flow

through cuvette which was specifically adapted for use in a Beckman Model B Spectrophotometer. In the use of the cuvette, 10 ml of the sample was sufficient to rinse out the previous sample and to obtain constant readings. Comparison of filtering through paper, or centrifuging to remove the dye-protein complex indicated a difference of about 0.6%. This difference was found to be due to the dye absorbed by the filter paper, and was relatively constant for dye concentrations which varied from 0.3 to 1.5 mg per ml.

Also a range of milk temperatures from 46° to 130° F. was studied and was found to have no significent effect on dye binding capacities.

Ashworth and Seals (1957) found that the most direct convenient standard curve was obtained by plotting the light absorption values of the dye left, after filtering, against protein concentration found by the Kjeldahl method.

Treece, et al., (1959) collected milk samples from Holstein, Guernsey and Jersey cows of different ages and in different stages of lactation for studies with dye binding methods for protein determination. Samples were collected at three different times, approximately three months apart, from different cows and from two different experiment stations. All samples were analyzed in duplicate for protein content by the Kjeldahl method and by the orange G dye binding

method. The correlation found between the two methods was 0.98. Approximately 2/3 of the samples when tested by orange G fell within $\pm$ 0.05 percentage units of the corresponding Kjeldahl value. This indicates the reliability of the orange G method for protein determinations on heterogenous samples of milk.

The precision of the orange G dye binding method has been increased by Ashworth, et al., (1960). The results of 345 comparisons of protein determined by the new technique with that determined by the macro-Kjeldahl method showed a correlation coefficient of 0.98, with a standard deviation from regression of 0.078% protein. From 224 duplicate determinations it was observed that 19 out of every 20 single determinations fell within 0.06% protein of the average for duplicate readings.

Large variations in the amounts of butterfat and lactose were found to be without effect on the protein values determined by the dye binding techniques.

Orange G dye shows more stoichicmetry in its reaction with milk proteins than does amido black 10 B, although the latter gives a higher dye-binding capacity (DBC), according to Ashworth and Chaudry (1960). Values for the DBC of the major milk proteins were determined at three levels of protein and for both dyes. In general there was a parallel trend in the amount of each dye bound, indicating that the same protein groups

were involved. Values for DBC with amido black decrease more rapidly with increasing protein dye ratios than did the corresponding values for orange G.

The estimation of protein content appeared to be improved when regression lines were used to express the relation between the amount of dye reacting (either bound or unbound) and the per cent protein. An explanation lies in the fact that the regression allows for some change in DBC over the relatively narrow range of protein concentration generally employed. Thus empirical corrections for poor stoichiometry can be made which allows the dye binding methods to become highly useful, practical tests for milk protein.

Flourimetry as a Method of Determining Protein Content of Milk.

A commercial spectrophotoflourimeter was used by Fox, et al., (1963) to determine the relationship between the intensity of the flourescence of milk in the ultraviolet region and its protein content. It was concluded that the intensity of flourescence of milk in the ultraviolet region could be used to make accurate and rapid determinations of the protein content of milk.

Dye Binding of Serum Proteins

Scheurlen (1959) stated that serum protein could be photometrically determined at concentrations of 30 - 400 γ/ml by making use of the wavelength shift of the absorption maximum

of bromophenol blue in the presence of the proteins. The optical density increment of serum albumin was found to be approximately ten times that of globulin; nevertheless, it was shown that individual serum proteins could be estimated by the method with an error of about 1%.

Osborn (1960) studied the binding of acid dyes (light green, lissamine green, bromocresol green) by proteins denatured on filter paper under various conditions. The uptake of dye, measured by elution, was found to vary according to the method of denaturation, the nature of the dye solvent, and with the duration and temperature of the subsequent washing procedure. Also, different denaturing agents give different albumin globulin dye uptake ratios.

The dye binding capacity of eleven electrophoretically separated serum proteins was studied by suspending the separated fractions in three different dye solutions (Strickland, et al., 1959). These workers stated that reliance on dye binding capacities could be expected to detect only gross abnormalities in serum protein distribution.

Lissamine green has been shown to be an excellent dye for the staining and quantitative estimation of separated protein fractions in paper electrophoresis, (Brackenridge, 1961). The grade of paper, weight of protein applied, separated and combined protein denaturation, staining time, temperature, con-

centration of denaturant and type of protein, were all found to influence the weight of dye absorbed per unit weight of applied protein, and all must be rigidly standardized for valid quantitative results.

Brackenridge (1960) also studied the interactions between human serum proteins and lissamine green SF 150 (light green SF) on cellulose acetate. A pH of 6.0 was optimal for the combined operations of elution and measurement of absorbance. Dye binding by protein was optimal at pH 2.7 and decreased as the ionic strength increased. The optimal temperature for dye binding by albumin was 30° C. Dye binding by albumin and four globulin fractions was linear with respect to concentration.

Colorimetric Determination of Protein Content of Wheat and Wheat Flour.

Zeleny (1941) developed a method of utilizing certain physico-chemical properties of the gluten proteins for determining the protein content of wheat.

The proteins of wheat flour were readily peptized by a very dilute alkali. When an alkaline solution containing the peptized flour protein was neutralized, the gluten proteins were precipitated. If, however, the protein solution was sufficiently dilute, and its hydrogen-ion concentration after neutralization was controlled by a suitable buffer, the gluten proteins would not separate from the solution but would nevertheless appear as

a turbid and stable colloidal suspension. The degree of turbidity of such a suspension was used as a measure of the gluten protein content of the flour.

Through experimentation, this turbidity method was developed and the results were well correlated with Kjeldahl protein determinations of wheat flour samples.

This method was also adapted to whole wheat grain by Zeleny (1942). The fact that this method differentiates between gluten and non-gluten offsets part of its errors and this makes it as good a measure of baking quality of wheat as Kjeldahl protein values.

Biuret Test for the Determination of Protein Content of Wheat and Wheat Flour.

Pinckney (1949) applied the biuret reaction to the quantitative estimation of protein in wheat and wheat flour. The protein, peptized by 0.05 N potassium hydroxide, was treated with copper sulfate stabilized by a small amount of glycerol in alkaline solution.

The color intensity of the reddish-violet product, which was proportional to the protein concentration, was determined with a colorimeter. Approximately 1/10 of the protein remained unpeptized, and therefore was not directly measured. Nevertheless, biuret color values were closely correlated with crude protein values as determined by the Kjeldahl method. It was stated that biuret values could be evaluated in terms of either total protein or peptized protein, by means

of a graph, table, or formula derived from the biuret and Kjeldahl protein values of test samples. Peptized protein has also been referred to as "Biuret Gluten".

Jennings (1961) reported that the use of potassium sodium tartrate in place of glycerol in the biuret reagent used by Pinckney (1940) resulted in a 13% increase in sensitivity. Also, extraction of the protein and color development occur simultaneously in an alkaline copper tartrate solution.

The main factor causing imperfect correlation between Kjeldahl nitrogen content and the color produced by the biuret test was the variation in the latter of the absorbance coefficients of the extracted proteins.

Williams (1951) reported that the biuret method had several advantages over the Kjeldahl method for determining the protein content of wheat and wheat flour. These advantages included less expensive equipment and elimination of the use of concentrated and corrosive chemicals. The results obtained, using the biuret method, showed excellent correlations with those of the Kjeldahl method.

Colorimetric Procedure for the Determination of Protein Content of Wheat and Barley.

Feinstein and Hart (1959) developed a method for protein analysis in which the sample was extracted with dilute alkali. After the extract was centrifuged, an aliquot of the supernatant fliud was reacted with a sulfosalicylic acid solution to produce a slightly opalescent liquid. The optical density of

this liquid was then determined in a photoelectric colorimeter and the optical density readings were translated in % protein by means of a calibration curve. The colorimeter readings were found to be affected very little by factors other than protein. This method also gave a high correlation with Kjeldahl protein values.

Enari, et al., (1960) reported the following procedure as a rapid protein test for barley. To a one gram sample of barley flour, add 100 ml of 0.05 N NaOH; shake the mixture for 45 minutes and filter. Transfer 1 ml of filtrate to each of the three colorimeter tubes; to two of the tubes add 9 ml of sulfosalicylic acid solution (30 g/l.) and to the third tube add 9 ml of H_2O. The % transmission was read at 540 mu on a colorimeter, and an equation was calculated using the % transmission and Kjeldahl protein.

Phthalein Dyes for the Determination of Soybean Oil Meal Quality.

Frolich (1954) reported that phenolphthalein dye was absorbed in a linear relation according to the amount of heat applied to soybean oil meal. The unheated meal had the lesser amount of dye absorbed, while overheated samples absorbed higher amounts of dye.

This worker studied a variety of dyes, but found that only phthaleins, such as phenolphthalein, phenol red, bromothymol

blue, and cresol red reacted in this characteristic way. Cresol red was the most stable of these dyes. A correlation between heat treatments and cresol red dye absorption was also found with samples of rape seed meal, linseed meal and dried milk.

Olomucki and Bornstein (1960) conducted several tests using Frolich's (1959) dye absorption method. This study included twenty-five samples of solvent extracted soybean meal which were examined by the dye absorption method and biologically, by rate of growth and efficiency of feed conversion by growing chicks. In all cases evaluations of the meal by the dye absorption method agreed with results of the biological trials.

Dye Binding of Grain and Feedstuffs.

Udy (1954) has shown that acid and basic groups of protein molecules can be measured quantitatively on fractions of wheat protein by dye-binding techniques. These fractions were essentially soluble proteins, gluten proteins, a combination of the two, and proteins nondispersible in dilute acetic acid.

The acid equivalents of dye bound by 1 gram of protein for the four fractions of protein were obtained for several varieties of wheat. The different varieties failed to show any significant variation within any given protein fraction.

Differences in dye binding capacity for given fractions were within experimental error ($\pm$ 0.1 x 10^{-4}) except for

fraction D. The protein of fraction D was nondispersible in dilute acetic acid and contained about 98% starch which also binds a small amount of dye (4.6 mg dye/g. of starch). Flours from Comanche, Rio, Elgin, and Golden varieties of wheat were found to give 84, 87, 91, and 91% dispersion of their total protein, respectively, in dilute acetic acid. It was concluded that the reaction of certain dyes with wheat protein could be a useful analytical tool for the quantitative estimation of the protein content of whole wheat and wheat flour.

Udy (1956) reported that the amount of dye bound per gm of sample could be used to provide an accurate estimate of protein content. This estimate was based on the concentration of unbound dye, as measured colorimetrically at a wavelength of 470 mu.

For the experimental conditions employed, the equation relating protein content, P, (N x 5.7), to the concentration of unbound dye, C, was $P = 44.47 - 50.00\ C$ for 128 samples of wheat representing more than 50 varieties containing from 6.2 to 16.0% protein. For 218 samples of straight grade flour containing from 4.6 to 15.2% protein, the equation was $P = 40.92 - 45.64\ C$.

Udy (1956) further stated that the correlation coefficients between bound dye and Kjeldahl protein content were 0.992 and 0.997 for wheat and flour respectively; the standard errors

of estimate of protein content from the concentrations of unbound dye were 0.22% for wheat and 0.20% for flour.

Starch and bran were also found to bind significent amounts of dye. Although the starch content was not accurately known for a given flour sample, an average value of 67% starch gave consistent results. A 10% fluctuation in this value was equivalent to less than 0.2 mg of bound dye, and the amount of dye bound by the protein when calculated as the differences between total dye bound and that bound by starch was still within the error of estimate.

Udy (1956) also reported that the broad absorption band of orange G solutions in the region of 485 mu were exceptionally stable, and that color intensities followed Beer's Law under these conditions.

Bautista, et al., (1961) conducted a comparative study of the use of amido black 10B, lissamine green SF 150 and orange G to estimate protein content of wheat and wheat flours. Amido black 10 B showed fewer differences than orange G in absorption by proteins of wheat and wheat flour. Lissamine green SF 150 gave the highest correlations with Kjeldahl protein and the same standard curve could be applicable to both wheat and wheat flour proteins. The deviations between different standard curves were well within the experimental error.

Bunyan (1954) investigated the orange G binding properties of a range of common protein foods with particular reference to the degree of variation to be found between samples of a particular type of protein meal. A series of meat meals and whalemeat meals showed good correlations between Kjeldahl protein and dye binding capacities except for a few completely atypical samples. Among fish, soybean and ground nut meals, no atypical samples were found and high correlations were found between the two methods.

The fact that none of the regression lines passed through the origin suggests that protein was not the only constituent binding the dye. Even so, orange G binding might be used to estimate the protein content of fish, soya and ground nut meals, were it not for the large variation between meals of the same type. In the meat meal and whale meat meal series, dye binding methods could supplement the crude protein determination by enabling the discovery of abnormal meals.

According to Bunyan (1959), the data from miscellaneous protein sources gives an idea of the values to be expected for each source and could be extended to cover a range of samples of each type of protein.

Miller (1961) reported preliminary data of Kjeldahl protein and % transmission of unbound dye for various feed ingredients. The feed ingredients analyzed included corn,

sorghum grain, oats, barley, wheat shorts and middlings, dehydrated alfalfa, cottonseed oil meal, soybean oil meal. In all instances, there was a linear relationship of Kjeldahl protein to % transmission obtained by dye binding. Corn and sorghum grain were the only ingredients of which a sufficient number of samples was available for analysis to permit calculation of a correlation with any degree of confidence.

The corn and sorghum grain samples were all analyzed for Kjeldahl protein and for dye absorption, using the Udy Analyzer and the Foss Prometer.

It was reported that in comparison of Kjeldahl protein and % transmission of the Udy Analyzer, corn gave a correlation coefficient of 0.84 and a standard deviation of 0.93%, while when Kjeldahl results were compared with those of the Foss Prometer gave a correlation coefficient of 0.86 and a standard deviation of 0.83% were obtained. When comparing the Kjeldahl and Udy Analyzer results for sorghum grain, the correlation coefficient was 0.968 with a variance of 1.67%. When comparing Kjeldahl with the Foss Prometer results, the correlation coefficient was 0.96 with a variance of 1.49%. Each set of data had different regression equations (Miller, 1962).

From the literature, one can conclude that dye binding techniques have been investigated with various protein sources and have demonstrated their adaptibility for analysis of protein content. There also appears to be a worldwide interest

in using dye binding techniques. There is little evidence nevertheless, of the use of dye binding techniques for the quantitative determination of the protein content of formula feeds. There have been investigations conducted, however, using dye binding techniques for the quantitative analysis of the protein content of materials which may be feed ingredients. This indicates that dye binding techniques could be useful in the evaluation of incoming ingredients and in in-process control of the protein level in formula feeds.

MATERIALS AND METHODS

Kjeldahl Protein Determination[1]

A slight modification of the AACC (1957) method was used for Kjeldahl protein determination. One gram of material (wet weight basis) was weighed into a 500 ml Kjeldahl flask and mixed well with 10 grams of a mixture of potassium and sodium sulfate (60:40), 0.5 g mercuric oxide and a small amount of pumice. The final mixture was then digested with 20 ml of concentrated sulfuric acid on a 500-watt burner until clear (30 to 45 minutes). The cooled digest was diluted with tap water to 250-300 ml. The solution was then made alkaline by addition of 50 ml of sodium hydroxide solution of 1.47 sp. gr., containing 100 g of sodium thiosulfate per liter, and immediately distilled in 50 ml of 0.1253 N sulfuric

1. In this manuscript, Kjeldahl protein refers to Kjeldahl nitrogen content of samples multiplied by the factor 6.25.

acid. When titrated[2] with 0.1253 N sodium hydroxide, one ml of sodium hydroxide was equivalent to 1% protein. An automatic burette was set up so that % protein could be read directly. This reading was corrected by a factor of (6.25 for feed materials) / (5.7 for wheat) in order to obtain % protein of feed materials.

Dye Absorption for Protein Determination

For the dye adsorption tests for quantitative protein estimation, all samples to be analyzed were finely ground in a Hobart mill.

The subsequent analysis were then made using equipment from the Udy Analyzer Company. The samples (weight dependent on the protein content) were weighed and placed in the reaction tube with 40 ml of the reagent dye solution. The combined sample and dye solution were reacted for three minutes on the reaction period. The solution was then transferred to a squeeze-type polyethylene bottle which had a fiber glass filter disc placed in the dropper cap. The filtrate was transferred dropwise into the special flow-through cuvette

2. A mixture of sodium alizarin sulfate and methylene blue was used as indicator.

of the colorimeter. The % transmission of the filtrate was read on the scale of the colorimeter, previously calibrated at 43% transmission with a reference dye solution which was made by mixing equal parts of the reagent dye solution and distilled water.

The reagent dye solution used was purchased from the Udy Analyzer Company and was acid orange 12 in a phosphate buffering system at pH 2.0. Udy (1963) now uses this dye which has only one sulfonic group in preference to two on orange G which has two. Theoretically, for a given amount of protein there would be twice as much dye absorbed when acid orange 12 is used and the analysis would thus be more sensitive to changes in protein.

The use of a small fiber glass filter disc which is completely inert to the dye solution avoids dye losses due to absorption by cellulose filters. The short-light-path flow-through cuvette makes it unnecessary to dilute the filtered dye solution before taking readings. In this method the amount of dye which is not bound by protein is measured.

Chick Starter Rations

The ingredients used for all experimental rations analyzed were obtained from the Kansas State University pilot feed mill. The experimental chick starter rations were formulated to total ten pounds and were mixed in the laboratory.

The formula for the experimental chick starter is shown in Table 1.

Each ration was designed to have a single variable as shown in Tables 7 and 8. The ingredients for each ration were weighed on a single pan, direct reading balance with scale readings to 5 grams and were mixed for five minutes in a twin-shell blender. Each ration was sampled three times and each sample was then analyzed for protein by both the Kjeldahl method and the dye absorption technique. A 500 mg sample was used for analysis with dye absorption and a 900 mg sample was used for the Kjeldahl protein analysis.

In Table 7, basal refers to a ration which includes the macro ingredients shown in Table 1, whereas in Table 8, basal refers to a ration which contains all the micro and macro ingredients. The Kjeldahl protein and % transmission are recorded as the mean of three samples of the same ration.

Turkey Prestarter, Starter and Finisher Rations

The formulae of the turkey prestarter, starter and finisher rations are given in Tables 2, 3, 4, respectively. The macro ingredients of each formula were weighed on a Richardson batch scale system and the micro ingredients were weighed on the single pan direct reading balance. The ingredients were mixed on a horizontal ribbon mixer for three minutes in batches of 500 pounds. The mixed feed was then transferred by a bucket elevator and screw conveyor to the bin above

the pellet mill. The feed was gravity fed into a variable speed screw feeder on the pellet mill. The mash samples were obtained during the pelleting operation at the top of the screw feeder, the pellet samples were obtained as the pellets were being sacked, following the cooling operation.

The sample size for the dye binding analyses was reduced to 300 mg instead of the 500 mg sample size used for the chick starter formula. The sample size for the Kjeldahl protein determination was reduced to 600 mg as compared to 900 mg for the chick starter ration.

All data presented in Tables 9, 10, and 11, are means of duplicate analyses of the samples. The data reported in Table 11, were obtained from samples of a turkey finisher formula. This formula was calculated to be an 18% protein ration, while the turkey prestarter and the turkey starter formulae were calculated to be 29 and 28% protein, respectively.

Reformulating Rations

Three rations were reformulated by using the protein value as determined by dye absorption of the major ingredients. The formula for the swine lactation ration used in this study is given in Table 5. The grain sorghum and soybean oil meal samples were analyzed for protein content by the dye binding techniques. The ration was then reformulated by use of the

protein values obtained from these analyses. A 15.5% protein ration was calculated. In the reformulation, 13 pounds of soybean oil meal in the original formula was replaced by sorghum grain. The feed was then produced by the same procedure as outlined for the turkey rations and the finished feed was sampled ten times during the sacking operation. Duplicate analyses were run on each sample by both the dye binding and Kjeldahl protein methods.

A poultry formula, Table 6, was also reformulated according to protein values obtained from the analysis of the corn, sorghum grain and soybean oil meal by the dye binding method. This ration was formulated to contain 16% protein, and to obtain this level, 8 pounds of soybean oil meal was replaced by 4 pounds of corn and 4 pounds of sorghum grain. This feed was produced, sampled and assayed as outlined above.

A chick starter formula, Table 7, was reformulated as previously outlined. The amounts of corn, sorghum grain and soybean oil meal were adjusted to obtain a 20% protein ration. This test was replicated at four times. The feed was produced and sampled as outlined previously. Duplicate analyses were run on each sample for Kjeldahl protein content.

DISCUSSION AND RESULTS

Omitting or Doubling Ingredients

The experiment as designed in Table 8 had several objectives. The basal ration contained 3% fish meal, 3% dehydrated alfalfa meal, 2% distiller's dried solubles, equal amounts of

corn and sorghum grains, and enough soybean oil meal to make a 21% protein formula.

It was anticipated that dye binding might detect errors, such as omission of an ingredient or over-addition of an ingredient in a ration. Therefore, one might consider the use of dye binding techniques as an in-process quality control measure.

Different readings were obtained, Table 8, when ingredients such as soybean oil meal or fish meal were changed in amount. The readings changed significantly, becoming higher or lower, but the cause remains unexplained. These ingredients were the major protein sources. Doubling or omitting the dehydrated alfalfa or distiller's dried solubles was not detected in the readings. This can be explained by the protein content of these ingredients being very near the protein content of the final ration. Also these ingredients were added in small amounts and therefore contributed only a small amount of protein to the final formula.

Addition of Micro Ingredients

Another part of this experiment was to determine the effect of the addition of the micro-ingredients to the basal ration. The analyses by dye binding indicated less deviation from the basal than did the Kjeldahl protein. The addition of premix B and trace minerals caused an increase in Kjeldahl protein values. The premix in this case could be a source of additional nitrogen and the trace minerals may have acted

as catalysts. The addition of the micro ingredients had no obvious effect on dye absorption (% transmittance).

Substitution of Ingredients

Another phase of this study was the substitution of sorghum grain for corn, or visa versa, in the ration. When the grain source was 100 or 75% corn and 0 or 25% sorghum grain, respectively, the % transmittance reading was 0.8 units higher than that for the basal, Table 8, which contained 50% corn and 50% sorghum grain. When corn provided 0 or 25% and sorghum grain provided 100 or 75%, respectively, the % transmittance reading was 3.2 units higher and 3.4 units lower than the basal. The Kjeldahl protein content remained at 21% during these comparisons. Therefore, a change in the ratio of the grains in a ration may cause the % transmittance also to be altered, due to differences in dye absorption by the different grains. These results illustrate a limitation of the use of dye binding techniques as an in-process quality control analysis for formula feeds.

The results of a second study, shown in Table 9, did not substantiate the differences obtained in Kjeldahl protein by the addition of the trace minerals. The amount of trace minerals was doubled, but no differences in Kjeldahl protein were evident. Also, as found previously, the addition of the micro ingredients did not affect dye absorption.

As the soybean meal was replaced by fish meal in the

ration, the % transmission decreased and the Kjeldahl protein remained the same. The same was true when soybean oil meal was replaced by meat and bone meal. This also indicates differences in dye binding by these protein sources and a limitation of the dye binding technique for in-process qualit control estimations of protein content.

The analysis of the laboratory rations did indicate, however, that dye binding could be useful for protein estimations with the limitations noted previously.

Dye Binding of Pelleted Formulae

At this time, turkey rations were being produced in the pilot feed mill and were available for further studies of this technique. Samples were obtained from each of 16 batches of turkey prestarter which were manufactured, Table 10. Each batch was assumed to be a uniform, homogeneous mixture from the same bulk ingredients. In addition to determining the usefulness of dye binding for in-process protein analysis, samples were collected of mash and pellets to determine the possible effect of pelleting on the amount of dye bound by the final formula. The correlation coefficient between Kjeldahl protein and % transmission for mash was 0.82 as compared to 0.84 for pellets. The standard error estimate for the mash was 0.31 as compared to 0.30 for the pellets. The results of this study did not indicate significant differences

between dye binding and Kjeldanl protein of the mash and pelleted samples. These studies were continued on a turkey starter ration, Tables 3 and 11. The procedure remained the same except that three different conditioning temperature rises were randomly assigned to a total of 75 batches of the starter formula. Therefore, each temperature was tested on 25 batches of the turkey starter formula. The temperature of the unconditioned mash was 26°C and the conditioning temperature rises used were 16°, 32°, and 48° C. The temperature rises were attained by adding steam in the conditioning chamber of the pellet mill before pelleting.

The data obtained, Table 11, indicate there may be a temperature effect on dye binding. The higher the temperature in this study, the lower the correlation of dye binding to Kjeldahl protein. Also, the unconditioned mash had the least standard error of estimate.

The analyses of the mash samples had a wider distribution than did those of the pellets. This could have been due to errors in sampling or the pellets could have been a more homogeneous mixture.

Sampling Locations at the Pellet Mill

Another experiment was initiated to determine the effect of sampling location on the homogeneity of the feed as determined by crude protein analysis. Each batch of feed was sampled four times at the pellet mill as follows: Top of the

auger, end of the auger, end of the conditioning chamber, and as the pellets left the pellet mill. These samples were taken simultaneously so as to sample the same portion of each batch. A total of ten batches was sampled and these samples were analyzed for Kjeldahl protein, Table 13. The analyzed data indicate very little difference due to sampling location.

The mean of the analysis of samples taken at the top of the auger and the mean of the analysis of samples of pelleted feed were the same; the standard deviation was less for mash taken at the top of the auger than for the pellets. These data do not indicate that there was a mixing of the ingredients during the conditioning and pelleting operation.

Recalculation of Formulae

The major ingredients of a swine lactation formulae were analyzed for protein content by use of dye binding techniques and the formula was recalculated using these values. The protein content of the sorghum grain was found to be 10.0% as compared to 9.0% used in the original calculation. The soybean oil meal contained an average protein content of 44.0%. In recalculating the formula, the sorghum grain content of the formula was increased from 72.4 to 75.0% of the ration and the soybean meal was decreased from 16.6 to 14% of the formula. The ingredients were weighed, mixed and pelleted as previously outlined. The final pelleted formula (1 ton) was sampled ten times during sacking.

The samples taken during sacking were analyzed for Kjeldahl protein content and by the dye binding method. The Kjeldahl protein mean was 15.9% with a standard deviation of 0.41% compared to a calculated protein content of 15.5%. The per cent transmission mean was 31.8 with a standard deviation of 0.93%. The correlation coefficient, comparing Kjeldahl protein and dye binding, was 0.98 and the standard error of estimate was 0.68. The 15.9% protein content was attained with less soybean oil meal than was previously calculated due to a higher level of protein in the sorghum grain.

In this ton of feed, 52 pounds of soybean oil meal were replaced by 52 pounds of sorghum grain. If the cost of soybean oil meal was $0.04 per pound and that of sorghum grain was $0.018 per pound, the change in ingredients would lower the ingredient cost by $1.14 per ton.

This study was continued on a poultry layer and breeder formula which contained more high protein ingredients than the swine formula. The sorghum grain, corn, and soybean oil meal were analyzed. The corn and sorghum grain contained 10.0% protein while the soybean oil meal contained 44.5% protein. The sorghum grain content was increased from 30.0 to 30.8% of the formula, the corn content was increased from 35.0 to 35.8% of the formula, and the soybean oil meal was decreased from 11.6 to 10% of the formula. This formula (2 tons) was

sampled 20 times, during the sacking operation.

The samples taken during sacking were analyzed by the Kjeldahl method and by the dye binding method. The Kjeldahl protein mean was 16.7% with a standard deviation of 0.44%. The % transmission mean was 31.91 with a standard deviation of 0.70. The correlation coefficient between Kjeldahl protein and dye binding was 0.88 and the standard error of estimate was 0.34. The 16.7% protein content was attained with less soybean oil meal than was previously calculated because both the corn and sorghum grain furnished more protein than the previously calculated amounts.

In these two tons of feed, 64 pounds of soybean oil meal were replaced by 32 pounds of sorghum grain and 32 pounds of corn. If the cost of the soybean oil meal was $0.04 per pound as compared to $0.018 for sorghum grain and $0.021 for corn, there would have been a total of $0.65 saved per ton on this formula.

These results show that if the protein content of the feed ingredients are deviating from the average value, then an in-process protein analysis would help control the protein content and quality of the formula feed and might also save the manufacturer money in the manufacturing of formula feeds.

The major ingredients of a chick starter formula were analyzed for protein content by use of dye binding techniques and the formula was recalculated uring these values. This procedure was replicated four times at approximately 10 day intervals. The formula was mixed, conveyed, pelleted, cooled, and crumblized in each test. The feed was sampled every 4th 50 lb. sack during sacking for a total of ten samples per ton.

The samples were ground and duplicate analyses were run for Kjeldahl protein. The mean (u) and standard deviation (s) were calculated by use of the average of the duplicate analyses. The means, Table 16, of the first two tests were very near the calculated protein content as determined by dye binding techniques, while the means of the last two tests deviated from the calculated protein content. The protein content of the soybean meal as determined by dye binding techniques was below the expected protein content of soybean meal. This low protein content may be attributed to several causes, such as a heterogenous sample of the bulk material, or experimental error in the dye binding analysis used for determining the protein content.

The standard deviation (s) of the tests ranged from .45 to 1.12% protein with an average (s) of .78%. This indicates that if a feed manufacturer is to guarantee a formula to contain 20% protein, then one should calculate it to contain

20.8% protein. This will assure the manufacturer that he will maintain 20% or more protein in his feed, 70% of the time.

SUMMARY AND CONCLUSIONS

The use of dye binding techniques for the quantitative estimation of protein in formula feeds has been found to be applicable with a few limitations. When bulk ingredients are changed in a formula a new transmittancy curve must be calibrated in order to be able to use dye binding techniques for analysis of formula feeds. Ingredients of the same protein content have been shown to have different dye binding capacities. For example, a sample of 8.5% protein corn has a higher dye binding capacity than does a sample of 8.5% protein sorghum grain. Also as the high protein ingredients of the ration are substituted by other high protein ingredients the dye binding capacity of the formula feed may be altered.

The addition of micro ingredients, such as salt, ground limestone, dicalcium phosphate, trace minerals, and vitamin drug premix did not affect the dye binding capacities of the formula feeds in these studies.

The dye binding technique can detect certain changes in a ration such as leaving out an ingredient or putting in twice the amount if this causes a change in protein content. It cannot identify the source of the error. The Kjeldahl protein analysis also has the limitation, that it will give the total nitrogen content of a sample, but not the source of nitrogen.

The addition of heat during pelleting appears to have an effect on the total dye binding capacity of a formula feed. The effect might be attributed to denaturation of the protein or to other chemical or physical factors.

Analysis of samples taken at different locations at the pellet mill did not indicate a mixing effect from the pelleting operation.

Dye binding analysis of protein content of major ingredients was used to recalculate various formulae. Reformulation based on the protein content of the ingredients resulted in a final protein content close to the specified level and demonstrated the application of dye binding as an in-process quality control method for the controlling protein content.

SUGGESTIONS FOR FUTURE WORK

Further research needs to be conducted using dye binding techniques as a quantitative estimation of formula feed proteins.

Other work needs to be conducted to investigate the effect of heat treatment during pelleting on dye binding capacities of formula feeds. This could lead to new knowledge on changes in formula feeds during processing.

A transmittancy curve needs to be developed for a formula feed and then analyses should be obtained from the production of this formula feed and an evaluation made of the feasibility of dye binding as a quality control method for the protein

content of formula feeds. Kjeldahl protein values would need to be obtained until confidence had been established in dye binding techniques.

Research needs to be designed to obtain dye binding capacities for each feed ingredient that could be written into an equation. By using this equation and the amounts of ingredients added to a formula feed, one might calculate its potential dye binding capacities; this would contribute to the application of dye binding techniques for determining protein content of formula feeds.

A study should be designed to check the sample size, particle size of sample and reacting time of reagent dye solution with formula feed samples to obtain optimum dye binding values. From such a study an established rule might be developed so that if the protein content of a formula feed was increased, changes could be made in the sample size or the reactant time.

Studies designed to investigate the use of other dyes for their capacity to react with protein or amino acids of feed ingredients and formula feeds would be of value.

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APPENDIX

Table 1. Chick Starter Formula (21% protein)

Ingredient	Protein Value	Pounds
	%	
Macro:		
Ground yellow corn	9.3	30.0
Ground sorghum grain	8.4	30.0
Soybean oil meal	45.7	27.0
Dehydrated alfalfa	16.8	5.0
Fishmeal, menhaden	60.3	3.0
Distiller's dried solubles	26.9	2.0
Micro:		
Ground limestone		1.5
Dicalcium phosphate		1.0
Salt		0.5
Trace minerals[1]		0.05
Vitamin and drug premix[2]		1.0
		100.05

[1]Trace mineral mix was guaranteed to contain a minimum of 10% manganese, 10% iron, 12% calcium, 1% copper, 5% zinc, 0.3% iodine and 0.1% cobalt.

[2]Vitamin and drug premix supplied the following activity per pound of feed: vitamin D_3, 750 ICU; vitamin A, 1000 IU; niacin, 3 mg; riboflavin, 1 mg; calcium pantothenate, 2 mg; choline chloride, 10 mg; vitamin B_{12}, 3 µg; methionine, 230 mg; chlorotetracycline, 2.5 mg; bacitracin, 2.5 mg; Bifuran®, 227 mg.

Table 2. Turkey Prestarter Formula (29% protein)

Ingredient	Protein Value	Pounds
	%	
Ground yellow corn	9.0	15.0
Ground sorghum grain	8.0	14.5
Soybean oil meal	45.0	44.0
Dehydrated alfalfa	17.0	5.0
Fish meal, menhaden	60.0	5.0
Meat and bone scraps	50.0	5.0
Distiller's dried solubles	26.0	2.0
Dried whey	12.0	2.0
Animal fat		4.0
Salt		0.5
Trace minerals[1]		0.05
Dicalcium phosphate		2.0
Vitamin and drug premix[2]		1.0
Total		100.05

[1]Trace mineral mix was guaranteed to contain a minimum of 10% manganese, 10% iron, 12% calcium, 1% copper, 5% zinc, 0.3% iodine and 0.1% cobalt.

[2]Vitamin and drug premix supplied the following activity per pound of feed: vitamin D_3, 750 ICU; vitamin A, 2000 IU; niacin, 6 mg; riboflavin, 2 mg; calcium pantothenate, 4 mg; choline chloride, 220 mg; vitamin B_{12}, 6 µg; methionine, 227 mg; aureomycin, 2.5 mg; bacitracin, 2.5 mg; Amprol®, 25%, 227 mg; Histostat-50®, 227 mg; Santoquin®, 57 mg.

Table 3. Turkey Starter Formula (28% protein)

Ingredient	Protein Value	Pounds
	%	
Ground yellow corn	9.0	19.5
Ground sorghum grain	8.0	19.5
Soybean oil meal	45.0	41.5
Dehydrated alfalfa	17.0	5.0
Fish meal, menhaden	60.0	4.0
Meat and bone meal scraps	50.0	5.0
Distiller's dried solubles	26.0	2.0
Salt		0.5
Trace minerals[1]		0.05
Dicalcium phosphate		2.0
Vitamin and drug premix[2]		1.0
Total		100.05

[1]Trace mineral mix was guaranteed to contain a minimum of 10% manganese, 10% iron, 12% calcium, 1% copper, 5% zinc, 0.3% iodine, and 0.1% cobalt.

[2]Vitamin and drug premix supplied the following activity per pound of feed: vitamin D_3, 750 ICU; vitamin A, 2000 IU; niacin, 6 mg; riboflavin, 2 mg; calcium pantothenate, 4 mg; vitamin B_{12}, 6 µg; choline chloride, 220 mg; bacitracin, 2.5 mg; Amprol© 25%, 227 mg; Histostat©50, 227 mg; Santoquin©, 57 mg.

Table 4. Turkey Finisher Formula (18% protein)

Ingredient	Protein Value	Pounds
	%	
Ground yellow corn	9.0	34.5
Ground sorghum grain	8.0	34.5
Soybean oil meal	45.0	17.0
Dehydrated alfalfa	17.0	4.0
Meat and bone scraps	50.0	4.0
Distiller's dried solubles	26.0	2.0
Dicalcium phosphate		2.0
Ground limestone		0.5
Salt		0.5
Trace minerals[1]		0.05
Vitamin and drug premix[2]		1.0
Total		100.05

[1]Trace mineral mix was guaranteed to contain a minimum of 10% manganese, 10% iron, 12% calcium, 1% copper, 5% zinc, 0.3% iodine, and 9.1% cobalt.

[2]Vitamin and drug premix supplied the following activity per pound of feed: vitamin D_3, 750 ICU; vitamin A, 1500 IC; niacin, 3 mg; riboflavin, 1 mg; calcium pantothenate, 2 mg; choline chloride, 10 mg; vitamin B_{12}, 6 µg; methionine, 227 mg; aureomycin, 2.5 mg; bacitracin, 2.5 mg; Histostat-50®, 227 mg.

Table 5. Swine Lactation Formula (15.5% protein)

Ingredient	Protein Value	Pounds
	%	
Ground sorghum grain	10.0	75.0
Soybean oil meal	45.0	14.0
Dehydrated alfalfa	17.0	7.6
Dicalcium phosphate		1.0
Ground limestone		1.0
Salt		0.5
Trace minerals[1]		0.05
Vitamin and drug premix[2]		1.0
Total		100.15

[1]Trace mineral mix was guaranteed to contain a minimum of 10% manganese, 10% iron, 12% calcium, 1% copper, 5% zinc, 0.3% iodine, and 0.1% cobalt.

[2]Vitamin and drug premix supplied the following activity per pound of feed: vitamin D_3, 150 ICU; vitamin A, 1000 IU; niacin, 3 mg; riboflavin, 1 mg; calcium pantothenate, 2 mg; choline chloride, 10 mg; aureomycin, 4.5 mg; vitamin B_{12}, 4.5 µg; furazolidone, 57 mg.

Table 6. A P Layer and Breeder Formula (16% protein)

Ingredient	Protein Value	Pounds
	%	
Ground yellow corn	10.0	35.8
Ground sorghum grain	10.0	30.8
Soybean oil meal	44.5	10.0
Wheat middlings	15.0	9.0
Meat and bone scraps	50.0	2.0
Fish meal, menhaden	60.0	2.0
Distiller's dried solubles	26.0	2.0
Ground limestone		5.0
Dicalcium phosphate		1.0
Salt		0.5
Trace minerals[1]		0.05
Vitamin and drug premix[2]		1.0
Total		100.15

[1]Trace mineral mix was guaranteed to contain a minimum of 10% manganese, 10% iron, 12% calcium, 1% copper, 5% zinc, 0.3% iodine, and 0.1% cobalt.

[2]Vitamin and drug premix supplied the following activity per pound of feed: vitamin D_3, 750 ICU; vitamin A, 3000 IU; niacin, 3 mg; riboflavin, 1 mg; calcium pantothenate, 2 mg; choline chloride, 125 mg; methionine, 230 mg; aureomycin, 4.5 mg; vitamin B_{12}, 4.5 µg; furazolidone, 114 mg.

Table 7. Chick Starter Formula (20% protein)

Ingredient	Protein Value	Pounds
	%	
Ground yellow corn	Varied[1]	Varied[1]
Ground sorghum grain	Varied[1]	Varied[1]
Soybean oil meal	Varied[1]	Varied[1]
Dehydrated alfalfa	17.0	5.0
Fish meal, menhaden	60.0	3.0
Distiller's dried solubles	25.0	2.0
Ground limestone		2.5
Dicalcium phosphate		1.0
Salt		0.5
Trace minerals[2]		.05
Vitamin and drug premix[3]		1.0
Total		100.05

[1]The protein content of these ingredients was determined by dye-binding techniques and then calculated the amounts needed to obtain a 20% protein formula.

[2]Trace mineral mix was guaranteed to contain a minimum of 10% manganese, 10% iron, 12% calcium, 1% copper, 5% zinc, 0.3% iodine, and 0.1% cobalt.

[3]Vitamin and drug premix supplied the following activity per pound of food: vitamin D_3, 750 ICU; vitamin A, 1000 IU; riboflavin, 2 mg; calcium pantothenate, 4 mg; choline chloride, 20 mg; vitamin B_{12}, 2.5 µg; methionine, 57.5 mg; chlorotetracycline, 2.5 mg; bacitracin, 2.5 mg; Amprol®, 25%, 57.5 mg.

Table 8. Results of Analysis of a Chick Starter Ration by Kjeldahl and Dye Binding Methods.

Test	Variable[1]	Mean Kjeldahl Protein	Mean Transmittance
		%	%
1.	Basal (macro ingredients)	20.9	47.7
2.	- dehydrated alfalfa	21.2	47.8
3.	- fish meal	20.3	44.0
4.	- distiller's dried solubles	21.0	46.3
5.	- soybean oil meal	12.0	24.9
6.	- corn	26.5	65.8
7.	- sorghum grain	26.6	68.8
8.	25% soybean oil meal	14.6	20.0
9.	50% soybean oil meal	17.1	34.9
10.	75% soybean oil meal	19.5	40.9
11.	125% soybean oil meal	22.2	51.1
12.	150% soybean oil meal	23.6	57.7
13.	Double dehydrated alfalfa	20.8	46.2
14.	Double fish meal	22.4	49.9
15.	Double distiller's dried solubles	21.2	46.4
16.	100% corn	21.1	48.5
17.	75% corn, 25% sorghum grain	21.1	48.5
18.	25% corn, 75% sorghum grain	20.9	45.3
19.	100% sorghum grain	21.0	45.1
20.	Added ground limestone	20.6	47.4
21.	Added dicalcium phosphate	20.7	47.2
22.	Added salt	21.1	47.2
23.	Added trace minerals	21.7	48.0
24.	Added premix B	21.5	48.2

[1] Variable in each case indicates the specific change made from the basal ration.

Table 9. Results of Analysis of a Chick Starter Ration by Kjeldahl and Dye Binding Methods.

Test	Variable[1]	Mean Kjeldahl Protein	Mean Transmittance
		%	%
1.	Basal (complete formula)	20.6	47.0
2.	-limestone	21.6	48.8
3.	-decalcium phosphate	21.5	50.2
4.	-salt	20.9	49.0
5.	-premix B	21.4	52.3
6.	-trace mineral	20.9	49.0
7.	-double trace minerals	21.3	50.3
	Major protein sources:[2]		
8.	100% soybean oil meal	21.5	51.5
9.	75% soybean oil meal 25% fish meal	22.0	52.5
10.	50% soybean oil meal 50% fish meal	21.3	47.8
11.	25% soybean oil meal 75% fish meal	21.5	45.8
12.	100% fish meal	21.1	42.5
13.	75% soybean oil meal 25% meat and bone meal	21.4	52.7
14.	50% soybean oil meal 50% meat and bone meal	20.8	48.8
15.	25% soybean oil meal 75% meat and bone meal	20.5	45.3
16.	100% meat and bone meal	19.8	46.7

[1] Variable in each case indicates the specific change made from the basal ration.

[2] Major protein source supplied a designated amount of protein to maintain the specified % protein of the formula and in the following the % of this amount of protein was supplied by the designated sources of protein.

Table 10. Results of Analysis of a Turkey Prestarter Ration by Kjeldahl and Dye Binding Methods.

Test	Mash		Pellets	
	Mean Kjeldahl Protein	Mean Transmittance	Mean Kjeldahl Protein	Mean Transmittance
	%	%	%	%
1.	29.33	40.00	28.96	39.75
2.	29.33	38.50	28.69	37.75
3.	29.84	42.00	29.46	40.00
4.	29.15	39.75	29.05	38.25
5.	28.83	38.00	28.60	36.75
6.	29.24	38.75	28.41	37.50
7.	30.98	41.75	28.05	37.25
8.	28.96	39.50	28.32	38.00
9.	28.60	38.25	29.51	37.00
10.	29.64	41.75	30.97	41.50
11.	30.05	39.75	29.10	37.50
12.	30.70	42.00	30.15	41.00
13.	30.15	41.50	29.88	41.00
14.	29.15	39.25	28.32	37.00
15.	29.15	39.00	29.43	39.50
16.	29.05	38.75	28.33	38.25
Mean	$u=$ 29.50	$u=$ 39.96	$u=$ 29.08	$u=$ 38.60
Standard Dev.	$s=$ 0.45	$s=$ 1.88	$s=$ 0.62	$s=$ 2.58
Correlation Coefficient		$r=$ 0.82	$r=$ 0.84	
Standard error of est.		$s_{b}=$ 0.31	$s_{b}=$ 0.30	

Table 11 a. Results of Analysis of a Turkey Starter Ration by Kjeldahl and Dye Binding Methods at a 16°C Temperature Rise.

Test	Mash		Pellets	
	Mean Kjeldahl Protein X %	Mean Transmittance Y %	Mean Kjeldahl Protein X %	Mean Transmittance Y %
1.	25.95	35.50	28.30	38.75
5.	29.10	40.00	28.00	38.75
6.	27.70	39.00	27.80	38.50
7.	26.70	37.00	27.00	36.75
15.	28.00	38.00	29.80	40.25
16.	30.20	38.50	29.10	38.25
20.	28.90	39.00	27.20	36.25
26.	32.80	45.25	29.00	40.25
27.	32.10	48.75	28.80	40.50
29.	29.30	42.00	28.90	40.00
33.	29.40	41.75	27.70	39.00
34.	31.20	44.25	28.20	38.25
35.	27.80	37.50	26.10	37.50
42.	29.60	42.25	28.00	39.50
43.	19.00	26.50	26.90	36.50
47.	32.20	47.25	29.20	40.50
49.	28.90	39.75	28.70	40.25
50.	30.00	43.50	28.70	40.25
54.	26.10	36.50	27.50	38.25
58.	26.80	37.50	28.00	39.00
59.	24.80	33.40	26.20	35.00
61.	27.80	39.00	27.70	38.75
71.	31.40	44.75	28.90	41.00
73.	31.00	37.00	29.10	40.75
75.	31.10	44.25	29.00	39.25
Mean:	u= 28.67	u= 40.44	u= 28.22	u= 38.89
Standard Dev.:	s= 3.03	s= 5.06	s= 0.83	s= 1.67

Table 11b. Results of Analysis of a Turkey Starter Ration by Kjeldahl and Dye Binding Methods at a 32°C Temperature Rise.

Test	Mash		Pellets	
	Mean Kjeldahl Protein X %	Mean Transmittance Y %	Mean Kjeldahl Protein X %	Mean Transmittance Y %
2.	26.70	37.00	27.80	37.75
4.	28.50	40.00	27.80	37.00
10.	27.00	37.00	28.00	38.00
12.	27.40	38.00	27.80	38.40
14.	28.10	39.00	29.20	38.75
18.	28.00	41.50	28.30	39.00
19.	26.90	38.25	28.00	37.50
23.	31.30	48.50	29.60	41.75
28.	30.50	44.50	27.60	38.00
30.	30.40	45.00	28.90	40.25
31.	29.40	40.75	27.50	36.75
36.	26.10	36.50	26.00	35.25
39.	28.90	39.50	28.10	38.00
40.	24.90	35.00	28.00	39.50
41.	30.90	44.75	27.80	39.00
48.	31.80	43.00	28.00	38.95
51.	31.20	46.50	27.40	38.75
55.	31.60	51.00	29.10	41.00
57.	32.00	52.00	29.20	42.25
62.	29.30	41.75	28.10	37.00
64.	30.40	42.75	27.90	37.75
65.	30.60	45.25	28.20	39.10
67.	29.90	41.75	28.10	39.00
69.	31.60	45.25	27.60	36.50
72.	30.70	44.75	27.90	39.75
Mean	$\bar{x}$= 29.36	$\bar{y}$= 42.37	$\bar{x}$= 28.07	$\bar{y}$= 38.60
Standard Dev.	s= 2.01	s= 4.44	s= 0.73	s= 1.62

Table 11c. Results of Analysis of a Turkey Starter Ration by Kjeldahl and Dye Binding Methods at a 48°C Temperature Rise

Test	Mash		Pellets	
	Mean Kjeldahl Protein X %	Mean Transmittance Y %	Mean Kjeldahl Protein X %	Mean Transmittance Y %
3.	26.90	37.50	27.10	37.75
8.	27.30	37.50	27.20	35.00
9.	27.60	39.10	27.40	37.25
11.	29.80	40.75	27.80	37.25
13.	27.40	38.25	26.10	38.50
17.	29.20	42.00	29.40	41.00
21.	30.10	41.75	28.50	38.00
22.	33.90	51.75	30.60	43.25
24.	28.00	39.25	28.50	38.75
25.	32.70	49.25	27.30	38.00
32.	31.00	45.25	27.00	38.50
37.	28.70	39.50	27.70	36.75
38.	31.70	44.75	28.30	38.00
44.	27.60	38.10	27.20	38.25
45.	30.30	40.50	28.50	39.50
46.	30.90	44.50	28.90	41.00
52.	28.70	40.25	28.70	39.50
55.	28.70	44.75	28.30	40.00
56.	31.80	48.75	28.30	38.75
60.	31.10	46.50	28.20	39.00
63.	28.90	42.50	28.40	39.00
66.	30.00	44.75	27.90	37.25
68.	30.50	44.75	28.40	39.75
70.	29.80	43.25	28.30	29.75
74.	29.00	40.60	28.10	38.25
Mean:	$\bar{X}$= 29.66	$\bar{Y}$= 42.63	$\bar{X}$= 29.08	$\bar{Y}$= 38.68
Standard Dev.:	s= 1.85	s= 3.84	s= 0.89	s= 1.66

Table 12. Correlation Coefficients of Kjeldahl and Dye Binding Methods of Turkey Starter Formula.

Treatment	Correlation Coefficient	Standard Error of Estimate
Unconditioned Mash	0.9280	0.2283
16° rise, pelleted	0.8595	0.3882
32° rise, pelleted	0.8153	0.4574
48° rise, pelleted	0.7582	0.3852

Sample Regression Equations	
Unconditioned Mash	$Y = 1.810X - 11.08$
16° rise, pelleted	$Y = 1.623X - 3.51$
32° rise, pelleted	$Y = 1.815X - 14.35$
48° rise, pelleted	$Y = 1.415X - 1.06$

Table 13. Results of Analysis of Samples for Kjeldahl Protein Taken from a Turkey Finisher Formula at Four Different Sampling Locations in the Pellet Mill.

Test	Top of Auger	End of Auger	Conditioned Mash	Pellets
	%	%	%	%
1.	17.8	18.4	18.9	17.9
2.	17.7	17.8	18.8	17.7
3.	18.5	19.0	18.8	18.0
4.	18.3	17.6	17.7	18.3
5.	18.3	17.0	18.8	17.7
6.	18.1	18.5	17.5	18.6
7.	18.1	18.4	18.9	17.9
8.	18.2	18.8	18.5	18.4
9.	17.7	18.3	18.0	17.5
10.	17.5	18.5	19.4	17.7
Mean:	$u = 18.02$	$u = 18.23$	$u = 18.53$	$u = 17.96$
Standard Deviation:	$s = 0.33$	$s = 0.57$	$s = 0.60$	$s = 0.44$

Table 14. Results of Kjeldahl Protein as Compared to Dye Binding Techniques for Protein Analysis of Swine Lactation Formula.

Test	Kjeldahl Protein	Transmittance
	%	%
1.	16.10	32.40
2.	15.90	31.40
3.	15.87	31.00
4.	15.60	31.25
5.	16.60	33.25
6.	16.00	31.75
7.	15.79	31.40
8.	15.79	31.20
9.	15.46	30.90
10.	16.67	33.50
Mean:	$u = 15.90$	$u = 31.80$
Standard Deviation:	$s = 0.41$	$s = 0.93$

Correlation Coefficient $r = 0.98$

Standard Error of Estimate $s_b = 0.68$

Table 15. Results of Kjeldahl Protein as Compared to Dye Binding Techniques for Protein Analysis of A P Layer and Breeder Formula.

Test	Kjeldahl Protein %	Transmittance %
1.	16.17	30.60
2.	16.78	32.25
3.	17.05	32.00
4.	16.83	32.25
5.	16.67	32.15
6.	15.90	30.75
7.	17.16	32.25
8.	15.83	31.90
9.	17.32	32.90
10.	16.17	30.40
11.	17.32	32.90
12.	16.67	30.60
13.	16.72	31.90
14.	17.54	33.20
15.	16.72	32.10
16.	16.72	32.00
17.	15.90	31.30
18.	16.72	32.20
19.	16.78	32.00
20.	16.45	32.00
Mean:	$u = 16.72$	$u = 31.91$
Standard Deviation:	$s = 0.44$	$s = 0.70$

Correlation Coefficient: $r = 0.88$
Standard Error of Estimate: $s_b = 0.34$

Table 16. Results of Kjeldahl Protein Analysis of Chick Starter Formula.

Test	(Ton basis)	Number of Samples	Calculated Protein Content		Protein Analyses	
			Original	Adjusted	Mean Average	Standard Deviation
			%	%	%	%
1	24 lbs. of soybean meal was replaced with 12 lbs. of corn and 12 lbs. of sorghum grain.	20	20.42	20.04	20.20	0.89
2a[1]	No treatment	10	21.18	-----	21.47	0.45
2b[1]	72 lbs. of soybean meal was replaced with 36 lbs. of corn and 36 lbs. of sorghum grain.	10	-----	20.03	19.97	0.79
3[2]	No treatment	10	20.01	-----	20.59	1.13
4[2]	No treatment	20	20.05	-----	20.87	0.64

[1]The original formula was used without adjustment and in test 2b the formula was calculated to contain 20% protein according to protein content as determined by dye binding techniques.

[2]The protein content determined by dye binding analyses of the major ingredients did not vary from the calculated protein content.

IN-PROCESS CONTROL OF PROTEIN LEVELS IN FORMULA FEEDS

by

KERMIT EDWARD ADELGREN

B.S., Kansas State University, 1956

AN ABSTRACT OF A MASTER'S THESIS

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KANSAS STATE UNIVERSITY
Manhattan, Kansas

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ABSTRACT

This research was planned to investigate the feasibility of adapting dye binding techniques to in-process protein analysis of formula feeds.

A group of rations was formulated, each containing a different variable. These rations were analyzed for Kjeldahl protein and dye binding. Different ingredients of the same crude protein content were found to have different dye binding capacities. Thus a new curve would have to be established for a formula feed, if the ingredients were altered greatly from the original formula, in order to use dye binding for determining the final protein content.

The addition of micro ingredients, such as salt, ground limestone, dicalcium phosphate, trace minerals and vitamin drug premix, did not affect the dye binding capacities of the formula feeds.

The dye binding technique will detect a change in a ration, such as leaving out an ingredient or putting in twice the amount, if it alters total protein content, but it cannot identify the source of the error. The Kjeldahl protein analysis has the same limitation, as it will give a total nitrogen content of a sample, but not the source of the nitrogen.

Studies were also conducted on complete rations in the pilot feed mill. Turkey prestarter and starter formulae

were available for these tests. The turkey starter rations had heat treatments involving 16°, 32°, and 48°C temperature rises. The results of these tests indicate that there may be a temperature effect on dye binding. As the temperature of conditioning and pelleting increased, the correlation between Kjeldahl protein and dye binding decreased. The highest correlation between Kjeldahl and dye binding was found for the mash.

The analysis of samples taken from various locations at the pellet mill gave no indication that there was a mixing effect at the pellet mill.

The major ingredients of two formulae were analyzed (by dye binding techniques) and the formulas were reformulated to the designed protein content according to protein content of these ingredients. The protein of the grain used in these formulae was higher than the average value.

Therefore, grain could replace part of the soybean oil meal in these formulae without changing the % protein of the formulae. Both formulae were thus produced at a savings as compared with use of average values of protein content. Also the protein content of the final feed was nearer the amount specified by the formula. This demonstrates the feasibility of dye binding for in-process quality control analysis of protein content.

The dye binding analysis of protein content of major ingredients was used for four tests to calculate a chick starter formula. The standard deviation of the tests indicates that if a feed manufacturer desires to maintain a 20% protein formula 70% of the time, one should calculate the formula to contain 20.8% protein.

The use of the dye binding technique for the protein determination of feed ingredients and of formula feeds has certain limitations. But in spite of these limitations, the dye binding technique offers several possibilities for quick in-process protein analysis of formula feeds and of feed ingredients.