EVALUATION OF PEANUT MEAL AS A SOURCE OF PROTEIN FOR BROILERS

By

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ABSTRACT

Peanut meal is a rich source of protein which can be used to advantage in poultry rations in areas where this product is reasonably priced. It is commonly known that peanut meal is limiting in certain essential amino acids, and recently it was found that certain consignments could be contaminated with toxic substances, such as aflatoxin. Although peanut is an important crop in the Middle East, there is no information available concerning the nutritive value of the peanut meals produced in the area. This research was carried out to determine the nutritive quality of Iraqi peanut meals and their possible uses in modern broiler rations.

Proximate composition including calcium and phosphorous, methionine, cystine and lysine content, available lysine, and aflatoxin content of each of the three meal samples were determined. For the evaluation of protein quality, two bioassay procedures were used; protein efficiency ratio (PER) and practical broiler feeding tests.

The results of the PER experiments showed that PER of three samples of Iraqi peanut meals were similar, but lower than that of soybean meal. Furthermore, the supplementation of these meals with the amino acids, lysine, methionine, tryptophane, isoleucine, and valine resulted in a

significant improvement of their PER. However, this resultant nutritive value did not equal that of soybean meal.

Results of the broiler practical feeding experiments showed that peanut meal could replace up to 75 percent of the soybean meal, that is 24 percent of the total diet, if such diets are supplemented with 2 percent fish meal. Furthermore, lysine was found to be the most limiting amino acid in a diet containing peanut meal as the principle source of protein, that is 27 percent of the total ration. Supplementation of such rations with 0.09 percent methionine and 0.57 percent lysine improved the weight gains and feed efficiency and brought it up to the level of the corn-soybean meal diet.

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INTRODUCTION

The peanut (Arachis hypogaea) also called goober, pindar, groundnut, and earthnut, is used for the production of oil, hydrogenated fat, and protein foods and feeds. It is believed that the peanut originated in the tropics of South America. Among the leading countries in peanut production today are China, India, West Africa, and the United States.

Peanut meal or cake is the by-product of the production of oil from peanut seeds which have been first dehulled or shelled. According to the definition of the Association of American Feed Control Officials, peanut meal should not have more than 7 percent fiber. If it contains more than this amount, it is called peanut meal and hulls.

Feed constitutes about 68 percent of the total cost of broiler production in Lebanon (Ward and Fuleihan, 1962). Since protein supplements constitute about 30 percent of the broiler ration and are the most expensive items used, any attempt to reduce the cost of these supplements will reduce the cost of broiler production.

Peanut meal is a high protein feed available in Lebanon at a relatively low price and most of this meal is imported from Iraq. It is higher in crude protein and cheaper than the commonly used soybean meal. However,

peanut meal is poorer than soybean meal in lysine and methionine. In addition, certain batches of the meal have been found to be contaminated with toxic factors which render it inferior to soybean meal.

Many investigations have been made to determine the nutritive value of peanut meal and the extent to which it can replace soybean meal in poultry rations. However, little research has been conducted to evaluate peanut meals produced in the Middle East as a source of protein for poultry. Furthermore, no data are available on the amino acid and aflatoxin contents of these meals.

Iraq is the leading peanut meal producer in the Near East; the average yearly production of this meal in Iraq is estimated at 6000 tons. Since Iraq is the main source of peanut meal for Lebanese feed manufacturing plants, the objective of the present work was to evaluate the quality of Iraqi peanut meal shipped to Lebanon. This objective was fulfilled by analysing the samples for proximate composition including calcium and phosphorous, methionine, cystine, lysine, available lysine, and aflatoxin. In addition, PER studies and large scale animal feeding experiments were conducted. In these latter experiments, the possibilities of replacing soybean meal partially or completely with peanut meal were studied. Also investigated were the effects of amino acid supplementation, singly or in combination, on body weight gains, feed efficiency, vital organ weights and

carcass composition of broilers.

REVIEW OF LITERATURE

Amino Acid Composition

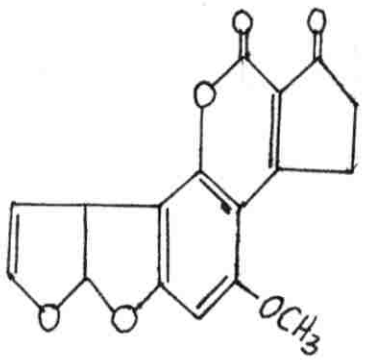
Several chemical, microbiological, and chromatographic adsorption methods have been used for the determination of amino acids. Block and Weiss (1956) in a review article have described detailed procedures for these methods. Several workers have reported the methionine, lysine and cystine contents of peanut meals (Orr and Watt, 1957; Shurpalekar et al., 1962; Tasker et al., 1962). These workers obtained figures that ranged from 218-225 mgm./gm. N for lysine, 55-62 mgm./gm. N for methionine and 93-100 mgm./gm. N for cystine. Bunyan and Price (1960) reported that the average available lysine content of six samples of peanut meal was 147 mgm./gm. N using the Bruno and Carpenter (1957) method of available lysine determination.

Toxicity Factors in Peanut Meal

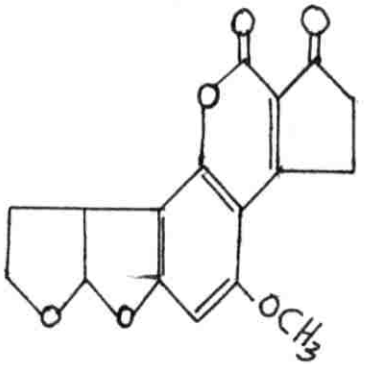
Leiner (1962) reported that the toxicity factors in raw peanut were trypsin inhibitors, hemmaglutinin and goitrogens. Blount (1961) has indicated that a toxic factor in Brazilian peanut meal was responsible for the turkey "X" diseases. Sargeant et al. (1961) reported that there were toxic substances in peanut coming from Brazil, Nigeria,

French West Africa, and Gambia. Later the same laboratory (Sargent et al., 1961) found that the toxicity was due to an infection with a toxin producing strain of Aspergillus flavus which produces, in small amounts, a highly toxic factor for which the name aflatoxin is now being used.

Chang et al. (1963) were able to identify two compounds in the aflatoxin mixture whose structure is given below. They named these aflatoxin B_1 and B_2.



B_1



B_2

These workers compared the toxic properties of aflatoxin B_2 with those of aflatoxin B_1 by biological assay procedures. Their data indicate that both compounds have toxic properties manifested by depression in growth, reduction in liver weight and histopathologic lesions in the liver. However, the biological potency of aflatoxin B_2 was found to be less

than that of aflatoxin B_1. It was suggested by these workers that the point of unsaturation in the B_1 compound, absent in the B_2, is an important contributing factor to the higher toxic potency of aflatoxin B_1.

Asplin and Carnaghan (1961) conducted an experiment on ducklings and chicks to study the effect of feeding certain toxic batches of peanut meal. They found that both ducklings and chickens were susceptible to the toxic principle in certain samples of peanut meal. However, it was shown that ducklings were more susceptible to the toxic principle in these meals than chickens. Harding et al. (1963) reported that pigs were also poisoned when fed rations containing toxic peanut meals. Allcraft et al. (1963) showed that cows, fed rations containing toxic peanut meal, excreted in the milk a toxic factor having a biological effect in ducklings similar to that caused by aflatoxin. Upon precipitation of the protein fractions of the milk, it was shown that the toxin was present only in the rennet-precipitated casein fraction which also included the fat. No toxin was found in the protein-free filtrate. Furthermore, there was none found in livers or blood from cows or pigs receiving rations supplemented with toxic peanut meal. Eggs produced by pullets receiving toxic peanut meal were also found to be free of the toxin. Gardiner (1962) conducted an experiment concerned with a differential species effect of various levels of Brazilian peanut meal on growth and mortality of

poults and chicks. The growth rate of the poults fed each level of the peanut meal was markedly reduced and was inversely related to the level of peanut meal in the diet. In the chicks receiving high levels of peanut meal (37 and 55%), the weights were significantly lower than those receiving 18 percent peanut meal. The difference in weights of chicks between the control group and those receiving 18 percent peanut meal approached significance at the 5 percent level. There were no significant differences in chick mortality among treatments.

Protein Evaluation

Several extensive reviews on methods of protein evaluation in foods have appeared in the literature (Allison, 1949; Allison, 1955; Frost, 1959). Osborne et al. (1919) introduced the concept of PER which was defined as grams of body weight gain per gram of protein consumed. The PER was determined at several levels of protein intake and the maximum value obtained was considered the PER of the test protein. However, Campbell (1963) stressed the need for 10 percent protein in the diet and ad libitum feeding to get maximum PER values. He stated that the rate of growth of an animal provides a simple way of evaluating dietary protein. If the diet is limiting in one or more of the essential amino acids, growth will be depressed or stopped entirely. Thus growth is a good index to evaluate dietary protein

quality.

The duration for the assay period was studied by several workers. Assay periods varied from 1 to 12 weeks but most workers used 4-week assay periods. Chapman et al. (1959), Morrison and Campbell (1960), and Sure (1955) found that PER values decreased as the duration of the experiment increased. They concluded that optimum PER values are obtained by using a 4-week period. Chapman et al. (1959), and Derse (1960) proposed standardized methods for PER determination with respect to protein level, duration of assay, method of feeding, use of an appropriate dietary standard, and strain, age and sex of the animals.

Although the PER assay method is simple, convenient and widely used, it is subject to several criticisms. Campbell (1963) outlined these criticisms as follows:

1 - The assumption that the gain in body weight is constant in composition is not necessarily true.

2 - The results may vary with level of protein in diet and with food intake.

3 - PER makes no allowance for maintenance but assumes that all protein is used for growth.

4 - PER does not permit the evaluation of proteins which do not support growth.

Peanut Meal in Chick Starter and Broiler Rations

A number of papers have appeared on the nutritive value of peanut meal and the extent to which it can be used in place of soybean meal in practical poultry rations. In the first part of this review, the work on chick starter rations will be covered while the second part will deal mainly with broiler rations.

Hammond (1944) stated that as much as 15 percent of peanut meal could replace equal quantities of soybean meal with satisfactory results in a chick starter diet composed of wheat, soybean meal, alfalfa leaf meal, vitamin and mineral supplements. Using a basal diet containing milo, soybean meal, fish liver oil, iodized salt, bone meal, mineral mixture, and animal protein factor (APF), Blaylock et al. (1950) found that peanut meal supplemented with at least 0.3 percent DL-methionine and 0.2 percent L-lysine was a satisfactory source of protein for the growth of chicks to 6 weeks of age. The chicks were slightly heavier when the amounts of methionine and lysine were doubled, but the difference was not significant. In studies with mixtures of soybean meal and peanut meal, methionine was found to be the first limiting factor. When the mixture was supplemented with both methionine and APF concentrates, the chicks were slightly heavier than those which received the methionine alone. Altschul et al. (1948) conducted an

experiment with chicks raised in batteries and used a 20 percent protein diet containing corn, wheat bran, ground oats, meat scraps, fish meal, dehydrated alfalfa meal, calcium carbonate, corn gluten meal, vitamin and mineral mixtures. They found that peanut meals supported chick growth equally as well as commercial screw-pressed soybean meal when used as the source of protein equivalent to 5.5 percent of the total 20 percent protein diet. Solvent extracted peanut meal had the same nutritional value as hydraulic-pressed peanut meal.

Heuser et al. (1946) conducted an experiment on Single Comb White Leghorn chicks reared in batteries up to 8 weeks of age. They used rations containing corn, bone meal, limestone, manganese sulfate, vitamin mixture, soybean meal, peanut meal and fish meal. These workers found that soybean meal as the only source of supplementary protein in a chick starter ration was better than peanut meal. Furthermore, peanut meal could replace one half of soybean meal with little difference in results when using diets well fortified with fish meal. Grau (1946) working with 2-week old White Leghorn chicks and using a purified diet, found that the addition of a combination of methionine, lysine, tryptophane, and threonine produced optimum growth in chicks receiving 20 percent of protein in the diet as peanut meal.

Young (1952) conducted repeated experiments with cross-bred male broiler chicks fed a basal diet composed largely

of corn starch, and purified casein plus methionine and glycine. This worker failed to show any improvement in growth when the diet was supplemented with graded levels of arginine up to 2.06 percent. The addition of various amounts of alcohol extracted peanut meal replacing equivalent amounts of casein protein promoted a marked growth response over that obtained with the basal diet supplemented with levels of arginine equal to those supplied by the peanut meal. Thus, he suggested that alcohol extracted peanut meal supplied an unknown factor needed for chick growth which is deficient or lacking in a purified casein-starch diet.

In experiments with Barred Plymouth Rock chicks, Marvel et al. (1945) observed that peanut meal could replace all the soybean meal when 10 percent of distillers dried solubles was included in the diet. However, Tarver and Driggers (1958) indicated that peanut meal (55% protein) could replace 50 percent of the soybean meal in modern broiler rations containing animal proteins. Douglas and Harms (1958) conducted battery trials on cross-bred broiler chicks using peanut meal at the rate of 0, 25, 50, 75, and 100 percent in place of soybean meal. The diets containing high levels of peanut meal were supplemented with L-lysine and/or methionine hydroxy analogue (MHA). Replacement of soybean meal with peanut meal resulted in depressing broiler growth rates. There was a trend for a greater depression of growth as the level of peanut meal was increased in the

ration. The feed efficiency was significantly depressed when peanut meal replaced soybean meal in the diet. The addition of L-lysine and/or MHA did not overcome the depression in growth rate or feed efficiency resulting from the addition of peanut meal. Driggers and Tarver (1958) used a 55 percent peanut meal in corn-soybean type rations fortified with DL-methionine, L-lysine, and fish meal with condensed fish solubles. These workers found that peanut meal could replace half of the soybean meal without affecting body weight of broilers when the diet was adequately supplied with methionine. They observed that peanut meal could replace half of the soybean meal if the diet was supplemented with fish solubles and 0.2 percent lysine. Furthermore, they found that peanut meal could replace all the soybean meal with satisfactory results if the diet was supplemented with fish solubles and 0.4 percent lysine. Douglas and Harms (1959) also studied the possible replacement of soybean meal with peanut meal and their results indicated that when peanut meal replaced soybean meal completely, it significantly decreased broiler weights at 8 weeks of age. However, when 25, 50, and 75 percent of soybean meal was replaced by peanut meal body weights decreased but the differences were not significant. Replacing 75 and 100 percent soybean meal with peanut meal significantly depressed feed efficiency. The addition of 0.15 percent MHA and 0.075 percent lysine did not significantly increase rate

of growth of chicks receiving 50 percent of their protein supplement as peanut meal. The addition of 0.25 percent lysine significantly increased weights of chicks receiving 100 percent of their protein supplement as peanut meal, while the addition of 0.20 percent MHA did not result in a significant increase in body weight. The supplementation of diets containing 50 percent peanut meal in place of soybean meal with 0.20 percent MHA, 0.15 percent lysine and 0.15 percent glycine produced growth equal to that when no peanut meal was added. Peanut meal-corn diets supplemented with 0.30 percent MHA, 0.35 percent lysine and 0.15 percent glycine produced weights equal to those obtained with all soybean meal-corn diets.

Waldroup and Harms (1963) showed that when peanut meal was used as the principal source of protein, the diet appeared to be deficient in lysine, methionine and tryptophane. Supplementing the peanut meal diet with these amino acids significantly improved performance of chicks raised on these diets. However, the growth rate of chicks fed the diet in which soybean meal was the major protein source was seldom approached by those fed the supplemented peanut meal diets. When the protein level of the peanut meal diet was increased so that levels of lysine, methionine, and tryptophane at least equivalent to the soybean meal diet were fed, performance of the chicks was significantly improved but was not comparable to that of chicks raised on the soybean meal diet.

This indicates that the poor performance of chicks raised on the peanut meal diet was due to an amino acid limitation and not to any inhibiting factor in the peanut meal; since increased levels of the peanut meal resulted in increased growth. The failure of some samples of peanut meal to support good growth indicates that variation exists between different samples of peanut meal, which could affect its use in broiler diets.

Hoie and Sannan (1961) in Norway found that peanut cake meals were inferior to soybean meal when used as partial or total substitutes for soybean protein, alone or in combination. It was suggested by those workers that the poor results obtained from the peanut meals may be to some extent due to a low content of lysine and may partly be due to a high content of coarse particles as well. Fangauf et al. (1958) conducted experiments in Germany to investigate the use of extracted peanut meal as replacement for soybean meal. The use of peanut meal resulted in faster growth of chicks but no differences were observed in feed conversion. Feed consumption was increased which shows according to these authors a preference of the chicks for peanut meal. The same workers (Fangauf et al., 1962) conducted experiments on broiler chicks to test the use of peanut meal and toasted soybean meal as vegetable protein sources. Chicks fed the peanut meal ration showed the same feed conversion, but significantly lower weight gains than

the normal ration which contained peanut meal and soybean meal. With the soybean meal ration, the deterioration of feed conversion and gain in weight were not significant compared to the normal ration. A combination of both meals gave the best results. It is possible that differences in peanut meals used may have contributed to discrepancies in results obtained by these workers.

Daghir et al. (1964) conducted three experiments with cross-bred broiler chicks raised to 8 weeks of age to study the effects of replacing soybean meal partially or completely with Iraqi peanut meal and the effects of amino acid supplementation of such rations on body weight gains, feed efficiency, vital organ weights, and carcass composition. In rations without animal protein, it was found that peanut meal could be added up to 8.5 percent of the total ration without a significant drop in body weight gains or feed efficiency. Lysine was found to be the most limiting amino acid in a diet containing peanut meal as the principle source of protein. Supplementing such a diet with both lysine and methionine improved body weight gain but did not bring it up to the level of the corn-soybean meal diet. High protein peanut meal was found to adequately replace 50 percent of the soybean meal in broiler rations supplemented with fish meal. Supplementation of such rations with 0.20 percent L-lysine-HCl and 0.05 percent MHA did not produce any significant improvement in weight gains or feed

conversion. Levels of 25.50 and 34.0 percent peanut meal produced a significant increase in heart weight expressed in grams per 100 grams of body weight. The peanut meal had no significant effect on moisture, fat or protein contents of the dressed carcass.

MATERIALS AND METHODS

Three different samples taken from three different batches of Iraqi peanut meal shipped to Lebanon at different seasons of the year and one sample of soybean meal from the United States were obtained. These samples were used for proximate analysis including calcium and phosphorous, microbiological assays, available lysine, aflatoxin determination and PER determinations. From now on, references will be made to samples A, B, and C, which stand for the first, second and third shipments of peanut meal respectively.

Proximate Analysis

Percent water, ether extract, crude fiber, ash, calcium and phosphorous were determined using the methods described by the Association of Official Agricultural Chemists (AOAC), 1960. Nitrogen was determined using a modified Kjeldahl method and crude protein was calculated by multiplying percent nitrogen by 6.25. Nitrogen free extract (NFE) was calculated using the following formula:

$$\% \text{ NFE} = 100 - (\% \text{ water} + \% \text{ ether extract} + \% \text{ crude fiber} + \% \text{ crude protein} + \% \text{ ash}).$$

Microbiological Assays

Lysine, methionine and cystine were determined by

using a modified microbiological assay procedure described by Block and Weiss (1956).

A sample containing about 0.5 gm. of protein was weighed accurately. After refluxing with 60 mls. of 20 percent (V/W) HCl for 8 hours (2 hours for cystine assay), the hydrolysate was transferred to an evaporating dish and allowed to evaporate to 3-5 mls. on a hot plate in order to remove most of the HCl. To the remaining residue, 60 mls. of distilled water was added and the pH was adjusted to 4.0 to precipitate the humins. The hydrolysate was cooled for an hour or more, then filtered through Whatman No. 1 filter paper. After the pH of the filtrate was adjusted to 6.8 by using dilute NaOH, the filtrate was transferred to a 250 ml. volumetric flask and brought to volume with distilled water. One ml. of toluene was added to prevent spoilage and the flask was kept refrigerated for further use. Using values found in the literature for peanut meal, the hydrolysate was diluted to contain about 20 mcg. lysine per ml. in case of lysine assay, 5 mcg. of methionine per ml., and 5 mcg. of cystine per ml. Assay tubes (previously sterilized) were set up in triplicates, containing 0.3, 0.6, and 1.0 ml. of diluted hydrolysate and the volume was made up in each tube to 1.0 ml. with distilled water. Standard amino acid solutions were prepared according to AOAC (1960) methods. The standard tubes (previously sterilized) were set up in triplicates to contain 0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml.

of working standard solution containing the amounts of amino acids stated above, and the volume was made up in each tube to 1.0 ml. with distilled water. Tubes were then placed in a wire rack and covered tightly with a layer of aluminum foil after which they were sterilized by autoclaving at 15 psi. for 15 minutes.

Leuconostoc mesenteroides P60 (ATCC 8042) was used as the assay organism. The organisms were maintained as stab cultures by weekly transfer to solid medium having the following composition:

Peptonized milk	-	10 gms.
Tryptone	-	10 gms.
Agar	-	10 gms.
Tomato juice	-	200 mls.
Distilled water	-	800 mls.

Ten ml. portions of the medium were autoclaved at 15 psi. for 15 minutes and allowed to solidify in the refrigerator. The basal media for the assay of the amino acids were obtained in dry form from Difco Laboratories, Detroit 1, Michigan, U.S.A. These were prepared according to the manufacturer's specifications and later sterilized at 15 psi. for 10 minutes.

Culture tubes containing sterilized growth medium were inoculated with the proper microorganisms from stab cultures and incubated at 37°C for 5-6 hours. After centrifugation to spin down the cells, the supernatant was decanted and the cells resuspended in sterile isotonic sodium chloride solution. A sufficient volume of basal

medium was inoculated at the rate of one drop of saline suspension of the bacteria per 5 ml. medium. One ml. of inoculated basal medium was added to each of the assay and standard tubes under asceptic conditions and incubated at 37°C for 72 hours. Two mls. of 0.004 percent bromothymol blue solution were added to each of the assay and standard tubes and was titrated with 1.0 N. NaOH using a microtitrator.

The standard curve for each amino acid was drawn by plotting the average volume of 1 N. NaOH used for titrating each set of triplicate tubes against the concentration of amino acid in the tube. By extrapolation from the respective standard curves, the concentration of each amino acid in the tubes containing the sample aliquots was determined.

The amino acid contents of each sample were calculated as mgm. amino acid (a.a.) per gm. of nitrogen as follows:

$$\text{Mgm. a.a./gm. N.} = \frac{\text{dilution factor x mcg. a.a./ml.}}{1000 \times \text{gm. N sample}}$$

Available Lysine Determination

The determination of available lysine was done using a modified method described by Carpenter et al. (1955) and Bruno et al. (1957).

A sample containing about 50 mgm. of nitrogen was weighed accurately, transferred to a 100 ml. round bottom

flask fitted with a ground glass stopper and suspended in 8 mls. of 10 percent (W/V) sodium bicarbonate. A solution of 0.3 ml. 2,4-dinitrofluorobenzene (DNFB) in 12 mls. of ethanol was added to the flask which was agitated gently in a mechanical shaker for 2 hours (only the ethanol was added to the blank). The ethanol was then evaporated by unstoppering the flask and holding it in boiling water until there was no further effervescence. Twenty four mls. of 8.1 N HCl were added by stages to reduce frothing, and the materials refluxed on a hot plate for 16 hours. After cooling, the contents of the flask were filtered with water washings and made up to 200 mls. with distilled water. The solution was then refrigerated overnight to help in the precipitation of excess DNFB. After refrigeration, one gram of sodium sulfide ($Na_2S.\ 9H_2O$) per 100 mls. was added to the hydrolysate which was then allowed to stand at room temperature for 5 minutes. Twenty mls. of the acid hydrolysate was added to a separatory funnel and extracted twice with 50 ml. portions of ether. The aqueous portion was transferred into 100 ml. volumetric flask and traces of ether were evaporated in boiling water. The residue was made alkaline with 2 N NaOH solution (after the titration of a dummy aliquot + phenolphthalein) and 20 mls. of buffer were added and the mixture was brought to volume with distilled water. Ten ml. aliquots were taken from these solutions and were poured into the same separatory funnels. To each, 0.05 ml. methoxy

carbonyl chloride was added and the mixture was shaken for ten minutes, after which 0.75 ml. of concentrated HCl was added to each. Two portions of 10 mls. each were extracted with ether and the ether extract was collected in 50 ml. Erlenmeyer flask and evaporated. The residue was dissolved in 10 ml. 1 N HCl with warming, after which it was cooled and the absorbancy was read at a wave length of 435 mu. The extinction was compared with those of E-DNP-lysine-HCl (dinitrophenyl lysine hydrochloride) standard aliquots containing 12-28 micrograms per ml.

Calculation:

$$\text{Mgm. lysine/gm. N} = \frac{\text{mcg./ml.}}{1000} \times 1000 \frac{\text{Mol. wt. lysine}}{\text{Mol. wt. DNP-lysine-HCl}} \times \frac{100}{\% \text{ N} \times \text{sample}} .$$

$$\text{Mgm. lysine/gm. N} = 41.71 \times \frac{\text{mcg./ml.}}{\% \text{ N} \times \text{sample}} .$$

Aflatoxin Determination

The detection of aflatoxin in three different samples of Iraqi peanut meal was determined by using a method described by the Tropical Products Institute (T.P.I.), 1962.

1. Preparation of thin layers of aluminum oxide (chromatoplates).

A thin layer chromatography apparatus[1] was used.

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[1] C. Desaga, G. m. b. H., Heidelberg, Germany.

Thirty grams of aluminum oxide G was mixed with 60 mls. distilled water in an Erlenmeyer flask. The quantity was enough to coat 5 glass plates 20 x 20 cm. each. The coated plates were heated in an oven at 100°C for 2 hours, after which they were cooled and stored in a vacuum dessicator.

2. Extraction and chromatography of the toxin.

Twenty grams of defatted peanut meal were extracted with methanol for 4 hours in a 100 ml. Soxhlet extractor (siphon rate 6/hr.). The extract was concentrated to 50 mls. and transferred to a separatory funnel. The extraction flask was rinsed with 25 mls. distilled water and added to the separator. An additional 5 mls. of water were added to the flask to remove the remaining solid material and transferred to the separator. The flask was washed with 25 mls.of chloroform and transferred to the separator. The separator was shaken gently after which the chloroform phase was run off through a bed of anhydrous sodium sulfate. The extraction was repeated three additional times using 3 x 25 ml. chloroform and extracts were bulked and then concentrated to 35 mls. Five µl. and 20 µl. aliquots of this concentrate were spotted near to one edge of a chromatoplate and were developed with 1.5 percent methanol in chloroform until a solvent path of 10 cm.

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from the base line has been obtained. The resultant chromatogram was examined under ultra-violet light ($\nearrow$ 3650 A^{o}) and the presence or absence of a blue/ purple fluorescent spot at Rf. 0.5-0.6 was observed. When no fluorescence was visible in this region, the residual chloroform extract was concentrated to 5 mls. and 15 µl. of this concentrate was chromatographed as before.

3. Level of toxicity.

The toxicity level of the meal being examined was classified on the basis of the presence or absence of fluorescence at Rf. 0.5-0.6 after the chromatography of the aliquots referred to previously.

	Toxicity level (ppm)	
Aliquot	No fluorescence observed	Fluorescence observed
5 ul. out of 35 ml.	<2.0	>2.0
20 ul. out of 35 ml.	<0.5	0.5-2.0
15 ul. out of 5 ml.	<0.1	0.1-0.5

4. Check sample of commercial peanut meal.

A standard sample of peanut meal was obtained from England containing 10 ppm. aflatoxin. Twenty grams of the standard sample received were extracted using the same procedure as above, diluting the dry chloroform

extract thus obtained to 60 ml. with chloroform (solution A). Five mls. of solution A were diluted to 35 mls. with chloroform and then 5 µl. and 20 µl. were spotted on a chromatoplate developing the plate as described previously. If two fluorescent spots at Rf. 0.5-0.6 were obtained, the sample was considered falling in the very high category (i.e. over 2.0 ppm). If only one spot was observed corresponding to the 20 µl. loading, the category of toxicity was considered high (i.e. 0.5-2.0 ppm). If no spots were observed, the remainder of 35 mls. was concentrated to 5 mls. and 15 µl. from the concentrated sample were then spotted on a further chromatoplate. A blue fluorescent spot of Rf. 0.5-0.6 following developing of the plate indicated that the category of toxicity of the sample was medium (0.1-0.5 ppm). If no fluorescent spot at Rf. 0.5-0.6 was observed following this part of the procedure, the sample was considered to have contained less than 0.1 ppm and in consequence would have been classified as falling in either the low or the negative levels of toxicity.

Animal Experiments

Four experiments were conducted at the Agricultural Research and Education Center (AREC) of the American University of Beirut in the Beka'a, Lebanon. The experiments

were performed between March 1964 and November 1964.

Chicks used in experiments I, II and IV were male day-old cross-bred broilers (Cornish x White Plymouth Rock), while those used in experiment III were straight-run broiler chicks of the same cross. Chicks in experiments I and II were started in wire-floored, thermostatically controlled 5-deck electric brooder batteries. Each deck was divided into two equal parts and each part was used as one pen. Room temperature ranged between 60°F and 80°F, and light was distributed uniformly among different decks through the use of side-wall bulbs for 24 hours daily.

PER was determined in experiment I and II using a modified procedure of Campbell (1963). Male day-old chicks of the same strain (Cobb) were raised to 4 weeks of age. The percentage composition of the basal diet was corn starch, 85; mineral mixture, 5.3; corn oil, 5.0; non nutritive cellulose, 3.5; vitamin mixture, 1.0; choline chloride, 0.2. To the basal diet, sufficient peanut or soybean meal was added at the expense of corn starch to provide 10 or 20 percent protein. In experiment II, lysine, methionine, tryptophane, isoleucine and valine were incorporated in diets containing peanut meal while soybean meal diets were not supplemented. The levels of amino acid used were determined on the bases of equalizing the amino acid pattern in the peanut meals used to that of soybean meal.

In each of experiments I and II 80 chicks were divided into 16 groups of equal average weights. A randomized block design was used and both chick groups and experimental diets were randomly assigned to each pen. The composition of the experimental diets used in experiments I and II are shown in Tables 1 and 2 respectively.

The possible toxicity of peanut meal samples was tested in experiments I and II by the method suggested by Campbell (1963) for use with rats. The growth of chicks fed the test diet at 10 and 20 percent protein levels for 4 weeks was compared with that of chicks fed similar levels of protein from soybean meal.

Two hundred straight-run day-old chicks were used in experiment III while 250 male day-old chicks were used in experiment IV. In each experiment, chicks were divided into 10 groups of equal average weights.

Experimental diets were formulated according to feed composition tables compiled by Hubbell (1963). Those used in experiment III are shown in Table 3 while those used in experiment IV are shown in Table 4. The rations were pelleted by pressure in a local feed manufacturing plant. In experiment III, soybean meal was replaced by peanut meal at levels of 0, 8, 16, 24 and 32 percent of the total ration. All other ingredients used were kept constant. Since the peanut meal used was about 6 percent higher in crude protein than soybean meal, the protein level of the diets increased

Table 1 - Composition of experimental diets (%) - Experiment I.

Ingredients	Diet number							
	1	2	3	4	5	6	7	8
Vitamin mixture[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mixture[2]	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
Non-nutritive cellulose	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Corn starch	66.2	65.0	65.4	61.7	47.4	45.0	45.8	38.4
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Peanut meal A	18.8				37.6			
Peanut meal B		20.0				40.0		
Peanut meal C			19.6				39.2	
Soybean meal				23.3				46.6

[1] The vitamin mixture was prepared at the AREC. It furnishes the following per lb. of diet: vitamin A, 4540 IU; vitamin D_3, 681 ICU; vitamin E, 10 mg; menadione, 2 mg; PABA, 30 mg; inositol, 60 mg; folic acid, 1.5 mg; biotin, 100 mcg; thiamine, 2 mg; niacin, 40 mg; pantothenate, 10 mg; vitamin B_{12}, 10 mcg; pyridoxine, 2 mg; ascorbic acid, 100 mg.

[2] The mineral mixture was prepared at the AREC. It furnishes the following per lb. of diet: Ca, 5.0 gm; P, 1.90 gm; Mn, 50.46 mg; I_2, 1.16 mg; Fe, 18.46 mg; Cu, 1.72 mg; Zn, 0.11 mg; Co, 0.17 mg; K, 0.02 gm; Mg, 299 mg.

Table 2 - Composition of experimental diets (%) - Experiment II.

Ingredients	Diet number							
	1	2	3	4	5	6	7	8
Vitamin mixture[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mixture[2]	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
Non-nutritive cellulose	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Corn starch	66.2	65.0	65.4	61.7	47.4	45.0	45.8	38.4
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Peanut meal A	17.98				35.96			
Peanut meal B		19.30				38.60		
Peanut meal C			18.90				37.80	
Soybean meal				23.30				46.60
Lysine	0.50	0.46	0.45		1.00	0.92	0.90	
Methionine	0.07	0.07	0.05		0.14	0.12	0.10	
Tryptophane	0.04	0.03	0.03		0.08	0.06	0.06	
Isoleucine	0.16	0.13	0.14		0.32	0.26	0.28	
Valine	0.05	0.02	0.03		0.10	0.04	0.06	

[1] For composition see same footnote in Table 1.

[2] For composition see same footnote in Table 1.

Table 3 - Composition of experimental diets (%) - Experiment III.

Ingredients	Experimental diets				
	1	2	3	4	5
Yellow corn	59.75	59.75	59.75	59.75	59.75
Soybean meal	32	24	16	8	0
Peanut meal	0	8	16	24	32
Fish meal	2	2	2	2	2
Alfalfa meal	2	2	2	2	2
Bone meal	2.75	2.75	2.75	2.75	2.75
Limestone	0.75	0.75	0.75	0.75	0.75
Salt	0.50	0.50	0.50	0.50	0.50
Vitamin and mineral mixture[1]	0.25	0.25	0.25	0.25	0.25
Calculated composition[2] (%)					
Protein	21.12	21.60	22.08	22.56	23.04
Methionine	0.41	0.38	0.36	0.33	0.31
Cystine	0.32	0.33	0.34	0.35	0.36
Lysine	1.19	1.06	0.95	0.83	0.71
Productive energy (Cal./lb.)	893	909	924	939	954

[1] Vitamin and mineral mixture was obtained from Nopco Chemical Company as Nopcosol M-5. It furnishes the following per lb. of diet: vitamin A, 1750 USP; vitamin D_3, 500 ICU; vitamin E, 0.5 IU; riboflavin, 1.5 mg; niacin, 10 mg; pantothenic acid, 2.5 mg; choline chloride, 100 mg; vitamin B_{12}, 2.5 mcg; manganese, 27.2 mg; zinc, 12.5 mg; iodine, 0.5 mg; Fe, 9.1 mg; Cu, 0.9 mg; Co, 90.8 mcg; zinc bacitracin, 2.0 mg; BHT, 56 mg.

[2] Figures are based on the feed composition tables provided by Hubbell (1963) except in case of peanut meal which was analysed by the author.

Table 4 - Composition of experimental diets (%) - Experiment IV.

Ingredients	Experimental diets				
	1	2	3	4	5
Yellow corn	52.75	64.75	64.75	64.75	64.75
Soybean meal	33.0	-	-	-	-
Peanut meal	-	27.00	26.91	26.48	26.39
Fish meal	2.0	2.0	2.0	2.0	2.0
Alfalfa meal	2.0	2.0	2.0	2.0	2.0
Limestone	0.75	0.75	0.75	0.75	0.75
Bone meal	2.75	2.75	2.75	2.75	2.75
Salt	0.50	0.50	0.50	0.50	0.50
Vitamin and mineral mixture[1]	0.25	0.25	0.25	0.25	0.25
Corn oil	6.0	-	-	-	-
MHA	-	-	0.09	-	0.09
L-lysine-HCl	-	-	-	0.52	0.52
Calculated composition[2] (%)					
Protein	21.11	21.23	21.37	21.48	21.52
Methionine	0.32	0.27	0.36	0.27	0.36
Cystine	0.28	0.24	0.24	0.24	0.24
Lysine	1.59	1.07	1.07	1.59	1.59
Productive energy (Cal./lb.)	990	1002	1002	998	997

[1] For composition see same footnote in Table 3.

[2] See same footnote in Table 3.

with an increase in peanut meal. In experiment IV, however, the diets were kept approximately equal in protein and energy by using the proper amounts of corn and corn oil. In the same experiment, diet 3 was supplemented with MHA, diet 4 was supplemented with L-lysine-HCl while diet 5 was supplemented with both amino acids. This was done in order to bring the level of these amino acids up to that present in the soybean meal diet.

At the termination of experiments III and IV, four average weight birds from each pen were dressed and eviscerated; the hearts, livers and spleens of these birds were removed and weighed immediately. For carcass analysis, 4 halves of these birds from experiment III and 2 halves from experiment IV were ground in an electric meat grinder and a sample was taken from the pooled half carcasses. Analytical methods described by AOAC (1960) were used.

Chicks in experiments III and IV were raised in floor pens using infra-red bulbs for brooding purposes. The house was divided into 10 pens, 5 pens on each side with a middle pathway. Chicks were brooded for 4 weeks and kept in the same pens till 8 weeks of age. Light was provided for 24 hours using ceiling light bulbs. They were vaccinated with Newcastle disease water vaccine at 8 days of age. In experiment III, few birds had an attack of coccidiosis at 6 weeks of age and a sulfa drug was administered to all. In experiment IV, however, some chicks showed respiratory

troubles at 4 weeks of age and so all were treated with antibiotics.

In all the experiments, water and feed were allowed ad libitum. Water was changed and waterers were cleaned daily. Chicks were wing-banded at one day of age, and individual chick weights were recorded at one week intervals with pen feed consumption calculated at the same time. A randomized block design was used in all experiments and both chick groups and experimental diets were assigned randomly to each pen. Two pens were allocated for each treatment and the data analysed statistically according to Snedecor (1956) and multiple comparisons were made using the method of Duncan (1955).

EXPERIMENTAL RESULTS

The proximate composition including calcium and phosphorous of three Iraqi peanut meal samples and one soybean meal sample are presented in Table 5. Values for methionine, cystine and lysine of the same samples along with available lysine are presented in Tables 6 and 7 respectively.

The aflatoxin contents of the peanut meal samples are presented in Table 8 which includes results obtained by the author and those obtained by a commercial laboratory in England. Aflatoxin levels obtained by the above laboratory are based on thin layer chromatography using Kieselgel plates with $CHCl_3$/MeOH 95:5 as developing solvent and accepting the T.P.I. limit of fluorescence as 0.006 u.

Table 5 - Proximate composition (including calcium and phosphorous) of three Iraqi peanut meals and one soybean meal.

Sample	Water (%)	Crude protein (%)	Ether extract (%)	Crude fiber (%)	Ash (%)	N.F.E. (%)	Calcium (%)	Phosphorous (%)
PM[1]-A	9.51	52.83	1.75	4.38	6.44	25.09	0.18	0.55
PM-B	8.92	50.48	1.33	4.40	5.95	28.92	0.24	0.54
PM-C	8.06	50.88	0.99	4.76	7.90	27.41	0.24	0.54
SM[2]	10.91	43.23	0.79	6.47	6.28	32.32	0.29	0.57

1 Peanut meal.

2 Soybean meal.

Table 6 - Amino acid content of three Iraqi peanut meals.

Sample	mg./gm. N		
	Methionine	Cystine	Lysine
Peanut meal - A	48.6	51.0	342.0
Peanut meal - B	53.5	53.4	358.4
Peanut meal - C	56.9	65.4	402.8
Soybean meal	75.5	62.4	633.5

Table 7 - Available lysine content of three Iraqi peanut meals.

Sample	Available lysine mg./gm. N
Peanut meal - A	91.7
Peanut meal - B	151.3
Peanut meal - C	138.2
Soybean meal	273.4

Table 8 - Aflatoxin content of three Iraqi peanut meals.

Sample	Toxicity level	
	Author	Walton Oaks Laboratory[1]
Peanut meal - A	>2 ppm	1.2 ppm
Peanut meal - B	0.1-0.5 ppm	0.8 ppm
Peanut meal - C	>2 ppm	0.5 ppm

1 The Walton Oaks Experimental Station, Vitamins LTD, Dorking Road, Tadworth, Surrey, England.

Experiment I

This experiment was conducted to study the protein quality of three samples of Iraqi peanut meal and one soybean meal by using the 28 day PER assay. For this experiment, the growth response of day-old chicks was measured.

Average weight gain, feed consumption and PER values for the different meals are presented in Table 9. As the level of soybean meal was increased from 10 to 20 percent protein, the weight gain and feed intake of the chicks increased while the PER decreased. In chicks fed the peanut meals, weight gain and feed intake increased as the level of the protein increased from 10 to 20 percent. However, these values were lower than those of the birds fed soybean meal. PER values of the different peanut meals increased as the level of the protein increased from 10 to 20 percent.

The data on 0-4 week weight gain and PER were analysed statistically and the results are shown in Tables 10 and 11. Significant differences at the 1% level of probability were found among treatments in both body weight gain and PER. Means of body weight gain were separated using Duncan's (1955) multiple range test. It is observed that peanut meals fed 10 percent protein did not differ significantly nor did they differ when they were fed at 20 percent protein. Peanut meals fed at 20 percent protein gave superior response to same meals fed at 10 percent. Soybean meal fed at 10

percent protein did not differ significantly from any of the peanut meals fed at 20 percent protein. Soybean meal fed at 20 percent protein was significantly higher than all other treatments.

When PER values were separated by Duncan's multiple range test, PER values of the peanut meals did not differ significantly whether fed at 10 or 20 percent protein. However peanut meals fed at 20 percent had higher PER values than those fed at 10 percent. The reverse was observed in the case of soybean meal where PER values at the 10 percent protein level were higher than those at 20 percent. Soybean meal fed at either 10 or 20 percent protein did not differ significantly in PER. Body weights at weekly intervals are presented in Table 12. These data demonstrate that the nutritive value of the protein of peanut meal was lower than that of soybean meal both at 10 and 20 percent protein levels. Furthermore, they show that the protein quality of the three different samples of peanut meal was similar.

The comparison of growth of chicks fed soybean meal or peanut meal at 10 and 20 percent levels of protein as a test for possible toxicity is presented in Figure 1. The slope of the line showing the increase in weight gain with the increase in protein level for soybean meal was found to be 27.3 which is much higher than slopes obtained for peanut meal A, B and C which were 9.3, 10.3, and 9.9 respectively.

Table 9 - Average weight gains, feed consumption of chicks fed 10 and 20 percent protein diets and PER values of three Iraqi peanut meals and one soybean meal - Experiment I.

Level and source of protein in diet[1]	Average gain 4 weeks (gms.)	Average feed intake 4 weeks (gms.)	PER 4 weeks
10% Soybean meal	115 ± 15.1[2]	392	2.92 ± 0.234[2]
10% Peanut meal A	37 ± 2.8	243	1.52 ± 0.240
10% Peanut meal B	37 ± 4.8	230	1.57 ± 0.219
10% Peanut meal C	27 ± 2.8	179	1.54 ± 0.120
20% Soybean meal	388 ± 11.7	766	2.55 ± 0.169
20% Peanut meal A	130 ± 10.4	346	1.86 ± 0.099
20% Peanut meal B	140 ± 14.2	388	1.80 ± 0.141
20% Peanut meal C	126 ± 10.5	338	1.85 ± 0.071

[1] Ten chicks per diet.

[2] Mean ± Standard Error.

Table 10 - Analysis of variance of 0-4 weeks average weight gains and PER - Experiment I.

Source of variance	d.f.	M.S.	
		0-4 weeks weight gain	PER
Replication	1	189.00	0.040
Treatment	7	27012.00**	0.524**
Error	7	102.00	0.047

** Significant at 1% level of probability.

Table 11 - Separation of means by Duncan's multiple range test - Experiment I.

	Body weight gains (gms.)							
Treatment	10% PM[1] C	10% PM A	10% PM B	10% SM[2]	20% PM C	20% PM A	20% PM B	20% SM
Means[3]	27	37	37	115	126	130	140	388

	PER							
Treatment	10% PM A	10% PM C	10% PM B	20% PM B	20% PM C	20% PM A	20% SM	10% SM
Means[3]	1.52	1.54	1.57	1.80	1.85	1.86	2.55	2.92

1 Peanut meal.

2 Soybean meal.

3 Means not underlined by same continuous line are significantly different at the 5% level of probability.

Table 12 - Average body weight in grams at weekly intervals - Experiment I.

Level and source of protein in diet	Age in weeks				
	0	1	2	3	4
10% Soybean meal	41	70	107	134	156
10% Peanut meal A	42	60	70	74	78
10% Peanut meal B	42	61	68	74	78
10% Peanut meal C	42	58	64	64	68
20% Soybean meal	42	86	175	298	430
20% Peanut meal A	42	68	94	129	172
20% Peanut meal B	41	72	101	138	181
20% Peanut meal C	42	66	93	128	168

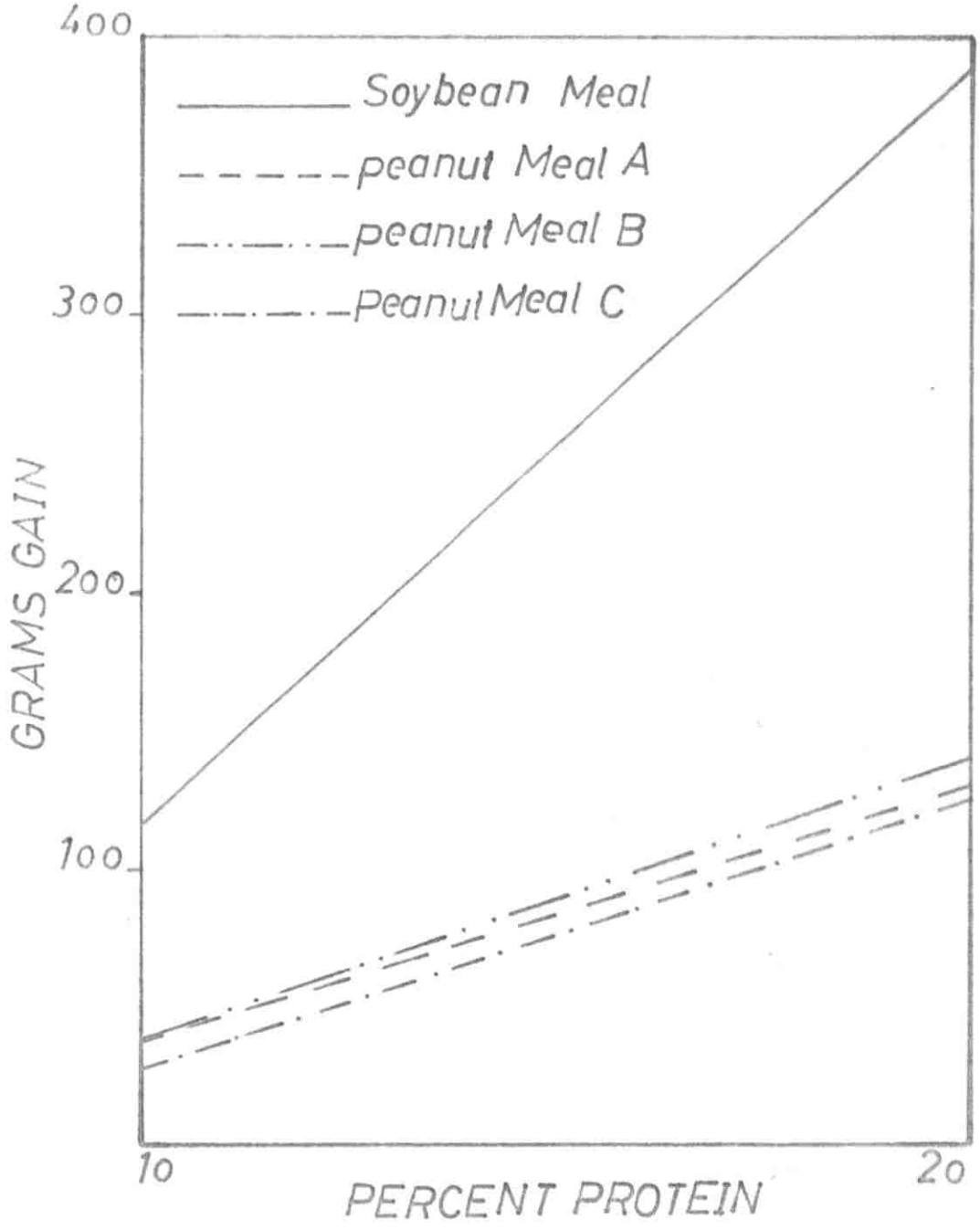

Figure 1. Difference in 4-week body weight gains of chicks fed 10 and 20 percent protein from soybean meal and peanut meals.

Experiment II

This experiment was conducted to study the effect on protein quality of supplementing the peanut meals with methionine, lysine, tryptophane, isoleucine and valine.

The average weight gain, feed consumption and PER values for the different meals are presented in Table 13. As the level of soybean meal was increased from 10 to 20 percent protein in the diet, the weight gain and feed intake of the chicks increased, while PER decreased. In case of the peanut meal samples, weight gain and feed intake of the chicks also increased as the level of the peanut meal protein increased from 10 to 20 percent. PER, however, decreased with an increase in the protein level from 10 to 20 percent, but were lower than that of the soybean meal. The data on 0-4 week weight gain and PER were analysed statistically. The results are shown in Tables 14 and 15. Statistical differences occurred among treatments at the 1% level of probability. The means of the weight gain of chicks are separated by Duncan's multiple range test and shown in Table 15. Peanut meal samples fed at 10 percent protein did not differ significantly from the soybean meal fed the same protein level. This indicates that at 10 percent protein, amino acid supplementation of peanut meal groups gave similar results to the soybean meal group. When peanut meals B and C were fed at 20 percent protein

level they differed significantly from the soybean meal fed at 20 percent protein. Peanut meal A, however, fed at the 20 percent level did not differ significantly from soybean meal fed at the same protein level. Amino acid supplementation of peanut meal A fed at 20 percent protein improved gain to the same extent as soybean meal which was not the case with peanut meals B and C.

PER values were also separated by Duncan's multiple range test and are presented in Table 15. Peanut meal B fed at 20 percent protein gave the poorest PER, but was not significantly lower than peanut meal A or C fed at 20 percent protein. Peanut meal B at 10 percent level of protein gave significantly higher PER than the same meal fed at 20 percent protein level. This was not true in the case of peanut meals A or C. Soybean meal fed at 20 percent protein gave a significantly higher PER value than peanut meals B or C fed at the same levels, but was not significantly different from peanut meal A fed at the same protein level. Here again, soybean meal fed at the 10 percent protein level gave the highest PER.

Body weights at weekly intervals are presented in Table 16. These data demonstrate that the nutritive value of peanut meal is lower than that of soybean meal both at 10 and 20 percent protein levels even though they were supplemented with certain amino acids bringing the total level of these amino acids in the peanut meals equal to that

present in the soybean meal. They also demonstrate that samples B and C were similar in nutritive value, while sample A was superior and approaches soybean meal in value when properly supplemented with its limiting amino acids.

Comparison of growth of chicks fed soybean meal or amino acid supplemented peanut meal at 10 and 20 percent levels of protein as a test for possible toxicity is presented in Figure 2. The slope of the line showing the increase in weight gain with the increase in protein level for soybean meal was found to be 29.6, while that obtained for peanut meal samples A, B and C were 25.6, 18.8, and 19.4 respectively.

Table 13 - Average weight gains and feed consumption of chicks fed 10 and 20 percent protein diets supplemented with several amino acids, and PER values of three Iraqi peanut meals and one soybean meal - Experiment II.

Level and source of protein in diet[1]	Average gain 4 weeks (gms.)	Average feed intake 4 weeks (gms.)	PER 4 weeks
10% Soybean meal	114 ± 20.1[3]	385	2.94 ± 0.094[3]
10% Peanut meal A + a.a.[2]	76 ± 17.1	314	2.40 ± 0.094
10% Peanut meal B + a.a.	72 ± 10.7	302	2.34 ± 0.141
10% Peanut meal C + a.a.	64 ± 8.0	288	2.24 ± 0.219
20% Soybean meal	410 ± 29.9	802	2.55 ± 0.071
20% Peanut meal A + a.a.	332 ± 15.5	766	2.17 ± 0.120
20% Peanut meal B + a.a.	260 ± 18.2	690	1.88 ± 0.070
20% Peanut meal C + a.a.	258 ± 17.5	658	1.95 ± 0.070

[1] Ten chicks per diet.

[2] Amino acids - for exact levels see Table 2.

[3] Mean ± Standard Error.

Table 14 - Analysis of variance of 0-4 weeks average weight gains and PER - Experiment II.

Source of variance	d.f.	M.S.	
		0-4 weeks weight gain	PER
Replication	1	1387	0.070
Treatment	7	36234**	0.230**
Error	7	1521	0.015

** Significant at 1% level of probability.

Table 15 - Separation of means by Duncan's multiple range test - Experiment II.

Body weight gains (gms.)

Treatment	10% PM[1] C	10% PM B	10% PM A	10% SM[2]	20% PM C	20% PM B	20% PM A	20% SM
Means[3]	64	72	76	114	258	260	332	410

PER

Treatment	20% PM B	20% PM C	20% PM A	10% PM C	10% PM B	10% PM A	20% SM	10% SM
Means[3]	1.88	1.95	2.17	2.24	2.34	2.40	2.55	2.94

1 Peanut meal + amino acids for all PM treatments.

2 Soybean meal.

3 Means not underlined by the same continuous line are significantly different at the 5% level of probability.

Table 16 – Average body weight in grams at weekly intervals - Experiment II.

Level and source of protein in diet	Age in weeks				
	0	1	2	3	4
10% Soybean meal	40	70	104	128	154
10% Peanut meal A + a.a.[1]	40	60	80	97	116
10% Peanut meal B + a.a.	40	62	80	92	110
10% Peanut meal C + a.a.	40	61	76	91	104
20% Soybean meal	40	89	170	268	450
20% Peanut meal A + a.a.	40	80	152	251	373
20% Peanut meal B + a.a.	40	78	149	205	300
20% Peanut meal C + a.a.	40	76	120	208	298

[1] Amino acids - for exact levels see Table 2.

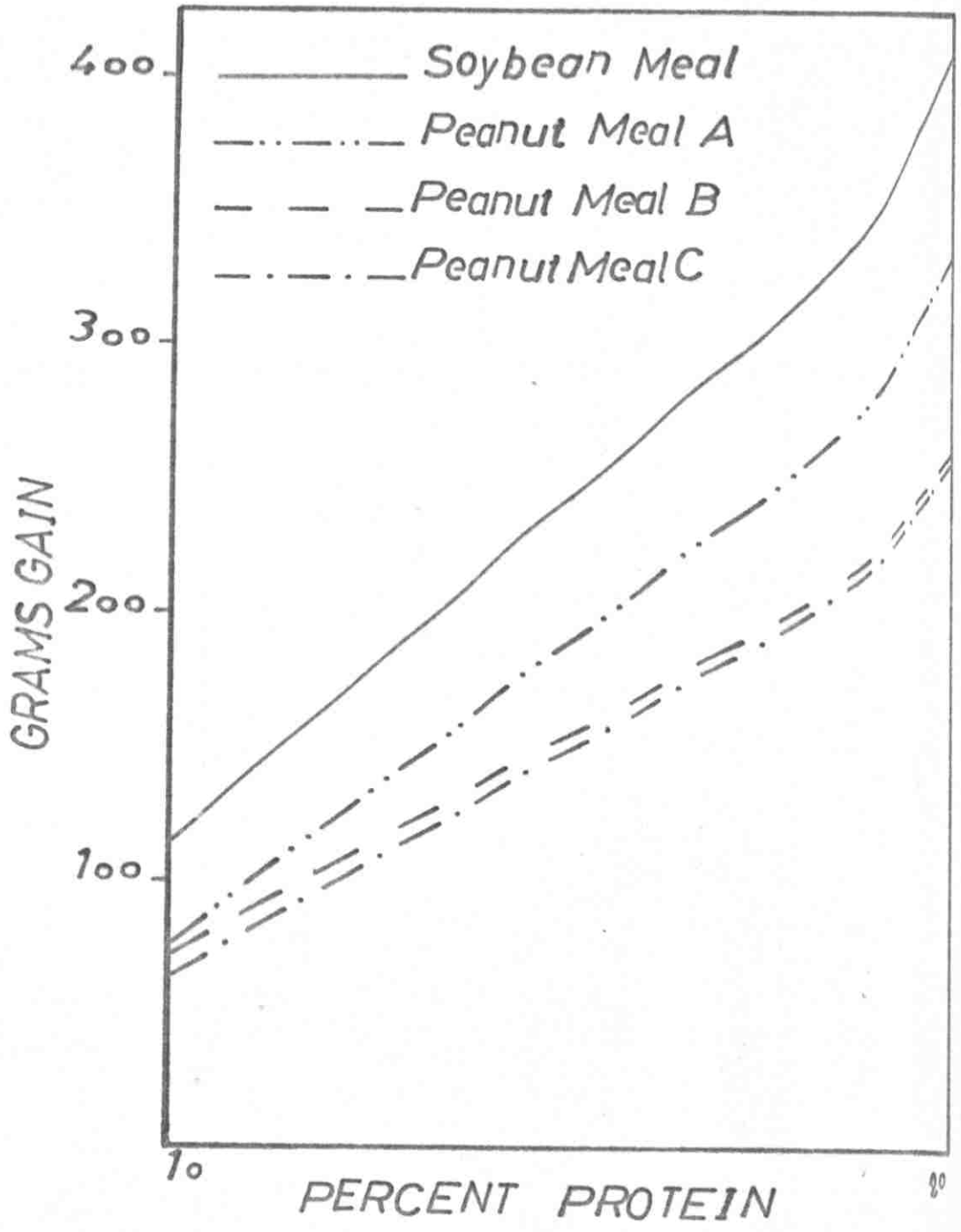

Figure 2. Difference in 4-week body weight gains of chicks fed 10 and 20 percent protein from soybean meal and amino acid supplemented peanut meals.

Experiment III

This experiment was conducted to study the effect of replacing soybean meal partially or completely with peanut meal in a practical broiler ration on body weight gains, feed efficiency, vital organ weights and carcass composition of broiler chicks raised to 8 weeks of age.

The effects of replacing different levels of soybean meal by peanut meal on body weight gains, feed efficiency, vital organ weights and carcass composition are shown in Table 17. As the level of peanut meal was increased, body weight gains decreased from 1448 to 1177 grams. In the same way, feed per gain increased with an increase in peanut meal levels. The statistical analysis of the data on live weight gains and feed per gain are shown in Tables 18 and 19. Chicks receiving 32, 24, and 16 percent peanut meal did not differ significantly in body weight gains. This is shown by the separation of body weight gain means by Duncan's multiple range test. Furthermore, chicks receiving 24, 16, 8, and 0 percent peanut meal did not differ significantly in body weight gains, but there was a significant difference in body weight gain between those receiving 0 or 8 percent peanut meal and those receiving 32 percent peanut meal.

Feed intake decreased with an increase of peanut meal in the diet. Table 17 shows that organ weights and carcass composition of the birds receiving the different treatments

were very similar. Body weights at weekly intervals are shown in Table 20. Differences in body weight began to appear at one week of age and continued to the end of the experiment. These differences between the 0 and 32 percent level of peanut meal were very striking early in life or about 3-5 weeks of age, but diminished as the birds got older; for example at 3 weeks groups receiving 32 percent peanut meal weighed one third less than groups receiving 0 percent peanut meal. This ratio, however, dropped to one fifth at 8 weeks of age. This may indicate that the effects of amino acid deficiency and/or toxic principles decrease as the chick gets older.

Table 17 - Effect of different levels of peanut meal on body weight gains, feed per gain, organ weights and carcass composition - Experiment III.

	Peanut meal added (%)				
	00.0	08.0	16.0	24.0	32.0
Body weight gain (gms.) (0-56 days)	1448	1425	1339	1306	1177
Feed consumption (gms.)	3413	3368	3243	3228	3085
Feed/gain	2.35	2.36	2.43	2.48	2.62
Mortality[1]	1/40	6/40	1/40	1/40	0/40
Organ weights[2]					
Heart	0.49	0.51	0.50	0.50	0.52
Liver	1.89	1.98	1.64	1.90	2.10
Spleen	0.21	0.23	0.21	0.19	0.20
Carcass composition (%)					
Moisture	67.26	66.90	65.40	66.52	66.43
Fat	10.05	11.04	12.19	11.34	11.96
Protein	18.57	17.79	17.62	18.47	17.99

[1] Chicks dead at 8 weeks out of 40 started.

[2] Grams per 100 grams body weight.

Table 18 - Analysis of variance of 0-8 weeks average live weight gain and feed per gain - Experiment III.

Source of variance	d.f.	M.S.	
		0-8 weeks live weight gain	Feed/ gain
Replication	1	3063	0.005
Treatment	4	23375**	0.023
Error	4	2371	0.004

** Significant at 1% level of probability.

Table 19 - Separation of means by Duncan's multiple range test - Experiment III.

	Body weight gains (gms.)				
Treatment	32% PM[1]	24% PM	16% PM	8% PM	0% PM
Means[2]	1177	1306	1339	1425	1448

[1] Peanut meal.

[2] Means not underlined by same continuous line are significantly different at the 5% level of probability.

Table 20 - Effect of different levels of peanut meal on average body weights (grams) at weekly intervals - Experiment III.

Peanut meal added (%)	Age in weeks								
	0	1	2	3	4	5	6	7	8
00.0	40	78	166	308	486	710	964	1246	1488
08.0	40	78	163	291	465	654	879	1180	1466
16.0	40	73	148	273	436	630	883	1134	1379
24.0	40	70	140	256	406	592	828	1084	1346
32.0	40	62	116	204	337	518	735	978	1217

Experiment IV

This experiment was conducted to study the effects of completely replacing soybean meal with peanut meal in a practical broiler ration and to test the effects of supplementation of the peanut meal with methionine hydroxy analogue (MHA) and L-lysine-HCl singly or in combination on the same criteria as those studied in experiment III.

The effect of replacing completely soybean meal with peanut meal and of supplementing the peanut meal diet with methionine and lysine singly or in combination on body weight gains, feed efficiency, vital organ weights and carcass composition is shown in Table 21. The statistical analyses of the data on live weight gains, feed efficiency and fat and moisture content of carcasses are shown in Tables 22, 23, 24 and 25 respectively. Chicks receiving corn-soybean meal diets and those receiving peanut meal supplemented with 0.09 percent methionine and 0.57 percent lysine had significantly higher body weight gains than all other experimental groups. Those receiving the peanut meal diet supplemented with 0.57 percent lysine had significantly higher body weight gains than those receiving peanut meal unsupplemented or supplemented with 0.09 percent methionine. The addition of 0.09 percent methionine to the peanut meal diet did not result in any significant improvement in body weight gains over the unsupplemented peanut meal diet. The

addition of 0.09 percent methionine and 0.57 percent lysine to peanut meal diets significantly improved body weight gains which made this diet practically equal to that of the soybean meal diet. Feed per gain shows a similar trend to that of body weight gains. There were no significant differences in feed per gain between the peanut meal diet supplemented with both methionine and lysine or the soybean meal diet. No significant differences, however, were found in feed per gain among the peanut meal unsupplemented diet, the peanut meal diet supplemented with methionine, or the peanut meal diet supplemented with lysine alone.

Means of moisture and fat content of carcasses were separated using Duncan's multiple range test. Table 25 shows that moisture content of carcasses of birds receiving all-peanut meal diets supplemented with methionine was significantly higher than all other treatments. There were no significant differences in moisture content among those receiving unsupplemented peanut meal diet, peanut meal diet supplemented with both lysine and methionine or peanut meal diet supplemented with lysine alone. Those receiving the soybean meal diet had significantly lower moisture content than all other treatments. The fat content of carcasses of birds receiving the peanut meal diet supplemented with methionine was significantly lower than those receiving all other diets. No significant difference was found in fat content among groups receiving the unsupplemented peanut meal

diet, peanut meal diet supplemented with lysine and methionine, and peanut meal diet supplemented with lysine alone. Those receiving the soybean meal diet had significantly higher fat content than all other treatments studied. There were no significant differences in protein content of the carcasses or in organ weights of birds receiving the different treatments.

Body weights at weekly intervals are shown in Table 26. Here again differences in body weight among treatments began to appear at one week of age, and were most significant during the 3 to 5 week period. These differences continued and were fairly well pronounced by 8 weeks.

Table 21 - Effect of amino acid supplementation of corn-peanut meal diets on body weight gains, feed per gain, organ weights and carcass composition - Experiment IV.

	Dietary treatment				
	SM[1]	PM[2]	PM + MHA[3]	PM + L-lysine-HCl	PM + MHA + L-lysine-HCl
Body weight gain (gms.) (0-56 days)	1410	952	973	1147	1342
Feed consumption (gms.)	3006	2297	2317	2645	2832
Feed/gain	2.13	2.41	2.39	2.31	2.11
Mortality[4]	2/50	2/50	5/50	7/50	4/50
Organ weights[5]					
Heart	0.48	0.52	0.49	0.54	0.45
Liver	1.90	2.00	2.00	2.10	2.00
Spleen	0.19	0.16	0.18	0.17	0.18
Carcass composition (%)					
Moisture	66.18	67.97	71.52	67.46	67.84
Fat	13.75	11.63	8.52	12.00	11.94
Protein	17.33	16.44	16.77	16.96	16.53

1 Soybean meal.

2 Peanut meal.

3 Methionine hydroxy analogue.

4 Chicks dead at 8 weeks out of 40 started.

5 Grams per 100 grams body weight.

Table 22 - Analysis of variance of 0-8 weeks average live weight gain and feed per gain - Experiment IV.

Source of variance	d.f.	M.S.	
		0-8 weeks live weight gain	Feed/ gain
Replication	1	547	0.000
Treatment	4	87289**	0.039**
Error	4	3861	0.003

** Significant at 1% level of probability.

Table 23 - Separation of means by Duncan's multiple range test - Experiment IV.

	Body weight gains (gms.)				
Treatment	PM[1]	PM + MHA	PM + lysine	PM + lysine + MHA	SM[2]
Means[3]	952	973	1147	1342	1411

	Feed/gain				
Treatment	PM + lysine + MHA	SM	PM + lysine	PM + MHA	PM
Means[3]	2.11	2.13	2.31	2.39	2.41

1 Peanut meal.

2 Soybean meal.

3 Means not underlined by same continuous line are significantly different at the 5% level of probability.

Table 24 - Analysis of variance of moisture and fat content of carcasses - Experiment IV.

Source of variance	d.f.	M.S.	
		Moisture	Fat
Replication	1	0.0	0.0
Treatment	4	7.9**	5.9**
Error	4	0.1	0.05

** Significant at 1% level of probability.

Table 25 - Separation of means by Duncan's multiple range test - Experiment IV.

	Moisture (%)				
Treatment	SM[1]	PM[2] + lysine -HCl	PM + lysine -HCl + MHA	PM	PM + MHA
Means[3]	66.18	67.46	67.84	67.97	71.52

	Fat (%)				
Treatment	PM + MHA	PM	PM + lysine -HCl + MHA	PM + lysine -HCl	SM
Means[3]	8.52	11.63	11.94	12.00	13.75

1 Soybean meal.

2 Peanut meal.

3 Means not underlined by same continuous line are significantly different at the 5% level of probability.

Table 26 - Effect of amino acid supplementation of a corn-peanut meal diet on average body weight (grams) at weekly intervals - Experiment IV.

Dietary treatment	Age in weeks								
	0	1	2	3	4	5	6	7	8
SM[1]	37	68	142	263	412	644	909	1208	1447
PM[2]	37	58	92	136	210	339	523	761	988
PM + MHA	37	58	100	150	229	363	537	774	1009
PM + L-lysine-HCl	37	60	100	168	282	455	676	928	1184
PM + MHA + L-lysine -HCl	37	63	118	225	373	596	844	1114	1379

1 Soybean meal.

2 Peanut meal.

GENERAL DISCUSSION

Proximate Composition

The data presented in Table 1 show that the three different samples of Iraqi peanut meal are similar in composition. When compared to soybean meal, they are all higher in protein and ether extract and lower in fiber. Peanut meal samples varied in ether extract from 0.99 to 1.75 percent which indicates differences in the thoroughness of extraction, but this does not seem to be related to protein content. Since these samples were all processed and extracted in the same plant, differences in composition can not be attributed to different methods of processing, but rather to conditions used in the same process. Proximate composition of peanut meal taken from different sources is presented in Table 27. These have been obtained from Morrison (1957) for meals in the U.S.A. and Subrahmanyan et al. (1957) for meals in India. The only striking differences are the high fat percent and low fiber percent in the Indian meal sample.

Amino Acid Composition

Data presented in Table 2 show that peanut meal is lower in methionine, cystine, and lysine content than soy-

Table 27 - Proximate composition of peanut meals.

	Morrison (1957)	Subrahmanyan (1957)	Author (average of 3 samples)
Moisture (%)	7.0	11.0	8.8
Protein (%)	52.3	52.7	51.4
Fat (%)	1.6	8.9	1.4
Fiber (%)	6.9	1.0	4.5
Ash (%)	5.9	4.6	6.8
NFE (%)	26.3	21.8	27.1

bean meal, except for sample C of the peanut meal which is slightly higher than soybean meal in cystine content. There is also quite a difference in the methionine, cystine, and lysine content of the three samples of peanut meal. This could be due to different conditions during processing or storage time, specially in hot humid climates. Several workers have reported that there were differences in the lysine, methionine, and cystine content of different samples of peanut meal. Table 28 below shows figures from three references along with the average amino acid contents of the three meals studied in this thesis. Figures given by Orr and Watt (1957) are for meals from the U.S., while those by Shurpalekar et al. (1962) and Tasker et al. (1962) are for meals from India.

Available Lysine Content

The data presented in Table 3 show that the peanut meals studied contain nearly half as much available lysine

Table 28 - Amino acid composition of peanut meals.

	Mgm./gm. N		
	Lysine	Methionine	Cystine
Orr and Watt (1957)	223	55	94
Shurpalekar et al. (1962)	218	56	93
Tasker et al. (1962)	225	62	100
Average of peanut meals A, B and C	367	52	57

as soybean meal. Furthermore, it clearly indicates a big difference in the available lysine content of the three samples of peanut meal. This difference could be due to several factors. Temperature during processing, moisture content, reducing sugars present and oil content have all been shown to affect the available amino acid content, particularly available lysine.

Aflatoxin Content

Data presented in Table 8 show that our figures for aflatoxin do not fully agree with those obtained by the British workers, which indicates that the procedure for aflatoxin determination is not well standardized. Both results indicate, however, that two samples are fairly high in aflatoxin and hence Iraqi peanut meals can be infested with Aspergillus flavus, the mould responsible for the production of the toxin. Peanut meal samples from 12 different countries were analysed by Allcraft et al. (1962) and found to contain high levels of aflatoxin.

PER

In experiments I and II the weight gain and feed intake increased as the protein content of the diet increased from 10 to 20 percent in the case of both soybean meal and peanut meal. This is because the 20 percent protein diet satisfies the amino acid requirement of the chick more than the 10 percent protein diet. The lower feed intake of the chicks receiving peanut meal would explain in part the lower weight gains. In experiment I, weight gains of chicks receiving peanut meal were significantly lower than those receiving soybean meal which indicates that the nutritive value of peanut meal is poorer than that of soybean meal. In experiment II, the supplementation of the peanut meals with methionine, lysine, tryptophane, isoleucine, and valine to make it equal to that of soybean meal resulted in a considerable improvement in growth, but was not equal to that of soybean meal, except for sample A of the peanut meal where the weight gain was not significantly lower than that of soybean meal. This illustrates that the poorer growth obtained by feeding peanut meal is not only due to amino acid limitation but other factors could also be involved.

In both experiments, the PER of the soybean meal decreased as the level of the protein increased from 10 to 20 percent. The chick seems to make better use of the protein ingested when supplied at low levels and is parti-

cularly true when protein levels are barely meeting the requirement with protein wastage being reduced to a minimum. At adequate levels of protein (20%), the PER decreases since there is enough supply of protein for growth, and utilization of protein is reduced along with increased wastage. In case of unsupplemented peanut meal, PER increases as the level of protein increases from 10 to 20 percent. This agrees with the finding of Tasker et al. (1962) that PER of peanut flour increases from 1.84 to 1.98 as the level of peanut flour protein increases from 10 to 15 percent in the diet when rats were used. This is probably due to severe amino acid deficiencies in peanut meal, causing poor protein utilization at lower levels of intake. Increasing the protein level appears to have increased the levels of the limiting amino acids resulting in a better PER. The PER of the supplemented peanut meal obtained in experiment II followed the same trend as that of soybean meal; that is it decreased as the protein level increased from 10 to 20 percent. Furthermore, the PER of peanut meal improved considerably when it was supplemented with lysine, methionine, tryptophane, isoleucine, and valine, but was still significantly lower than that of soybean meal except for sample A. The average PER of unsupplemented peanut meal obtained when used at 10 percent protein level in the diet was 1.54 and the supplemented meals was 2.33. Several workers reported similar PER values (Anantharaman et al.,

1962 (1.48); Joseph et al., 1958 (1.65); Tasker et al., 1962 (1.84) using rats raised to 4 weeks of age and fed 10 percent protein diets.

Slopes of the lines presented in Figure 1 showing the increase in weight gain with the increase in protein level for peanut meals are not the same as that for soybean meal. This indicates that the poor growth observed at the two levels of peanut meal protein could be due to amino acid limitation or the presence of toxic substances. Peanut meal diets were then supplemented with five most limiting amino acids to determine whether the growth depression was due to amino acid limitation or to the presence of toxic substances. The slopes of the lines of supplemented peanut meals and that of soybean meal presented in Figure 2 show that the slope of peanut meal A (25.6) approached that of soybean meal (29.6). Furthermore, the slopes of the other two peanut meals B (18.8 and C (19.9) increased when these peanut meals were supplemented with five limiting amino acids. Since they did not become similar to that of soybean meal, differences in growth could be due to the presence of toxic substances such as aflatoxin. Similar results were obtained by Asplin and Carnaghan (1961) when samples of Brazilian groundnuts which were found to be highly toxic for ducklings, were fed to chicks. Body weights were 20 percent less than those fed the control diet at the age of 6 weeks. The control diet was identical to that of the experimental diet using a

non-toxic Indian peanut meal.

Practical Feeding Experiments

It was observed in experiment III that the highest live weight gain and the best feed efficiency were attained by chicks receiving the corn-soybean meal diet, but there was no significant difference among those receiving the corn-soybean meal diet and those receiving 8, 16 and 24 percent peanut meal diets. The poorest live weight gain and feed efficiency were attained by chicks receiving all-peanut meal diets. Similar results have been reported by Heuser et al. (1946), Tarver and Driggers (1958) and Daghir et al. (1964). All these workers showed that peanut meal could replace 50 percent of the soybean meal in modern broiler rations supplemented with animal protein without any significant effect on growth. Furthermore, Douglas and Harms (1959) showed that peanut meal containing 55 percent protein could replace 75 percent of the soybean meal, that is 24.7 percent of the total ration, without any significant effect on growth.

The supplementation of peanut meal with both methionine and lysine in experiment IV improved significantly body weight gains and feed efficiency and brought it up to the level of the corn-soybean meal diet. The addition of lysine to the peanut meal diet resulted in significant improvement in body weight gains and feed efficiency, while the addition

of methionine did not. However, chicks receiving a methionine supplemented peanut meal diet had better body weight gains and feed efficiency than those receiving unsupplemented peanut meal diets, but differences were not significant. Douglas and Harms (1959) reported similar findings using 0.25 percent lysine and 0.20 percent methionine. Furthermore, they showed that the addition of 0.3 percent methionine, 0.35 percent lysine and 0.15 percent glycine to an all-peanut meal diet produced weights equal to those obtained with all-soybean meal diets. Daghir et al. (1964) found that supplementing corn-peanut meal diets with both methionine and lysine improved body weight gains but did not bring them up to the level of the corn-soybean meal diet. This could be due to the low level of methionine and lysine used by those workers and the animal protein-free broiler ration employed. The positive response from methionine supplementation to peanut meal diets supplemented with lysine indicates a significant interaction between these two amino acids.

It is observed from Table 21 that the moisture content of carcasses of birds receiving all-peanut meal diets supplemented with methionine was significantly higher than all other treatments studied. The fat content of the same carcasses was significantly lower than those receiving all other diets. Those receiving all-soybean meal diets had significantly higher fat content than all other treatments.

Since all diets were made equal in calories and protein, this indicates that the all-soybean meal ration is more efficient from the standpoint of energy utilization.

SUMMARY AND CONCLUSIONS

Three Iraqi peanut meal samples, and one American soybean meal sample were analysed for proximate composition including calcium and phosphorous, methionine, cystine, and lysine content, available lysine, and aflatoxin content. Peanut meals were higher in protein and ether extract and lower in fiber than soybean meal. The most significant differences among peanut meals were in ether extract. The average available lysine content of the three samples of peanut meal (127 mgm./gm. N) was found to be nearly half as much as that of soybean meal (273 mgm./gm.N). Peanut meals tested were lower than soybean meal in methionine, cystine, and lysine content. There was also a difference in the methionine, cystine, and lysine content among peanut meal samples. Samples A and C of the peanut meal were estimated to be high in aflatoxin, while sample B was medium.

Four feeding experiments were conducted with cross-bred broiler chicks (Cornish x White Plymouth Rock). The first two were to determine PER of Iraqi peanut meals used in Lebanon, while the last two were practical feeding experiments to establish peanut meal levels to be used in practical broiler rations. In experiment I, PER of three unsupplemented peanut meal samples and one soybean meal sample was determined. Different levels of methionine,

lysine, tryptophane, isoleucine and valine were added in the second experiment to the same peanut meal samples bringing total levels of these amino acids to the same levels found in the soybean meal sample. Meals were fed at 10 and 20 percent protein levels in both experiments to evaluate the presence of toxic factors. PER of the three samples of peanut meal were similar but lower than that of soybean meal. The supplementation of peanut meal with five limiting amino acids to make its amino acid contents equal to that of soybean meal resulted in a significant improvement in PER, but not equal to that of soybean meal. This leads to the conclusion that the poor nutritive value of Iraqi peanut meal is not due to amino acid limitation alone, but also to the presence of toxic substances possibly aflatoxin.

The third experiment was conducted to study the effect of replacing soybean meal with 0, 25, 50, 75, and 100 percent peanut meal on live weight gain, feed efficiency, vital organ weights and carcass composition of broiler chicks raised to 8 weeks of age, while the fourth experiment was conducted to study the effect of completely replacing soybean meal with peanut meal and the supplementation of such rations with methionine and lysine singly or in combination on the same criteria as those of experiment III. Results indicate that high protein peanut meal replaces up to 75 percent of the soybean meal in broiler rations supplemented with 2 percent fish meal without a significant drop in body

weight gains or feed efficiency. Lysine was found to be more limiting than methionine in a diet containing peanut meal as the principle source of protein. Supplementation of such rations containing fish meal with 0.09 percent methionine and 0.57 percent lysine improved weight gains and feed efficiency and brought them up to the level of the corn-soybean meal groups.

Peanut meal had no significant effect on vital organ weights or protein content of the dressed carcasses of birds fed different levels of the meal. When a peanut meal diet was supplemented with methionine, the moisture content was significantly higher and fat content significantly lower than in the case of all other diets. Fat content of peanut meal fed birds was significantly lower than that of birds fed soybean meal.

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