PROTEIN EVALUATION OF PEANUT AND SESAME MEALS FOR THE CHICK

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PROTEIN IN PEANUT AND SESAME

AYYASH

AN ABSTRACT FOR THE THESIS OF

Bahij Ayyash for M.S. in Poultry Nutrition.

Title: Protein evaluation of peanut and sesame meals for the chick.

Peanut and sesame meals have a great potential in reducing the costs of poultry production due to their availability on the market and low price, compared with soybean meal. In attempting to evaluate these meals as protein supplements for the chick, different bioassays and chemical assays were used. The gross protein value of 4 peanut and 2 sesame meal consignments, and their net protein utilization (operative) values using the rat were determined.

The gross protein value as a growth method and net protein utilization as a nitrogen retention method give a fair indication of protein quality. The protein efficiency ratio of 2 sesame meals was also determined and possible toxicity in one of them investigated by the method of Campbell (1963).

The chemical assays consisted of proximate analysis, Orange G. binding capacity, determination of soluble (in 0.5 M NaCl) and albuminoid nitrogen, true protein, and available lysine.

The gross energy of 20 percent protein diets fed to rats was determined by a ballistic calorimeter and consequently the net dietary protein calories percent estimated. The diets used in the net protein utilization experiment were practical rations where peanut or sesame meals replaced completely soybean meal.

Gross protein values showed that peanut and sesame meals are poor protein supplements, since they ranged between 23 and 46 percent of casein. Protein efficiency ratio values and gain in body weight showed that sesame meal does not support growth at 10 percent protein level, and promotes little growth at 20 percent.

Adding 0.4 percent L-lysine-HCl to a ration containing 10 percent protein from sesame meal gave the

highest protein efficiency ratio when compared with sesame at 20 percent with and without lysine supplementation, or with soybean meal at the same level. Suspected toxicity in one sesame meal sample was disproved in the same experiment.

Net protein utilization (operative) with rats showed that peanut meal is fairly comparable with soybean meal. Available lysine values correlated highly with net protein utilization indicating that diets containing 55-69 percent corn and having 20 percent protein either from peanut or sesame meals may have lysine as the first limiting amino acid.

Nitrogen solubility was rather low especially for peanut meal and gave low correlation with gross protein value. True protein values paralleled closely the crude protein values, and peanut meals showed higher contents of non-protein nitrogen than sesame meals.

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I. INTRODUCTION

Peanut and sesame meals as sources of protein for poultry are of economic importance in the Middle East, since other protein supplements such as soybean, fish, and meat meals are imported from the U.S. and Europe. Local peanut and sesame meals are imported mostly from Iraq and are sold at relatively low prices.

Commercial feed manufacturing companies are beginning to include peanut meal in poultry feeds at the rate of 10 percent, thus supplying about 25 percent of the protein in the ration, without lowering the protein quality significantly. The remaining part of the protein comes mainly from soybean meal which is relatively adequate in its lysine content.

Many studies on proteins of peanut and sesame have been reported in the literature, but little is known about the local meals that are usually variable in composition and protein quality.

In this study, the commonly used methods for evaluating proteins were applied to some samples of these meals. Different consignments coming to Lebanon between 1963 and 1965 were used.

The methods used consisted of 3 animal assays and

some chemical assays. The animal assays were: Gross protein value (G.P.V.), protein efficiency ratio (P.E.R.) and net protein utilization (operative) (N.P.U.$_{(op)}$, using the rat. Gross energy of diets was also determined and the net dietary protein calories percent (N.D.p Cal%) as defined by Platt and Miller (1959), was calculated. The chemical assays consisted of proximate analysis, nitrogen solubility, true protein determination, orange G binding capacity and available lysine value (A.L.V.).

II. REVIEW OF LITERATURE

Limiting Amino Acids in Peanut and Sesame Meals

Douglas and Harms (1959), using a practical broiler ration reported that lysine is the first limiting amino acid in peanut meal. Millner and Carpenter (1963) found that methionine is the first limiting amino acid if peanut meal is fed as the only source of protein. McOsker (1962) studied toasted and untoasted peanut preparations and observed that in blanched but not roasted peanuts, the amino acids lysine, threonine and methionine were equally limiting, whereas for properly toasted peanuts, the sequence of amino acid limitation was lysine, threonine and methionine. Fisher (1964) found that in a 12 percent protein diet fed to chicks the first limiting amino acid is lysine, followed by threonine, then methionine, and at 24 percent level, methionine is the second limiting while threonine becomes adequate. Using solvent extracted groundnut meals of widely different origins, at 12 percent protein level, Fisher (1965) found that the three above mentioned amino acids were equally limiting and significant increase in growth of chicks was only obtained when all of them were supplied simultaneously at the rate of 0.4 percent L-lysine, 0.2 percent L-threonine and 0.3 percent DL-methionine.

Patrick (1953) found that lysine supplementation of a 30 percent sesame meal diet increased growth of chicks significantly. Kik (1960) using rats found that the biological value of sesame meal and seed was significantly improved when lysine and threonine were added. He observed that the addition of 0.2 percent L-lysine to a 9 percent sesame protein diet increased weight gain only by 4 percent and P.E.R. by 4.3 percent. However, further addition of 0.2 percent threonine produced an increase of 50 percent in gain and 32 percent in P.E.R. Threonine alone was not tried. The simultaneous addition of these 2 amino acids also increased the net utilization (true digestibility x biological value).

Smith and Scott (1965) using the free amino acid contents in chicks plasma as an availability measure of amino acids, found that the addition of 0.55 percent L-lysine to an 18 percent sesame meal protein diet raises the plasma level of this amino acid by 255 percent. Despite the evidence of growth assays which indicate no improvement upon the addition of methionine, histidine and threonine, the plasma level of these amino acids showed "apparent deficiencies". There is a relation between amino acid contents of protein and its level in the plasma. However, Sebrell and McDaniel (1952) found that different amino acids affected blood regeneration differently and sometimes variably. Longenecker and Hause (1958, 1959, and 1961)

were able to demonstrate with humans and dogs using column chromatography that there is poor correlation between venous level of plasma amino acids and the amino acid composition of an ingested protein. The authors developed a ratio where the requirements of each amino acid is taken into consideration, which showed that the sequence of plasma amino acid ratio is the same as that of amino acid limitation in the protein fed. Campbell (1963) states that plasma amino acid regeneration does not appear to be a suitable procedure for the evaluation of proteins. Consequently, the above amino acids in sesame meal that showed "apparent deficiencies" may not be really deficient.

Gross Protein Value

The G.P.V. method for measuring the protein quality of protein supplements was first developed by Heiman et al.(1939) who described it graphically by plotting the response of chicks to casein and to test protein. Numerically it is the amount of casein that would have given the same response as the test protein consumed over the amount of test protein and multiplied by 100. Robertson et al. (1940) expressed the G.P.V. as gain per gram of test protein consumed as a percentage of gain per gram casein; in both methods gain above basal protein group is considered. Anwar (1960) suggested that the basal protein consumed should be taken into account also. Consequently he subtracted from the numerator

term (extra gain/gm supplementary protein consumed) and from the denominator term (extra gain/gm casein protein consumed) the same quantity (gain of basal group/basal protein consumed), and considered this expression as a better indication of protein quality. Woodham et al. (1961) found that neither refinement nor simplification of the original method of Heiman produced discrimination in the nutritive value of protein of different samples of cottonseed and meat meals.

Casein is used as a standard protein though it is deficient in arginine for the chick even when fed as a supplementary protein source (Fisher et al., 1960). However, supplementing casein with this amino acid is not feasible when used in G.P.V. determinations. Carpenter and Ellinger (1955a) found a "highly significant relationship" between G.P.V. and A.L.V.[1] Boyne et al. (1961) and Anwar (1962b) found a high correlation between G.P.V. and A.L.V. indicating that G.P.V. is a method that evaluates a protein primarily through the availability of lysine for chick growth. Duckworth et al. (1963) determined the G.P.V. of 9 differently processed peanut meals, from 6 different countries, and found values ranging between 64 and 32 indicating that different processing methods and other factors related to soil,

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1. See section on A.L.V.

variety of peanut plant etc. have an effect on availability of lysine. Anwar (1962b) determined the G.P.V. of 10 peanut meals from different localities and found values ranging between 45 and 62.

Protein Efficiency Ratio

The method of P.E.R. was introduced by Osborne *et al.* (1919) to express the growth promoting value of proteins fed to rats. These workers found that P.E.R. values vary with the protein level of the diet. Bender (1956) found that P.E.R. values correlated with feed intake; the P.E.R. tends to become lower with decreased feed intake. Sure (1955) found that P.E.R. values tend to become lower as the time of the assay increases. He noticed that values for a 10-week period were much lower than those for a 4-week period. Chapman *et al.* (1959) tried to standardize the P.E.R. assay by using a 4-week assay period, placing one rat in each cage, feeding a 10 percent protein diet and correcting for strain variation by considering the P.E.R. of casein as 2.5.

Morrison and Campbell (1960) found that for plant proteins, the P.E.R. values drop slightly when the assay period is extended from 4 to 10 weeks using a 10 percent protein diet. In case of casein however, the drop was significant. It is concluded that for good quality

proteins, the decrease in P.E.R. value as the assay period becomes longer is more pronounced than for poor quality proteins.

Hegsted and Worcester (1947) found that there is a very high correlation between gain in weight and P.E.R., when testing 23 different proteins at 12 percent protein level. These authors concluded that gain in body weight can replace P.E.R. Sherwood and Weldon (1953) also reported that there is no advantage of P.E.R. calculation over gain in weight. However, the results of Chapman et al. (1959) and Morrison and Campbell (1960) show that there is a lower coefficient of variation for P.E.R. values than the corresponding weight gains. Using chicks, Hinners and Scott (1959) found slightly higher variance for P.E.R. than corresponding weight gains. Bender and Doell (1957) gave 2 major criticisms of the P.E.R. assay which are: First, it does not allow the evaluation of proteins that do not support growth; and second, it does not take into account the protein consumed for maintenance.

Hegsted and Chang (1965) discussed the invalidity of the P.E.R. assay when used as an indication of amino acid deficiency in a protein. They indicated that growth and nitrogen retention are not proportional to the limiting essential amino acids particularly lysine. The authors also pointed to the inability of the P.E.R. assay to measure the relative nutritive values of proteins.

Campbell (1963) suggested a method for detecting toxicity in protein sources. The method was described for rats, where besides the 10 percent protein group for P.E.R. determination, a 20 percent protein group is added. Foods giving a better response than casein at 10 percent protein level, should give at least an equal response at 20 percent level. However, foods giving a lower response, when supplemented with amino acids, to make them comparable with casein should give equal or better response at both levels. If these conditions are not met then toxicity is suspected.

Bunyan and Woodham (1964) ran P.E.R. on broiler chicks. The assay consisted of 4 days pre-experimental period when chicks were fed cracked wheat and maize only, then 10 days on a diet consisting of 21-22 percent crude protein. The test diet contained 18.6 percent protein; 12 percent coming from the test protein and 6.6 percent from barley, wheating, oat feed and dried yeast. It was fed for 4 weeks, and a standard protein of known nutritive value was considered. This type of P.E.R. assay is practical, but does not tell much about the protein source alone.

Net Protein Utilization

N.P.U. may be defined as "standardized" or "operative" according to whether measurements are made below or

above maintenance (Miller and Payne 1961). At maintenance level, N.P.U. is constant and directly related to amino acid score. However, as the protein concentration increases, increasing proportions of it are diverted from the anabolic pathway and oxidized. This fact is supported by the work of Platt and Miller (1958), and Miller and Payne (1961), who found a linear relationship between P Cal% and N.P.U. However, at a percentage of dietary calories derived from protein (P) greater than 40, the linear relationship derived by Miller and Payne (1961) does not hold. Morrison et al. (1962) using diets with "P" varying between 10 and 80 found that the relation is semi-logarithmic.

The original method was first described by Bender and Miller (1953a), who called it "net protein value" and defined it as N.P.U. = $\frac{B - B_k + I_k}{I}$ where B, I and Bk, Ik are the body nitrogen and nitrogen intake of the test and non-protein groups, respectively. The method was then developed by Miller and Bender (1955) who suggested the use of N/H_2O ratio in place of nitrogen determination by Kjeldahl. They developed a regression equation relating percent body nitrogen to moisture percent and age, so that if $y = \frac{100\ N}{H_2O}$ in gms and x = age in days then $y = 0.02x + 2.92$. The authors recommended the determination of y for each colony of rats. This equation is a modification of that of

Bender and Miller's (1953b) which is y = 0.024x + 2.92.

Bender and Miller (1953a) used groups of 4 rats each, one rat from each of 4 litters, placed each group in one cage and treated it as a unit. This design does not allow for statistical analysis. It was shown however, that 2 runs, the results of which agree within ± 4 N.P.U. units give a satisfactory estimate of the value. This N.P.U. method has the advantage over the balance technique used by Mitchell (1923) and Allison (1955) in being more practical and less tedious.

The rat has been used most extensively in N.P.U. determinations, however Ascarelli and Gestetner (1962) and recently DeMuelenaere et al. (1965) have used the chick. Working with the chick is not precise enough due to individual variation, inability to separate spilled feed from feces and large size of the chick as compared with the rat.

Bunyan and Price (1960) found $N.P.U._{(st)}$[1] value for 6 peanut and 8 soybean meals of 40 ± 2 and 52 ± 2 units respectively. Morrison et al. (1962) reported a value of 54 as $N.P.U._{(st)}$ of soybean flour and found a correlation coefficient of 0.85 between $N.P.U._{(st)}$ values of different proteins and the dietary lysine content with L-lysine-HCl concentrations varying from 0.32 to 0.72 percent.

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1. $N.P.U._{(st)}$ = N.P.U. standard.

Net Dietary Protein Calories Percent

The concept of N.D.p Cals% was introduced by Platt and Miller (1959) to assess the quality and quantity of a protein in practical human diets. N.D.p Cals% is dependent on the total metabolizable energy of the diet since protein quantity is expressed as a percent of the metabolizable calories.

The original term was net dietary protein value (N.D. pV.) which is the product of $N.P.U._{(op)}$ and protein level of the diet. When used to describe the protein content, the figure is expressed as N.D. p Cal%, which is $N.P.U._{(op)}$ multiplied by percent metabolizable calories coming from the protein.

Miller and Payne (1960 and 1961) found that expressing the quality and quantity of a protein in a diet in terms of N.D. p Cal% is fairly constant but when expressed as N.D. pV. it varies with the protein level. These authors determined $N.P.U._{(op)}$ for wheat gluten, casein, and beef, fed at concentrations from maintenance up to 45 percent protein. They found a linear relationship between $N.P.U._{(op)}$ and level of protein fed. It is concluded that N.D.p Cal% is a practical measure of the quantity and quality of a protein in a mixed diet where a higher amount of a poor quality protein can replace a certain amount of good quality protein.

Miller and Payne (1961) estimated metabolizable

energy from gross energy by using the equation, M.E./gm = G.E. x 0.95 - N percent x 0.075, applicable to rat and human.

M.E. and G.E. are metabolizable and gross energy respectively.

Orange G Binding Capacity

Udy (1956) was able to predict the protein content of wheat and flour from their orange G binding capacity with respective protein contents ranging from 6.2 to 16 percent and 4.6 to 15.2 percent. The correlation coefficients were 0.992 for wheat and 0.997 for flour.

Bunyan (1959) found a correlation coefficient of 0.99 relating orange G binding capacity to protein content in soybean and peanut meals and reported a regression equation of $Y = 0.217x + 28$ ($P = 0.001$) where Y = crude protein percent and x = mg dye bound/gm sample.

Olomucki and Bronstein (1960) tried to correlate the nutritive value of soybean meal with its binding capacity to cresol red. The authors found a "good correlation" between results obtained by the dye absorption test and chick growth, when soybean meal supplied 33 percent of the ration. The meals used received varying degrees of heat treatment and the under-heated samples absorbed the dye to a lesser extent.

Nitrogen Solubility

Almquist et al. (1935) suggested a solubility test for the estimation of protein quality, and that was the protein quality index (P.Q.I.) which is made up to 4 chemically determined values comprising copper precipitable, hot water soluble, phosphatungstic acid precipitable, and pepsin undigestible fractions of total nitrogen. Lund and Sandstrom (1943) separated proteins of the seeds into 5 fractions by successively extracting with water, 5 percent KCL, 70 percent ethanol at 70°C, and 0.2 percent KOH, while the residue was the fifth fraction. Evans and St. John (1945) found that the 0.2 percent KOH fraction gives a correlation with G.P.V.

Anwar (1962) found that solubility in 0.5 M NaCl of 10 groundnut meals tested, ranged from 21 to 68 percent and gave a correlation coefficient of 0.88 with G.P.V. Barnes and Woodham (1963) found a fairly good correlation between nitrogen solubility in 0.5 M NaCl and G.P.V. with chicks though there was some exceptional meals that had high G.P.V. and low solubility or vice versa. Values for nitrogen solubility obtained by these authors ranged between 18 and 77 percent with 6 samples out of 9 ranging between 40 and 63 percent.

Available Lysine Value

The determination of available lysine depends on the free amino groups in a protein. The test was developed by Carpenter and Ellinger (1955a) based on the Sanger reaction (Sanger 1945) which is the reaction of 2-4-dinitrofluorabenzene (D.N.F.B.) with the E-amino group of lysine. The implication is that since this E-amino group in lysine is free in intact proteins, it can react with certain groups of other constituents in the protein source, as the aldehyde group of sugars and forms linkages that are resistant to enzymatic digestion and D.N.F.B. reaction. The remaining amino groups therefore determine the availability of lysine for utilization.

The technique is restricted to proteins low in carbohydrates, since there is a lot of interference in color when carbohydrate contents are high, due to the formation of colored derivatives of other amino acids as arginine when they react with D.N.F.B. Bruno and Carpenter (1957) modified the procedure so that after the addition of methoxycarbonyl chloride, the solubility of E-dinitrophenyl-L-lysine is transferred from water to ether leaving behind ∝-dinitrophenyl-arginine. This leads to the interference of a colored histidine derivative which is soluble in ether. Carpenter (1960) tried to correct for this interference on the basis of recovery of added E-dinitrophenyl-L-lysine.

Bunyan and Price (1960) using the Bruno and Carpenter (1957) modification of Carpenter and Ellinger (1955a) procedure for the determination of available lysine found a value for peanut of 2.35 ± 0.14 gms/16 gm N_2 (147 ± 9 mg/ gm N_2).

III. MATERIALS AND METHODS

Introduction

Two growth experiments on chicks were carried out at the Agricultural Research and Education Center in the Beqa'a, to determine the protein quality of 4 consignments of peanut meal and 2 consignments of sesame meal. In the first experiment, the G.P.V. of these meals was determined, and in the second a P.E.R. was run for the 2 sesame meal consignments at 10 and 20 percent protein levels. In the P.E.R. experiment, possible toxicity was investigated for one sesame meal consignment, even though some authors have reported no toxic substances in sesame meal. The wide difference between the G.P.V. and $N.P.U._{(op)}$ values for one of the 2 sesame consignments relative to the other raised the question of possible toxicity in that consignment.

An $N.P.U._{(op)}$ using rats was determined for all meals in the Food Technology Department. Practical 20 percent protein rations were used.

Sampling the different consignments consisted of taking a portion from each sac in a batch, then mixing the portions together. The samples were placed in large tin cans with covers and stored at $0^{o}C$ in a walk-in cooler. The nomenclature and date of arrival of the different

consignments is given in Table 1.

Table 1. Nomenclature and date of arrival of peanut and sesame meal consignments.

Consignments[1]	Date of arrival
G.N. 04	November 1963
G.N. 05	February 13th 1964
G.N. 06	May 1st 1964
G.N. 07	February 22nd 1965
G.N. 09	August 4th 1965
S.M. 01	November 1st 1964
S.M. 02	April 8th 1965

[1] G.N. = peanut meal.
S.M. = sesame meal.

Animal Experiments

Gross protein value. Protein supplements of plant origin are usually deficient in lysine and when these are added to a basal cereal diet, the deficiency is aggravated. The G.P.V. method is designed to measure the relative amount of lysine available for growth. The protein supplement is added to a basal diet adequate in all nutrients except protein quality and quantity. The amount of protein contributed by the supplement is only 3 percent while the basal diet contributes 8 percent giving a total of 11 percent. Two controls are used, one is the basal diet, 8 percent protein, and the other is a positive control having 3 percent supplementary protein coming from casein.

Four hundred day-old male chicks (White Cornish male x White Plymouth Rock female) were placed in 4 batteries of 5 decks each allowing 20 chicks per deck. All chicks received the basal low protein diet to acquire an affinity for protein utilization at minimum diversion to pathways other than growth. At the end of a 14 days "depletion" period, 192 chicks were selected so that deviations from the mean body weight (79.9 gm) was not beyond $\pm$ 12 gm, with more than 95 percent not beyond $\pm$ 10 gm.

The experiment consisted of 8 treatments with 4 replicates each and 6 chicks per replicate. The composition of the experimental diets is given in Table 2.

After another 14-day period, chicks and feed were weighed and the following ratio as given by Anwar (1960) was calculated.

$$G.P.V. = \frac{T - C}{S - C} \times 100$$ where:

T = gain of test group over control per gram supplementary protein consumed.

C = gain of control group on basal diet per gram basal protein.

S = gain of control group on casein diet per gram casein protein.

In this ratio the protein consumed by the basal diet group is taken into consideration.

Table 2. Composition of diets used in G.P.V. experiment (%).

Ingredients	Basal	Casein	G.N.04	G.N.06	G.N.07	G.N.09	S.M.01	S.M.02
Corn	74.00	74.00	74.00	74.00	74.00	74.00	74.00	74.00
Dextrose	17.00	13.48	11.02	11.05	11.15	11.36	9.17	9.88
Alfalfa meal (17% protein)	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25
Fish meal (65% protein)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Steamed bone meal	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Limestone	0.50	0.50	0.50	0.50	0.50	0.50	-	-
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vit. mix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Test protein supplement	-	3.52	5.98	5.85	5.85	5.64	8.33	7.62
Determined protein (%)	8.95	11.95	11.95	11.95	11.95	11.95	11.95	11.95
Calculated %								
Ca	1.04	1.04	1.04	1.04	1.04	1.04	1.04	1.04
P	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Fat	2.70	2.70	2.70	2.70	2.70	2.70	2.70	2.70
Calculated P.E. Cal/ lb	1126	1114	1094	1094	1094	1096	1073	1077

1. The vitamin mixture provided the following per kg of diet calculated from manufacturers specifications: Vit. A 5500 I.U., Vit. D_3 1650 I.C.U., Vit. E 1.1 I.U., riboflavin 4.4 mg., niacin 22.0 mg., d-pantothenic acid 6.05 mg., choline chloride 220 mg., B_{12} 6.6 mcg., menadione sodium bisulfite complex 1.1 mg., manganese .6 mg., zinc .28 mg., iodine 12 mg., iron 2.0 mg., copper 20 mg., cobalt 20 mcg.

Protein efficiency ratio and detection of toxicity. The method of P.E.R. as modified by Campbell et al. (1963) was used. Besides determining P.E.R. at 10 percent protein level, a 20 percent protein level is used in toxicity detection. Toxicity is suspected if the straight lines joining the points indicating growth versus level of protein are not parallel, after supplementation with the amino acids that are deficient with respect to a standard protein supplement namely soybean.

One hundred and 44 chicks, out of 204 chicks similar to those of the G.P.V. experiment, were selected. Diets containing S.M. 01 and S.M. 02 as the sole source of protein were fed at 10 and 20 percent protein levels with and without lysine supplementation, together with one soybean meal fed at the same levels.

The total amino acid composition of soybean meal as compared with sesame meal indicates that sesame falls short mainly in lysine. Though isoleucine in sesame is lower than that of soybean, the difference is not critical. Consequently, lysine was supplemented at 0.4 percent for the 10 percent protein level and at 0.6 percent for the 20 percent protein level. The composition of the diets is given in Table 3.

The experimental period was 28 days, and both chicks and feed were weighed weekly. The ratios of growth over protein consumed, at 10 and 20 percent protein levels with

Table 3. Composition of diets used for P.E.R. determination (%).

Diet	Dextrose	Sesame meal	L-lysine-HCl	Soybean meal	Constant ingredients[1]
S.M.01 10%	56.90	28.10		-	15
20%	28.80	56.20		-	15
10% + L-lysine-HCl	56.90	27.70	0.4	-	15
20% + L-lysine-HCl	28.80	55.60	0.6	-	15
S.M.02 10%	59.60	25.40		-	15
20%	34.20	50.80		-	15
10% + L-lysine-HCl	59.60	25.00	0.4	-	15
20% + L-lysine-HCl	34.20	50.20	0.6	-	15
Soybean M 10%	62.58	-		22.42	15
20%	40.16	-		44.84	15

1. The constant ingredients item consists of: Alphacel 3.50, corn oil 5.00, mineral mixture* 5.30, vit. mixture† 1.00 and choline chloride 0.2 which adds up to 15 percent of the ration.

* The mineral mixture provided the following per kg: Calcium carbonate 8.8 gm, calcium phosphate 25.07 gm, copper sulphate 2.13 gm, ferric citrate 0.176gm, magnesium sulphate 0.265 gm, manganese sulphate 0.022 gm, potassium chloride 0.165 gm, potassium iodide 0.90 mg, sodium chloride 0.350 gm, disodium phosphate 0.615 gm, zinc carbonate 11.5 mg; and the vitamin mixture provided

† the following per kg of ration: Vit. A 10022 I.U., vit. D_3 1762 I.C.U., vit. E 22.0 I.U., inositol 132.2 mg, folic acid 3.3 mg, P.A.B.A. 66.1 mg, niacin 88.1 mg, calcium pantothenate 220 mg, riboflavin 8.8 mg, thiamine 4.4 mg, menadione sodium bisulfite 4.4 mg, ascorbic acid 220 mg, B_{12} 22 mcg, biotin 220 mcg, and pyrodoxine hydrochloride 8.8 mg.

and without lysine supplementation were calculated.

Net protein utilization (operative) with rats. There are 2 ways of running an N.P.U., depending on the level of protein used, the N.P.U. standardized at a level of 10 percent protein coming solely from the protein supplement, and the $N.P.U._{(op)}$ at a level that is normally found in a practical diet. In the case of $N.P.U._{(op)}$ nitrogen comes from more than one source, thus allowing for the supplementary effect of amino acids. N.P.U. is defined as the ratio of nitrogen retained together with nitrogen used for maintenance to nitrogen consumed (Bender and Miller, 1953). It is calculated from the formula:

$$N.P.U. = \frac{B - Bk + Ik}{I}$$

B is body nitrogen of a group receiving test protein;
Bk is body nitrogen of a group receiving a non-protein diet;
Ik is the nitrogen consumed by the non-protein group; and
I is the nitrogen intake of the test group.

At protein levels higher than that required for maintenance, some protein is diverted into energy pathways thus lowering the N.P.U. as defined above. The $N.P.U._{(op)}$ operative procedure makes allowance for this diversion.

Thirty two Sprague Dawly rats of mixed sexes were randomized into 8 groups of 4 each so that the average weight of all groups was within $\pm$ 2 gm.[1] Randomization

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1. 1st run 231 $\pm$ 2 gm, 2nd run 207 $\pm$ 1 gm.

was done after feeding a stock diet for 3 days to allow for adaptation. Each group was then put in one cage and diets fed for 10 days.

The rats were weighed daily to observe whether growth was regular. Feed was added daily in small amounts to have minimum spillage. Spilled feed was collected 3 times through the experimental period, and separated from feces. All weighings of consumed, spilled, and left over feed were calculated on oven dry basis.

Another experiment with the same diets and same technique was run after 6 months to confirm the results of the first. In this second run, the stock diet was fed for one week, and the initial weight of groups was lower than that of the first run. Temperature and relative humidity were low in the first run but reached to the limit of optimum tolerance in the second.

The composition of the diets giving a 20 percent protein level for the test groups is shown in Table 4.

The non-protein diet consisted of the following in percent: Corn starch 60, corn oil 15, glucose 15, salt mixture 5 and vitaminized starch[1] 5. All ingredients were ground to pass a 40 mesh seive before mixing.

At the end of 10 days rats were weighed and killed with chloroform. The head, thoracic, and abdominal cavities

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1. 96.66 percent corn starch well mixed with 3.33 percent vitamin mixture.

Table 4. Composition of diets used for $N.P.U._{(op)}$ determinations (%)

Ingredients	Diets						
	Soybean meal	S.M.01	S.M.02	G.N.04	G.N.06	G.N.07	G.N.09
Ground yellow corn	63.00	55.00	59.50	67.50	67.50	67.50	69.00
S.M.	-	37.50	33.00	-	-	-	-
G.N.	-	-	-	25.00	25.00	25.00	23.50
Soybean meal	29.50	-	-	-	-	-	-
Fish meal (65%)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Alfalfa meal (17%)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin mixture[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Steamed bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00	1.00	1.00	1.00

1. See Table 2 for composition.

were opened and the carcass dried in a forced air oven at 105 ± 5°C for 48 hours. The dried carcasses were then weighed and sampled for nitrogen determination. Nitrogen was also determined on diets on dry basis and $N.P.U._{(op)}$ calculated. The results are shown in Table 9.

Gross energy determination of twenty percent protein diets.

The gross energy as determined by a ballistic calorimeter was used to determine the gross energy of diets. The calorimeter was standardized by determining the amount of deflection of a galvanometer per gram sucrose. Sucrose was dried in a dessicator with H_2SO_4 for 24 hours. Knowing that sucrose has a gross energy of 3.95 Cal/gm the gross energy of the diets could be calculated from the amount of deflection. The diets were burned in an atmosphere of oxygen at 25 P.S.I. (40,000 lb/m^2) and their gross energy contents determined. A blank correction of 1.1 graduations including energy of thread was subtracted from each deflection value. Metabolizable energy was estimated from gross energy using the equation: $M.E. = D \times G.E. - K \times \%N_2$, where D is the coefficient of digestibility of energy and K is a factor which adjusts for the energy left in urine. D for man and rat is in the range of 0.95 and K in the range of 0.075 as given by Miller and Payne (1959). These same values are estimated for the chick. T.D.N. values given by Titus (1959, pp. 240-242) are used to estimate D, and energy constituents of chick urine to estimate K. (See Appendix

B page 59-61).

Chemical Tests

Proximate analysis. Nitrogen determination was done according to A.O.A.C. (1960) where potassium sulfate and mercuric oxide are used as catalysts and ammonia is received in 0.1 N acid, then excess acid is back titrated with 0.1 N base.

Orange G binding capacity. Orange G belongs to the family of azo dyes and is sold commercially in an impure form. The chemical name is p-diphenylanine-azobenzene disulfonic acid. This dye together with safranin have a special property of binding to proteins.

The method adapted is that of Bunyan (1959) where the amount of dye bound per gram sample, is determined by measuring colorimetrically the initial concentration of the dye added and the final concentration after binding.

Reagents used.

1. Buffer pH 2.2, made up of citric acid monohydrate 20.7 gm, disodium phosphate 1.44 gm and thymol solution (10% alcoholic) 0.125 ml in one liter.

2. Reference standard orange G solution. Orange G (B.D.H.) was recrystallized from 90 percent ethanol and dried for 3 hours at 80°C. The reference standard solution consisted of 1 mg of dried orange G/ml of buffer.

3. Working solution. Orange G as bought was dissolved in buffer pH 2.2, filtered through sintered glass and the volume adjusted to give an extinction at 470 mμ equal to that of the reference standard solution indicating that the dye as bought is about 70% pure. It was found necessary to dilute the working solution 250 times to measure the extinction with a Beckman Calorimeter with maximum transmission at 470 mμ. The reference standard solution was used to construct a standard curve giving a linear response up to 0.004 mg/ml (after dilution). Readings were made in one cm glass cells with the buffer solution set at 0.

Procedure. About 50 mg of actual protein were mixed with 25 ml of orange G working solution which keeps the concentration of the dye not lower than 0.5 mg/ml. The reaction was performed in 250 ml rubber stoppered Erlenmeyer flasks after shaking for one hour on a mechanical shaker.

After shaking, the contents were poured in sintered glass funnels of medium porosity and the filtrate collected rapidly without suction. An aliquot was then diluted 250 times and the extinction measured at 470 mμ. The amount of dye absorbed was found by difference.

Nitrogen solubility. Approximately one gm of ground peanut and sesame meal samples made to pass a 40-mesh seive were accurately weighed and transferred to a 250 ml Erlenmeyer flask. One hundred ml of 0.5 M NaCl was pippeted into

each flask at room temperature. The flasks were loosely stoppered, immersed in water whose initial temperature was 37°C, then shaken for 5 minutes.

Later, the flasks were firmly stoppered and shaken at an increased speed for 1½ hours, by the end of which the temperature dropped to 26°C (The temperature was supposed to be maintained at 37°C but an instrument for temperature adjustment, and shaking was not available).

The contents were then filtered through Whatman 541. Two, 25 ml portions of each filtrate were transferred to 500 ml Kjeldahl flasks, and nitrogen determined according to A.O.A.C. (1960) where the digestion mixture consisted of 14.25 gm K_2SO_4 and 0.75 gm HgO, ammonia received in 0.1 N H_2SO_4, but excess acid titrated with 0.01 N NaOH, using methyl red as indicator.

True protein determination. True protein was determined by the A.O.A.C. (1960) method where the albuminoid fraction of nitrogen is precipitated by cupric hydroxide, while the nitrogen that is soluble in hot water remained in solution. Nitrogen was then determined on the precipitated fraction and the result after multiplying by 6.25 was reported as true protein.

Available lysine determination. The method of Carpenter (1960) was used for the estimation of available lysine. The extinctions of the samples were compared with that of a standard solution of E.D.N.P.-lysine-HCl. A standard

curve was constructed with an approximate equation y = 63 x 0.4 where y = mcg/ml of E.D.N.P.-lysine-HCl and x = optical density. The standard curve gives concentration of E.D.N.P.-lysine-HCl in mcg/ml and mgm A.L.V./gmN_2 is equated to 41.71 $\frac{\text{mcg/ml}}{\text{sample wt x \%}N_2}$.

IV. RESULTS AND DISCUSSION

Animal Experiments

Gross protein value. The G.P.V. values for peanut meal were somewhat variable and ranged between 29 for G.N. 09 and 46 for G.N. 04, as shown in Table 5. Meal G.N. 09 had the lowest G.P.V., though its $N.P.U._{(op)}$ value as shown in Table 7 was as high as that of G.N. 04. A possible explanation for this difference is that G.N. 09 contains some toxic factors which affect the chick but not the rat.

The average amount of supplementary protein consumed by each chick over a 14-day period did not exceed 7 gm for any treatment. According to Heiman et al. (1939) it should not exceed 6.5 gm. The only value that exceeded 6.5 gm however, was that of casein. The growth response in this range must be linear for casein, since this protein is more balanced in amino acid composition than other proteins.

Statistical analysis of the gain in body weight as presented in Table 6 showed significance at the one percent level between the different treatments as expected. Growth was used as the criterion rather than the G.P.V. values since it is the independent variable, and protein consumption did not vary much. Four chicks died during the

Table 5. Average gain in body weight, protein consumed and G.P.V.

Meal	Av. body wt. gain (g)	Av. supplementary protein consumed (g)	G.P.V.
S.M. 02	51.68	5.18	23.4
G.N. 09	52.70	4.98	28.8
S.M. 01	55.38	5.21	33.4
G.N. 06	59.70	5.86	36.8
G.N. 07	64.48	6.08	45.4
G.N. 04	65.48	6.23	45.9
+Control	96.00	6.96	100.0
		Av. basal prot. cons.	
-Control	31.65	14.42	

Table 6. Analysis of variance of gain in body weight of chicks in G.P.V. experiment.

Source	D.F.	M.S.	S.D.
Total	187		
Between treatments	7	7088**	
Within treatments	180	391	19.77
Between batteries	3	787	
Within batteries	184	640	25.30
Between compartments & within treatments	24	362	25.30
Within compartments & within treatments	156	396	19.90

** Significant at the 1% level of probability.

Separation of means by Duncan's range test*

Treatments	-control	S.M. 02	G.N. 09	S.M. 01	G.N. 06	G.N. 07	G.N. 04	+control
	31.65	51.57	52.71	55.38	59.71	64.46	65.50	96.04

* Any 2 values not underlined by the same continuous line are significantly different.

experiment and the degrees of freedom became (192-4)-1 = 187.

Protein efficiency ratio and toxicity. The P.E.R. values ranged between 1.2 for a 10 percent protein diet whose nitrogen came from S.M. 01, and 3.2 for another 10 percent protein diet supplemented with 0.4 percent L-lysine-HCl, and whose nitrogen is coming from S.M. 02. Values are given in Table 8.

Contrary to expectation, after drawing the straight lines connecting the 10 and 20 percent protein level points versus growth (Figure 1), line S.M. 02 and S.M. 01 became parallel after L-lysine-HCl supplementation, indicating that meal S.M. 02 is not toxic. A possible explanation for this result is lower digestibility of S.M. 02 in a practical diet since S.M. 02 had rough consistancy, and the seeds were intact, while S.M. 01 was in the form of a powder. However, when S.M. 02 was mixed with purified ingredients in the P.E.R. experiment, its digestibility was improved.

Statistical analysis of the P.E.R. values of diets containing 10 and 20 percent protein from S.M. 02 showed no significant difference between the 2 levels, suggesting that neither of the 2 levels is optimal for highest P.E.R. It was not expected that sesame supplemented with L-lysine-HCl to induce growth similar to that of soybean meal at the 20 percent protein level, with close P.E.R. values. Seemingly, the addition of this amino acid at 0.6 percent

Table 7. Body weights (g) at 1, 2, 3 and 4 weeks of age of chicks on P.E.R. experiment.

Treatment	Wt_1	Wt_2	Wt_3	Wt_4	Wt_5
S_1 10	44	57	61	65	67
S_1 20	44	63	81	105	127
S_1 10^+ [1]	43	80	121	167	200
S_1 20^+ [2]	44	106	227	403	538
S_2 10	44	58	80	63	67
S_2 20	44	66	90	121	152
S_2 10^+	44	78	116	151	182
S_2 20^+	44	101	206	376	508
SOY 10	44	77	113	151	183
SOY 20	44	104	231	405	544

1. 10^+ = 10 percent protein + 0.4 percent L-lysine-HCl.
2. 20^+ = 20 percent protein + 0.6 percent L-lysine-HCl.

Table 8. Gain in weight of 4 weeks old broilers, P.E.R. values, analysis of variance of the means of gain and their separation by Duncan's range test.

	Gain in body weight and P.E.R.									
	S.M.01 10	S.M.01 20	S.M.02 10+	S.M.01 20+	S.M.02 10	S.M.02 20	S.M.01 10+	S.M.02 20+	SOY 10	SOY 20
Gain (g)	23	83	157	494	23	108	138	464	139	500
P.E.R.[1]	1.253	1.203	3.180	2.420	1.453	1.473	3.070	2.567	3.063	2.580

Analysis of variance

Source	D.F.	M.S.
Reps.	2	0.1278
Treat.	9	1.9193**
Error	18	0.0240
Total	29	

Separation of means by Duncan's range test*.

Treatments										
S.M.01 20	S.M.01 10	S.M.02 10	S.M.02 20	S.M.01 20+	S.M.02 20+	SOY 20	SOY 10	S.M.01 10+	S.M.02 10+
1.203	1.253	1.453	1.473	2.420	2.567	2.580	3.063	3.070	3.180

1. Values for all replicates are given in Appendix.
* Means not underlined by the same continuous line are significantly different.
** Significant at the 1% level of probability.

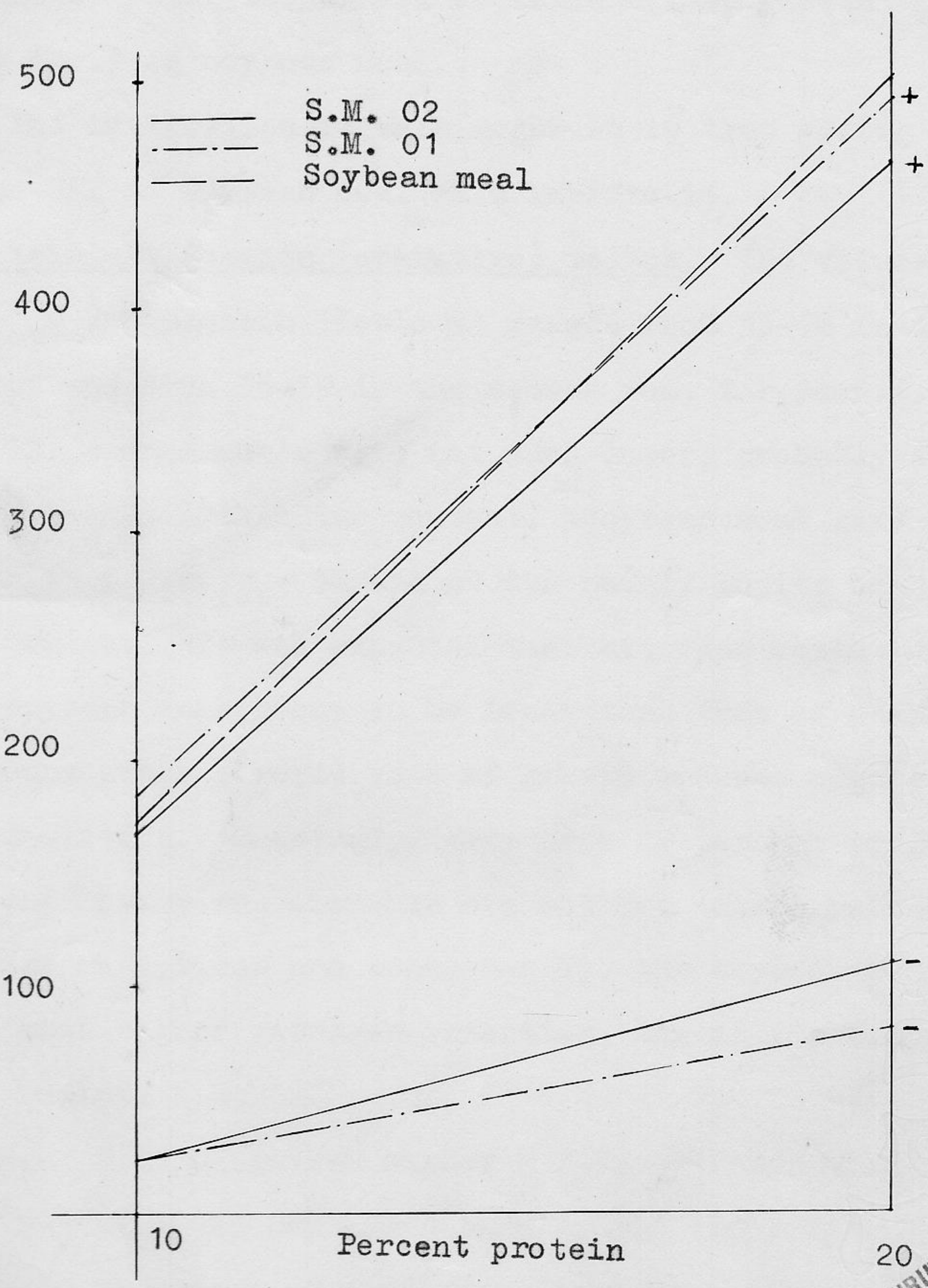

Figure 1. Gain in body weight of 4 weeks old broilers fed 10 and 20 percent protein with and without(±) L-lysine-HCl supplementation.

made sesame higher than soybean in total lysine content, thus counteracting the effect of unidentified growth factors found in soybean meal.

The implication in this argument is that adding L-lysine-HCl to soybean meal will improve it.

Net protein utilization (operative) values. The values of $N.P.U._{(op)}$ 20% protein (Table 9) ranged from 33-40 in the first run and from 35-39 in the second run, for peanut. Values for sesame meals were not much lower, probably due to their higher methionine content. Soybean meal gave a rather high $N.P.U._{(op)}$ 52 and 51 due mainly to its higher lysine content. It was expected that nitrogen retention of the soybean meal group to be lower than that of peanut meal groups since a rapid rate of growth induces higher fat accumulation. Seemingly, even at a 20 percent protein level, the lysine requirements are not met in the peanut and sesame meal diets and consequently, the soybean meal diet induced higher nitrogen retention, due to its higher lysine content.

Meal S.M. 02 gave a higher $N.P.U._{(op)}$ than that of S.M. 01, since as mentioned before, all ingredients were ground to pass a 40-mesh seive, which probably improved its digestibility markedly.

Gross energy contents and calculation of net dietary protein calories percent. One gram of dried sucrose when completely combusted gave an average deflection of 44.09 graduations

per gram or 44.09/3.95 = 11.162 graduations per calorie*.

The gross energy of diets was calculated according to their deflections/gm. The values did not vary much (3.976 $\pm$ 0.076 Cal/gm) among the 7 diets, as shown in Table 9, since all were nearly of the same protein level. The major constituent was corn which is high in nitrogen free extract, and fat does not exceed 3 percent for any of them, they all gave a figure in the range of 4 Cal/gm.

The product of $N.P.U._{(op)}$ by the percent energy supplied by protein with respect to metabolizable energy (p Cal%) for each diet is given in the same table. This figure gives a measure of the utilizable protein as a proportion of calories, and the higher the value the better is the diet. N.D.p Cal% is expected to be less variable than the $N.P.U._{(op)}$ values if the diets were high in fat, but since carbohydrates and proteins have nearly the same metabolizable energy, variation in N.D.p Cal% followed the same trend as $N.P.U._{(op)}$ values.

From an economic point of view, if the amount of reference protein/piaster for each diet is calculated it is found that soybean meal is more economical to use (Calculations are shown in Appendix C, page 61).

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* Average of 8 replicates.

Table 9. Net protein utilization (operative) and net dietary protein calories percent.

Diet	Percent N_2 (D.B.)[1]				N.P.U.$_{(op)}$		Calories/gm^2		p Cal%	N.D.p Cal%[3]
	Diets		Carcass							
	Exp.I	Exp.II	Exp.I	Exp.II	Exp.I	Exp.II	G.E.	M.E.		
S.M.01	3.798	3.758	9.820	9.547	33.0	34.1	4.27	2.82	33.7	11.3
S.M.02	3.578	3.503	9.648	9.241	35.0	37.2	4.41	2.94	30.4	11.0
G.N.04	3.680	3.645	9.796	8.647	40.5	39.7	4.49	2.99	30.8	12.4
G.N.06	3.797	3.779	9.739	8.739	33.3	35.2	4.42	2.93	32.4	11.1
G.N.07	3.784	3.710	9.948	9.074	37.2	35.9	4.54	3.02	31.3	11.4
G.N.09	3.720	3.677	9.878	8.834	39.7	38.9	4.49	2.98	31.2	12.3
SOY	3.851	3.819	10.731	9.938	51.7	51.5	4.58	3.04	31.7	16.4
Non-protein	0.030	0.030	10.463	10.349	-	-	-	-	-	-

1. D.B. = dry basis (Moisture in diets of exp. 1 varied between 10.0 & 11.7%).
2. G.E. = gross energy corrected for dry basis, M.E. = metabolizable energy estimated from the formula M.E. = 0.71 G.E. - 0.0546 $\%N_2$, (See Appendix "B" p. 60-61) (Diets of the first run were used).
3. Av. N.P.U.$_{(op)}$ in 2 runs x p Cal%.

Chemical Tests

Proximate composition. The proximate analysis of the different meal consignments helped in calculating protein, ether extract, calcium and phosphorous contents of the different diets. Sesame meal has about 4 percent ether extract as compared with peanut which is in the range of one percent. Though both meals are solvent extracted, sesame retained more fat. Another point to consider is that sesame meal is a good source of calcium and phosphorous as compared with peanut. The proximate composition is given in Table 10.

Orange G binding capacity and its correlation with crude protein content. The results of orange G binding capacity (Table 11) were highly correlated with the crude protein content of the 5 percent meal consignments, ($r = 0.98$).

The amount of dye bound per gram sample ranged between 82 and 103 mg. No correlation was found between G.P.V. and dye binding capacity.

The concentration of the dye in the working solution as adjusted to be 1.0000 mg/ml may not be accurate to the 4th decimal place, but the difference is not substantial. The 2 replicates for each sample were within $\pm$ 1 mg dye/gm sample. The possibility of substituting dye binding capacity for Kjeldahl remains to be investigated.

Nitrogen solubility in 0.5 M NaCl. There was considerable variation in the solubilities of the different consignments

Table 10. Proximate analysis of 5 peanut, 2 sesame meals, one soybean meal, and the basal ration used in G.P.V. experiment[1].

Diet	Moisture (%)	Crude protein (%)	Ether extract (%)	Crude fiber (%)	Ash (%)	N.F.E.[1] (%)	Ca (%)	P (%)
S.M.01	9.29	35.60	3.80	8.41	13.00	26.50	2.401	0.985
S.M.02	7.85	39.39	5.05	6.95	13.95	22.68	2.906	1.229
G.N.04	12.00	50.17	1.12	5.76	7.09	23.14	0.177	0.587
G.N.05	9.76	50.43	0.80	4.30	6.65	27.28	0.174	0.603
G.N.06	11.42	51.24	1.10	4.62	5.64	25.16	0.207	0.612
G.N.07	8.99	51.22	0.76	5.20	6.49	26.58	0.174	0.588
G.N.09	5.65	54.45	1.42	5.30	7.57	24.78	0.221	0.612
SOY	9.64	44.60	0.76	6.12	6.28	32.60	0.310	0.601
Basal ration	11.00	8.95	4.47	1.77	4.54	67.44	1.271	0.555

1. N.F.E. calculated by difference

Table 11. Orange G binding capacity of 5 peanut consignments.

mg dye absorbed/gm sample			N x 6.25
Rep. 1	Rep. 2	Average	
82.0	82.2	82.1	50.17
82.6	83.0	82.8	50.43
88.4	87.1	87.7	51.24
88.0	88.8	88.4	51.22
101.0	104.2	102.6	54.45

ranging between 18 percent and 53 percent as shown in Table 12. Nitrogen solubility as a measure of the protein value of the peanut consignments gave an r of 0.55 with G.P.V. However, when these values were correlated with $N.P.U._{(op)}$ values, an r of 0.88 was obtained.

Nitrogen in sesame is more soluble than that of peanut, but its protein quality is not higher. The comparison of nitrogen solubility with protein quality as determined by bioassays is fairly promising as the correlation coefficient with $N.P.U._{(op)}$ shows.

True protein or albuminoid nitrogen and amido nitrogen. True protein values paralleled closely the crude protein values (r = 0.987). The true protein content of sesame was higher than that of peanut. The ratio true protein: crude protein was high and ranged between 0.98 and 0.88. The amido nitrogen as found by difference ranged between 0.8 and 6.5 percent.

True protein content correlated poorly with G.P.V., and gave only an r = 0.43 with $N.P.U._{(op)}$.

Available lysine value. The A.L.V. values of peanut meals were nearly half as much as that of soybean meal. The main aim of the determinations was to correlate them with G.P.V. values and confirm the results but apparently there were other factors that interfered. The A.L.V. values however,

correlated nicely with $N.P.U._{(op)}$ ($r = 0.97$) suggesting that in 20 percent protein diets whose supplementary protein is coming from peanut or sesame meals, the first limiting amino acid is still lysine and not methionine. Values of A.L.V. are shown in Table 13.

G.N. 05 was omitted from the correlation calculations since neither its G.P.V. nor $N.P.U._{(op)}$ was determined.

Since G.N. 09 gave a comparatively low G.P.V. while its A.L.V. value was high, toxicity was suspected and was omitted from the correlation (G.P.V. vs A.L.V.) calculation, which gave an $r = 0.88$.

Table 12. Nitrogen solubility in 0.5 M NaCl, albuminoid N x 6.25 and amido N x 6.25.

Meal	N% solubility	Albuminoid N x 6.25	Amido N[1] x 6.25 (by difference)[2]	Albuminoid N x 100 / Crude N
S.M. 01	51.6	34.8	0.8	97.8
S.M. 02	49.3	37.6	1.8	95.4
G.N. 04	52.6	46.7	3.5	93.0
G.N. 05	28.3	44.6	5.8	88.5
G.N. 06	18.1	47.8	3.4	93.4
G.N. 07	37.7	46.2	5.0	90.2
G.N. 09	35.0	48.9	6.5	89.9

1. Amido nitrogen x 6.25 = (crude nitrogen - albuminoid nitrogen) x 6.25.
2. For crude protein see Table 9.

Table 13. A.L.V. mg/gm N_2.

Meal	S.M.01	S.M.02	G.N.04	G.N.05	G.N.06	G.N.07	G.N.09	SOY
A.L.V.	104.1	128.2	159.0	167.6	135.2	163.0	167.0	311.7
$N.P.U._{(op)}$	33.6	36.1	40.1	-	34.2	36.6	39.3	51.6

V. SUMMARY AND CONCLUSIONS

Summary

Four consignments of peanut meal and 2 consignments of sesame meal were evaluated as protein supplements for the chick. Animal experiments and chemical tests were conducted, results explained, correlated, and evaluated.

The animal experiments consisted of G.P.V. and P.E.R. with chicks and $N.P.U._{(op)}$ with rats. The chemical tests consisted of proximate analysis, orange G binding capacity, true protein, nitrogen solubility in 0.5 M NaCl, and A.L.V.

Results of G.P.V. ranged between 23 and 46 percent of casein with values of peanut meals higher than those of sesame meals. These values are rather low and the inclusion of these meals at high levels in a practical boiler ration is not feasible. Available lysine values did not have a high correlation ($r = 0.88$) with G.P.V. values probably due to toxicity in some of the peanut meals.

P.E.R. of the 2 sesame meals and one soybean meal, showed that sesame at 10 percent protein level does not induce growth. The explanation is that the lysine content of this meal at this protein level hardly covers the requirements for maintenance. Toxicity in one sesame meal was disproved in this experiment after it has been suspected

due to its low G.P.V. With the supplementation of L-lysine-HCl to 10 and 20 percent protein diets, growth and P.E.R. values of the sesame meal diets became equal to those of soybean meal.

Values of $N.P.U._{(op)}$ using rats at a 20 percent protein level, ranged between 33 and 40 with 51 for soybean meal. The diets were practical chick rations where peanut or sesame meals replaced all the soybean meal, and corn supplied about 5 percent of the crude protein. There was a high correlation between $N.P.U._{(op)}$ and A.L.V. ($r = 0.97$).

The gross energy of diets used in the $N.P.U._{(op)}$ experiment was determined with a ballistic calorimeter. Metabolizable energy was estimated from gross energy using T.D.N. of the different ingredients, to calculate coefficient of energy digestibility for the chick. Values of p Cal% and N.D.p Cal% were calculated using the $N.P.U._{(op)}$ values. The N.D.p Cal% values are not of absolute value for the chick, but of a relative significance since $N.P.U._{(op)}$ was not run on chicks. These values varied between 11.0 and 12.4 for the different meals. They are relatively high mainly due to the low coefficient of energy digestibility for the chick.

Orange G binding capacity gave a high correlation with the crude protein contents of peanut meals ($r = 0.98$). The possibility of substituting Kjeldahl by this test remains to be investigated. Values ranged between 82 and

104 mg dye/gm sample.

True protein values paralleled crude protein values with r = 0.987, and a ratio of true protein/crude protein between 0.88 and 0.98 was obtained. On the average, true protein content of sesame meals were higher than those of peanut meals.

Nitrogen solubility in 0.5 M NaCl ranged between 18 and 52 percent of total nitrogen with figures for sesame meals higher than the average for peanut meals. Nitrogen solubility of peanut meals alone correlated poorly with G.P.V. (r = 0.55) and gave an r of 0.88 with $N.P.U._{(op)}$ values.

It was thought that storing these meals under the conditions described in materials and methods would lower their protein quality after a long period of storage. However, the oldest meal (G.N. 04) gave the best results in G.P.V., $N.P.U._{(op)}$, and nitrogen solubility, indicating that storage under these conditions did not affect the protein quality adversely.

It was found that soybean meal is more economical to use in practical broiler rations when it is to be totally substituted by peanut or sesame meals. The comparison was based on the calculation of N.D.p gm/piaster for each diet.

Conclusions

The primary objective of this work was to know whether the protein quality varies markedly with different batches of peanut and sesame meals; what is their protein quality status as compared to soybean meal? What method is best to evaluate their protein quality? And what should be done besides proximate analysis to have a better picture of that quality?

It was found that there was significant variation between some consignments of peanut and sesame meals in their protein quality as measured by the G.P.V. method. There was not much variation in the results of $N.P.U._{(op)}$, since nitrogen retention methods are independent of growth and consequently less variable. A deficiency of one essential amino acid particularly lysine does not affect retention markedly, yet there was a high correlation between $N.P.U._{(op)}$ and A.L.V.

Since casein does not give better growth than soybean meal when fed to chicks, it can be concluded that the peanut and sesame meal consignments compare poorly with soybean meal as a protein supplement (23-46% of casein) as determined by G.P.V. L-lysine-HCl supplementation at the rate of 0.6 percent to a 20 percent protein diet from sesame meal promotes growth equal to that of soybean.

According to N.D.p Cal% calculations, it can be

concluded that the chick requirements of quality x quantity of protein is higher than that of the rat or humans mainly due to its faster rate of growth, and lower energy requirements.

The figures shown in Table 9 are equivalent to those of good European or American dietaries.

There was not much variation in the true protein content but large variation in nitrogen solubility was encountered. Since nitrogen solubility is a fair measure of the severity of heat treatment during processing, it can be concluded, that there is variation in the processing techniques. Determination of true protein does not have any advantage over that of crude protein, if meals of the same type and origin are to be compared, since these 2 values parallel each other.

According to the correlations determined, the best rated chemical assay is A.L.V. There is also some promise in developing the nitrogen solubility assay by trying solvents other than NaCl solution or by predigesting the meal with enzymes <u>in vitro</u>.

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APPENDIX

Appendix "A"

Table 14. P.E.R. values (3 replicates).

P.E.R. values	Rep. 1	Rep. 2	Rep. 3
S_1 10	1.18	1.43	1.15
S_1 20	1.24	1.23	1.14
S_1 10^+	2.81	3.20	3.20
S_1 20^+	2.40	2.32	2.54
S_2 10	1.20	1.50	1.66
S_2 20	1.48	1.48	1.46
S_2 10^+	2.91	3.04	3.59
S_2 20^+	2.50	2.50	2.70
SOY 10	2.82	3.24	3.13
SOY 20	2.48	2.60	2.66

Appendix "B"

Method of calculating metabolizable energy. It is assumed in this calculation that the coefficient of digestibility of nutrients can replace that of energy digestibility. Titus (1959, pp. 240-242) gives the T.D.N. values for:

Yellow corn	80 percent
Peanut meal	63 percent
Soybean meal	62 percent
Sesame	64 percent
Alfalfa	20 percent
Fish meal	69 percent

Multiplying these values by their corresponding percentages in the diets (20 percent protein diets whose composition is shown in Table 4 page 25) then adding and dividing by 100, a coefficient of 0.70 for sesame and soybean meal diets is obtained and 0.72 for peanut meal diets. An average of 0.71 was used in calculating metabolizable energy.

The gross energy of the nitrogenous part of the urine can be estimated from the gross energy of each of its nitrogenous constituents, and a factor can be obtained which when multiplied by nitrogen percent of diet it gives energy excreted.

According to Davis (1927)[1], the nitrogenous constituents of chick's urine are as follows:

Constituent	Percent (mg/100 ml)
NH_3	17.3
Urea	10.4
Creatine	8.0
Uric acid	63.0
Undetermined	1.3

The energy that has remained in these constituents is:

	Cal/gm M.W.	M.W.	Cal/gm
NH_3	0	17	0
Urea	152	60	2.533
Creatine	560	131	4.275
Uric acid	460	168	2.738

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1. Davis, R.E. 1927. The nitrogenous constituents of hen's urine. J. Biol. Chem. 74: 509.

The energy on the basis of urine nitrogen is:

	$\%N_2$	Cal/gm N_2	Cal/gm N_2 in urine
NH_3	82.35	-	-
Urea	46.65	5.43	0.619
Creatine	32.05	13.34	0.800
Uric acid	33.33	8.21	4.039
			5.458

Therefore M.E. = 0.71 G.E. - 0.0546 x $\%N_2$

The correction factor 0.0546 x $\%N_2$ is valid in adult chicks where growth is minimal since it is assumed that all nitrogen consumed is excreted, however it is of minor importance since it is small as compared with 0.71 G.E.

Appendix "C"

Table 15. Comparison of amounts of reference protein/piaster in $N.P.U._{(op)}$ diets.

Diet	N.D.p gm[1]/kg diet	Price in piasters/kg diet[2]	N.D.p gm/piaster
S.M. 01	79.3	28.52	2.78
S.M. 02	79.9	28.39	2.81
G.N. 04	91.8	28.15	3.26
G.N. 06	81.0	28.15	2.88
G.N. 07	85.7	28.15	3.04
G.N. 09	90.8	28.10	3.23
SOY	123.7	31.82	3.89

1. $N.P.U._{(op)}$ x (gm protein/kg diet).
2. The following current prices were considered - pts/kg: Yellow corn 25, soybean meal 40, sesame meal 28, peanut meal 28, fish meal 100, alfalfa meal 40, bone-meal 20, salt 5, vit. mix 420, and limestone 1.