PROTEIN QUALITY OF COTTONSEED CAKES PRODUCED IN THE MIDDLE EAST

by

Mahmood Vessal

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Approved:

James W. Cowan
In Charge of Major Work

Kurt Rottenstein

A. Khachadurian

Nuhad J. Daghir

W. W. Worzella
Chairman, Graduate Committee

American University of Beirut

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COTTONSEED PROTEIN QUALITY

Vessal

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ABSTRACT

Cottonseed meal is a rich source of protein and, if properly processed, can be used to supplement the diets of cattle, poultry and human beings. It is commonly known that cottonseed meal contains certain toxic substances, such as gossypol, which exert adverse physiological effects if taken by humans and other animals. Although cotton is an important crop in the Middle East, there is no information available concerning the nutritive value of the cottonseed meals produced in the area. This research was carried out to determine the nutritive quality of cottonseed meal samples obtained from Egypt, the Sudan, Iran and Syria.

Proximate composition, free, bound and total gossypol content and the amounts of methionine, cystine, lysine, threonine and tryptophane in each meal were determined. For the assessment of protein quality, two different bioassay procedures were used, namely: protein efficiency ratio (PER) and net protein utilization (NPU). In addition, the effect of supplementing three typical Middle Eastern dishes with two different levels of Sudanese cottonseed meal was studied.

The Egyptian cottonseed meal was found to be very high in free and bound gossypol and very poor in protein quality. Although the Sudanese sample was not the lowest in gossypol, it had the highest protein quality (PER = 1.48, NPU = 40.5). The protein quality of the other two samples was very low (PER less than 1.0) and they were considered to be of no practical importance as a protein source.

In most of the samples, the PER values were inversely proportional to the free gossypol content; however, the negative correlation,

when all samples were considered, was non-significant ($r = -0.80$, $P = 0.1$).

On the basis of the requirement of the growing rat for essential amino acids, threonine was found to be the most limiting in four of the five samples; lysine was most limiting in one of the Syrian samples.

Supplementation of two high legume Middle Eastern diets with 20 percent Sudanese meal resulted in a significant increase in the growth of experimental rats; no increase in rat growth was obtained with supplementation of a meat-containing diet.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Proteins are essential dietary components needed primarily for growth, maintenance and repair of body cells. For the development of a healthy individual, there should be in the diet not only a sufficient amount of protein, but also the protein should be of good quality.

Dietary proteins can be considered to come from two sources: (1) those of animal origin which usually provide the proper pattern of essential amino acids, and (2) those of plant origin which are usually limiting in one or more of the essential amino acids, and are thereby of lower quality than animal proteins. Due to the high cost of production, animal proteins are much more expensive than plant proteins.

Because of the low socio-economic status of a large segment of the population of the Middle East, the consumption of cheaper plant foods far surpasses that of more expensive animal products. It has been estimated that in this group of people less than 10 percent of the total caloric intake is from animal sources (2). It is known that by careful selection and blending of different plant proteins, mixtures may be obtained having equal or even higher quality than animal proteins. However, because of the lack of knowledge of selection and because of the low intake of animal products, protein malnutrition, especially among children, is prevalent in the Middle East.

In certain countries of the Middle East, such as Egypt, Syria and the Sudan, cotton is an important cash crop and is grown mainly

for its fibers. Oil expressed from the cottonseed provides a secondary return from this plant; the meal which remains after oil extraction is used mostly as fuel and fertilizer and, to a limited extent, for animal feeding. Cottonseed meal, however, is a rich source of protein and if properly processed could be used to supplement the diets of cattle, poultry and human beings. It is commonly known that cottonseed meal contains certain toxic substances, such as gossypol, which exert adverse physiological effects if taken by humans and other animals. It is only by carefully controlled processing that these toxic components can be removed and the meal made safe for consumption. In the Middle East, the methods used for the extraction of cottonseed oil are crude, resulting in high temperatures which lower the quality of the protein in the meal.

Little research has been conducted to determine the quality of the protein of cottonseed meals produced in the Middle East; also, there is little data available on the gossypol content of these meals. The purpose of the present research was to evaluate the quality of the protein of various samples of cottonseed press cake[1] produced in various regions of the Middle East. For the assessment of protein quality, two different animal assay procedures were used in addition to the determination of individual amino acids. The free and total gossypol content of each sample was determined and its relationship to protein quality established. In addition, the effect of supplementing several typical Middle Eastern dishes with cottonseed meal

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1. Throughout the text, the terms cottonseed meal, cottonseed press cake and cottonseed cake are used interchangeably.

was studied.

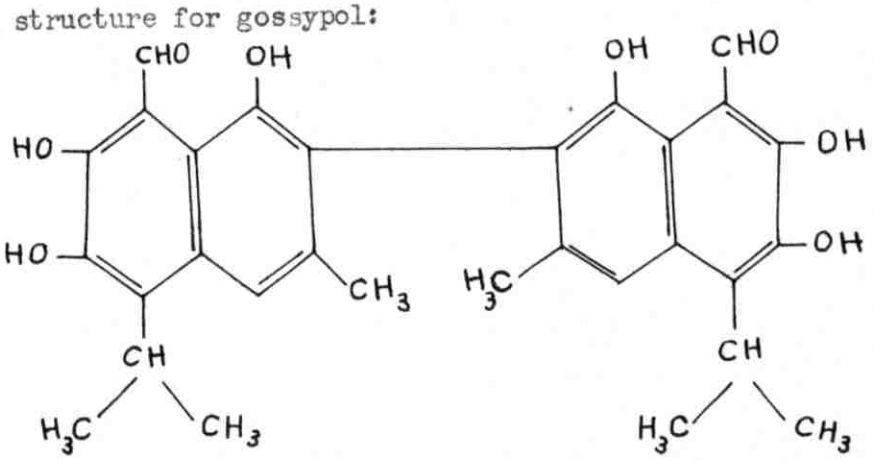
Information gained from this kind of study would be helpful to nutritionists and technologists in the area. The nutritionists, by knowing gossypol content and essential amino acid composition would be able to choose meals which would be suitable for use as dietary supplements. By altering the existing processing methods, the technologists can develop methods for the production of higher quality meals.

LITERATURE REVIEW

Cottonseed meal is rich in protein which provides a relatively good pattern of essential amino acids for monogastric animals. However, its use is limited by its content of certain toxic substances, such as gossypol, and also by the fact that protein quality is often adversely affected during the oil extraction process.

Toxicity and Protein Quality of Cottonseed Meal

Gossypol is a yellow pigment present in the pigment glands of the seeds of the cotton plant (8). Adams (1) postulated the following structure for gossypol:



This structure was later confirmed by Shirley and Dean (74) and by Edwards and Cashaw (33).

Chemically, gossypol acts as an aldehyde, as a polyphenol and has strong acidic properties. It is present mostly in a free form in the seed, but during the processing of the seed for oil extraction, some of the gossypol is bound to the epsilon-amino group of lysine, thus reducing the physiological availability of this amino acid.

Free gossypol is responsible for the toxicity of cottonseed meal in monogastric animals and for the discoloration of egg yolks; this substance can be extracted from the meal with acqueous acetone (8). Bound gossypol, which is considered to lower the protein quality of cottonseed meal by making lysine unavailable, can be extracted preferentially with a mixture of aniline and acetone (9).

Physiological Action of Free Gossypol

Eagle and associates (29, 30, 32), Lillie and Bird (54), and Harms and Holley (44) studied the toxic effects of gossypol on dogs, rats, mice, chicks, pigs and rabbits. These authors concluded that guinea pigs are the most sensitive animals to gossypol followed by rabbits, swine and dogs; the least sensitive are rats and poultry.

The fact that fluid accumulates in the body cavity of animals fed gossypol-containing diets suggests that gossypol increases membrane permeability (8). In addition, gossypol may produce in rabbits hypoprothrombinemia (44) and severe edema (76). Rigdon et al. (69) observed a kind of hemolytic anemia in young chickens due to gossypol poisoning. In addition, these workers observed the accumulation of yellowish brown pigments in the duodenal villi and sinusoids of the spleen and the presence of vacuoles in the liver.

To determine the permissible level of gossypol in poultry rations containing cottonseed meal, Heywang and Bird (46) fed to White Leghorn and New Hampshire chicks, different kinds of cottonseed meal containing different levels of gossypol. Their data on growth, feed consumption and feed efficiency showed that the free

gossypol content of the diet should not be greater than 0.016 percent for White Leghorn or 0.02 percent for New Hampshire chickens. However, Couch et al. (24) reported a dietary tolerance level of 0.06 percent free gossypol for New Hampshire chicks. This disagreement among authors may be explained on the basis of the work of Gallup and Reder (39) who reported that gossypol toxicity could be overcome by feeding high levels of protein in the diet. In addition, Hale and Lyman (43) observed that pigs could tolerate a gossypol level up to 0.015 percent when fed a diet containing 15.5 percent crude protein.

The presence of gossypol alone cannot account for all the toxic effects of cottonseed meal. Gallup (38) has suggested that there are, in cottonseed cake, toxic substances other than gossypol which reduce the nutritive value of the cake. In a study using rats, mice, guinea-pigs and rabbits, Eagle et al. (32) observed that severe toxic effects were produced when cottonseed pigment glands were added to the diets of fasting animals, whereas an equivalent amount of pure gossypol affected the animals very little.

The mechanism by which free gossypol causes tissue damage and retards growth is not fully understood.

Bound Gossypol and Protein Quality

Lyman et al. (57) found a relationship between bound gossypol and protein quality. Martinez and coworkers (58) reported that a level of 1 percent gossypol was effective in destroying lysine. They observed that gossypol at this level reduced the physiological avail-

ability of lysine by forming a complex with the epsilon-amino group of this amino acid. Smith and associates (75), working with weanling rats, found that bound gossypol significantly depressed growth rate and also the availability of lysine in the diet. After supplementation of these diets with lysine, a significant increase in growth was obtained.

Processing of Cotton Seed Meal

In developing a method for extracting oil from cottonseed, the objectives should be: (1) to reduce the level of free gossypol and (2) to maintain protein quality by avoiding high processing temperatures. Various methods of processing and the advantages and limitations of each method have been reviewed extensively by Altschul (7) and to certain extent by Bressani and Scrimshaw (16).

During the past few decades, extensive research has led to the development of the present processing methods which make possible the production of cottonseed meals of low gossypol content and high protein quality. Among the more active contributors to this research were Milligan and Bird (61) who used low temperature processing. Conkerton et al. (23) reported the reduction in lysine, arginine and sugar content of solvent extracted heat-treated cottonseed meals. Information concerning the mechanism of heat damage was presented by Condon et al. (22) and Kuiken (53). King and associates (52) developed a method for the extraction of oil and free gossypol from cottonseed meat using an acetone-petroleum ether-water azeotrope. In this procedure the protein quality of the meal is retained since high proc-

essing temperatures are avoided. There are numerous reports on the nutritional evaluation of meals produced by different processing methods (7, 15, 40, 41, 42, 63, 78).

Gossypol and Egg Discoloration

Eggs of hens fed cottonseed meal, when stored in the cold, developed an olive-green to black discoloration of the yolk and a pink discoloration of the albumen. The discoloration of the yolk has been studied extensively by several authors (47, 48, 55, 72, 79). These workers all concluded that this discoloration was due to oxidation reactions involving the gossypol which was transferred from the feed to the egg. This condition could be prevented by feeding iron salts, which would prevent the absorption of gossypol from the gut, or by dipping the eggs in heavy oils before storage. The pink discoloration of the white is thought to be due to the presence of cyclopropene fatty acids in the meal (28, 34, 56).

Cottonseed Flour for Human Consumption

The nutritive value of certain cottonseed flours was studied by Demayer and Vanderborght (25). In feeding trials with malnourished African children, these authors found that a kind of cottonseed flour containing 57 percent crude protein and 0.045 percent free gossypol was safe for human consumption and had a reasonably high nutritive value. On the basis of these findings, they recommended the use of such flours for the supplementation of poor diets in developing areas.

Frenk (35) used a cottonseed flour of the same protein and free gossypol content as the one described above for the supplementation of corn flour in the feeding of malnourished children in Mexico. He obtained superior results with corn meal supplemented with cottonseed flour as compared to corn meal alone.

At the Institute of Nutrition of Central America and Panama (INCAP), a vegetable food mixture for infants containing 38 percent cottonseed flour has been developed. The mixture contains up to 20 mg. free gossypol and 0.43 gm. total gossypol per 100 gm.; no ill effects were observed when this mixture was tested on children (73).

METHODOLOGY

Amino Acid Determination

There are several chemical, microbiological, and chromatographic adsorption methods in common use for the determination of amino acids. The details of all these procedures have been described by Block and Weiss (14); a review of microbiological methods for amino acid assays was presented by Sakr (71).

Protein Evaluation

Protein evaluation methodology has been reviewed extensively by several authors (3, 4, 5, 37) and recently, a critical review of protein evaluation procedures has been published by Campbell (17). This latter review includes a detailed discussion of the relative merits and drawbacks of each method. Since the methods used in this study were protein efficiency ratio (PER) and net protein utilization

(NPU) the present discussion will be restricted to these procedures.

1. Protein efficiency ratio (PER)

The rate of growth of an animal provides a sound basis for the evaluation of protein foods (17). If a food is limiting in one or more essential amino acid, the growth of the animal will be depressed; thus growth is a good index of protein quality.

The concept of protein efficiency ratio (PER) was introduced in 1919 by Osborne et al. (66) who defined this term as gm. gain per gm. protein consumed. At present, the PER assay is the most widely used of all protein evaluation methods. In its original form PER was determined at different levels of protein intake and the maximum value chosen as the PER of the test diet. However, Campbell (17) stresses the need for 10 percent protein in the diet to obtain maximum PER values and the need for ad libitum feeding.

Chapman et al. (20) demonstrated that the age of rats used for PER determinations should be between 20-23 days. They obtained highly significant differences in PER values between rats put on test at 22, 29, 36 or 45 days of age. Derse (26) recommends 21-day old rats for PER determinations.

The need for a short assay period was pointed out by Mitchell (62) in 1924. Sure (77) used a 10-week period for PER determinations. However, Chapman et al. (20), Morrison and Campbell (64) and Sure (77) observed a decrease in PER as the duration of the assay increased and concluded that 4-week period gave the best results.

The sex of the animals may affect the results of PER determina-

tions. As early as 1926, Hoagland and Sinder (49) showed that PER varied with the sex of the animals. Recently, Morrison and Campbell (64) demonstrated that female rats gave higher PER values at a lower dietary protein level than males. These workers concluded that, since the females did not gain as rapidly as males, and since the variation within groups of females was larger, a test using males was preferable. Variations in PER due to different strains of animals was established by the same authors.

The increase in PER values by the addition of water to purified diets was reported by Keene et al. (51), but studies made by Sabry et al. (70) did not confirm these results.

The need for a reference diet for comparison of test proteins was recognized by Friedman and Kline (36); these workers used casein as a reference standard, assigning it a PER value of 100. Chapman et al. (20) chose an arbitrary PER value of 2.5 for casein and suggested that all values for test proteins should be corrected using a factor calculated by dividing 2.5 by the PER obtained for the reference diet.

Standardized methods for PER determinations have been developed by Derse (27) and by Chapman et al. (20). These procedures are standardized with respect to dietary protein level, duration of assay, method of feeding, use of an appropriate standard, and to strain, age and sex of the animals. All these standardizations are essential for compafison of results obtained from different laboratories (17).

Although the PER assay is simple and is widely used in many laboratories, it is subject to several criticisms. Mitchell (62) and

more recently Bender and Doell (11) pointed out the following drawbacks of PER determinations:

a. PER determinations assume that the gain in body weight has a constant composition. This might not be true in many cases.

b. Results vary with food consumption and protein level in the diet.

c. PER assays consider no protein requirement for maintenance and assume that all the protein in the diet is used for growth.

d. PER does not permit the evaluation of proteins which do not promote growth.

All these criticisms were reviewed by Campbell (17) who refers to the results of several workers whose contributions were effective in alleviating the drawbacks of PER assays. He considers the PER assay simple and at the same time sufficiently accurate, if all experimental conditions are carefully standardized.

2. Net protein utilization

In 1953, Bender and Miller (12) introduced a method of protein evaluation which they called net protein utilization (NPU). It is the measure of the amount of consumed protein which is actually retained by the body. In this method the test diet, containing 10 percent protein, is fed to weanling rats for 10 days; a negative control group is fed a non-protein diet. At the end of the assay, the nitrogen content of the carcasses is determined and NPU calculated from the following formula:

$$NPU = \frac{\text{(body N of test group)} - \text{(body N of non-protein group)} + \text{(N consumed by non-protein group)}}{\text{(N consumed by test group)}}$$

Later, the same authors found that the nitrogen/water ratio in the rat carcass is constant and they devised a procedure to determine NPU by estimation of the water content of the body (13).

Bender (10), and Bender and Doell (11) found that NPU determinations are not affected by differences in food intake. However, Chapman et al. (20) could not confirm these findings and reported that NPU determinations are as variable as PER measurements.

Campbell (17) argues that NPU values are insensitive and variable for lysine deficient foods such as cereals, whereas Miller and Naismith (60) point out that this criticism is not of great importance in evaluating the protein quality of foods in uderdeveloped areas. They base their argument on the fact that, in developing areas, diets are generally limiting in the sulfur-amino acids rather than in lysine.

In a study of factors which may affect NPU determinations, Morrison et al. (65) and Campbell and coworkers (18) found that NPU values decreased when vitamins and minerals were deleted from the diets. A direct relationship between NPU values and the dietary lysine content was observed by the same authors.

Bender (10) found a high positive correlation between NPU and PER values of different foods. According to him, NPU and PER assays lead to the same order of classification of protein foods. These findings have been supported by other workers (18, 70).

Because of the similarity of results obtained among various protein evaluation methods, the researcher, normally chooses that method for which the facilities of his laboratory are best suited.

MATERIALS AND METHODS

Five samples of cottonseed cake were obtained; one each from the Sudan, Egypt and Iran and two from Syria. The samples from Iran and one of the Syrian samples were decorticated and the other three were undecorticated.

Proximate Analysis

Moisture, crude fat (ether extract), crude fiber, and ash were determined in duplicate using the methods described in A.O.A.C.[2] Handbook, 1960. Nitrogen was determined using a modified Kjeldahl method; percent crude protein was calculated by multiplying percent N by 6.25. Nitrogen free extract (N.F.E.) was calculated by the following formula:

$$\% \text{ N.F.E.} = 100 - (\% \text{ moisture} + \% \text{ ether extract} + \% \text{ crude fiber} + \% \text{ crude protein} + \% \text{ ash}).$$

Determination of Gossypol

Free and total gossypol were determined by the colorimetric method of Pons and Hoffpauir (67) in which gossypol reacts with aniline to produce a yellow color. A mixture of isopropyl alcohol and hexane (6:4 V/V) was used for the extraction of free gossypol and dimethyl formamide for the extraction of total gossypol. Color intensity was measured with a Unicam spectrophotometer (Model SP 600) at 440 mμ; gossypol values were extrapolated from a standard curve prepared using various concentrations of pure gossypol-acetic acid (50).

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2. Association of Official Agricultural Chemists.

Microbiological Assay of Amino Acids

Except for certain modifications, the microbiological assay procedures used were those described by Block and Weiss (14). The amino acids determined were methionine, cystine, lysine, threonine and tryptophane.

For the determination of all the above mentioned amino acids except tryptophane, a weighed sample containing approximately 0.5 gm. protein was refluxed with 60 ml. of 20 percent (V/V) HCl. With the exception of the cystine assay, for which a 2-hour hydrolysis period was used, the samples were refluxed for 8 hours. After hydrolysis most of the HCl was removed by evaporation on a hot plate. To the remaining residue, 60 ml. of water was added and the pH adjusted to 4.0 to precipitate the humins. The hydrolyzate was refrigerated for one hour, then filtered through Whatman No. 1 filter paper. The filtrate was neutralized with dilute sodium hydroxide to pH 6.8 and the volume made up to 250 ml. with distilled water.

For the tryptophane assay, an enzymatic hydrolysis procedure using pepsin, trypsin and erepsin was used (14). During hydrolysis, the samples were covered with a layer of toluene to prevent spoilage. The assay organisms used were: (1) Leuconostoc mesentroides P - 60 (ATCC[3] no. 8042) for lysine, methionine, cystine and threonine; and (2) Lactobacillus arabinosus 17 - 5 (ATCC no. 8014) for tryptophane. The lyophilized cultures of these organisms were revived as described by Sakr (71). The organisms were maintained as stab cultures by week-

3. American Type Culture Collection, Washington D.C.

ly transfer to a solid medium having the following composition:

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

Ten-mililiter portions of the medium were autoclaved for 15 minutes at 15 psi. and allowed to solidify in the refrigerator.

The basal media for the assay of the respective amino acids were obtained in dry form from Difco Laboratories, Detroit 1, Michigan, USA. These were prepared according to the manufacturer's specifications and later sterilized at 15 psi. for 10 minutes.

Culture tubes containing sterilized growth medium[4] 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. This suspension was used for the inoculation of the samples.

Standard amino acid solutions were prepared according to the A.O.A.C. methods; stock solutions were diluted to the following concentrations:

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4. The composition of the growth medium was the same as that of the solid medium except that agar was deleted.

Amino acid standard	mcg./ml.
L-lysine	20
L-methionine	5
L-threonine	10
L-cystine	5
L-tryptophane	2

Using values found in the literature for cottonseed meal, sample hydrolyzates were diluted in such a way that the amount of amino acids in the dilutions would be approximately equal to the amount of the respective amino acid in the standard.

The samples and standards were assayed under the same experimental conditions; all assays were carried out in triplicate. Serial amounts of the standard amino acid solutions (0. 0.2, 0.4, 0.6, 0.8 and 1.0 ml.) were placed in previously sterilized pyrex test tubes. For the unknowns, 3 different levels (0.3, 0.6 and 1.0 ml.) were used instead of 6; in all tubes the volume was made up to 1 ml. by adding distilled water. The tubes were randomized in a wire rack and covered tightly with a layer of aluminum foil. After autoclaving for 15 minutes at 15 psi., the tubes were allowed to cool. Each tube was then inoculated with 1 ml. of the basal medium which contained 4 drops of the saline suspension of the bacteria per 20 ml. After inoculation, the tubes were incubated at 37°C for 72 hours, at which time the lactic acid produced was titrated with 1.0 N NaOH using bromthymol blue as the indicator.

The standard curve for each amino acid was drawn by plotting

the average volume of 1.0 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 curve, the concentration of each amino acid in the tubes containing the sample aliquots was determined.

The amino acid content of each sample was calculated as gm. amino acid per 16 gm. N as follows:

$$\text{gm. a.a. /16 gm. N} = \frac{\text{mcg. a.a. / ml. x dilution factor}}{\text{1000 x N content of the sample}} \times \frac{16}{1000}$$

Animal Experiments

The experimental animals used were weanling (21-23 day-old) male, albino rats of the Sprague-Dawley strain[5]. After arrival by air, the animals were placed on a stock diet[6] for a recovery period of 1-2 days. Groups of ten animals were used for PER determinations, whereas eight rats per group were used for NPU and supplementation experiments. The animals were individually housed in mesh-bottom cages in an air-conditioned room held at 70 ± 2°F and relative humidity of 60 percent. In all experiments, animals were assigned to diets according to a randomized block design. Animals received food and water ad libitum; food consumption and weight gains were determined weekly.

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5. Obtained from Animal Suppliers (London) Ltd.

6. Obtained from Vitasni Feed Company, Beirut, Lebanon.

The basal diet had the following composition on an air dry basis:

Ingredients	Percentage
Corn starch	80
Corn oil	10
Non-nutritive cellulose (Alphacel)[7]	5
Mineral mixture (USP XIV)[7]	4
Vitamin mixture[7]	1

For the preparation of the test diets, sufficient cottonseed cake was incorporated into the basal diet, at the expense of corn starch, to provide 10 percent protein. The amount of crude fat (ether extract) contributed by the cakes was calculated and subtracted from the amount of corn oil added; in order to keep the ratio of other ingredients constant, the decrease in the amount of corn oil was compensated for by adding a respective amount of Alphacel. The reference standard diet was prepared from the basal diet in which casein[8] served as the sole source of protein.

Protein efficiency ratio (PER) was determined, with slight modifications, by the standardized procedure described by Chapman et al. (20). Corrected PER values were calculated as follows:

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7. Obtained from Nutritional Biochemicals Cooperation, Cleveland, Ohio, USA.

8. Obtained from General Biochemicals, Chagrin Falls, Ohio, USA.

$$\frac{2.5}{\text{determined PER of casein}} \times \text{PER (unknown)}$$

At the end of the assay period, the livers of the rats were removed and their weights recorded.

Determination of NPU was based on the method of Bender and Miller (12) and Miller and Bender (59) with certain modifications. A group of animals fed a non-protein basal diet served as a negative control for the calculation of maintenance nitrogen. After 10 days on diet, the animals were sacrificed with chloroform; incisions were made in the thorax, skull and abdomen of each animal and the carcasses were dried in a forced draft oven at 210°F for 2-3 days. Each dried carcass was ground separately in a meat mincer two or three times to ensure a homogeneous mixture. Kjeldahl nitrogen was determined on 2-3 gm. of the ground material. Net protein utilization was calculated from the following formula (12):

$$NPU = \frac{B - (BK - I_K)}{I}$$

in which B and B_K are the total body nitrogen of the animals on the test and non-protein diets respectively, and I and I_K are the nitrogen intake of the two groups.

Supplementation Experiment

Three typical Middle Eastern diets were prepared and the effect on rat growth of supplementing each diet with various levels of cottonseed cake was studied. Two of the diets are common to low

income groups in Lebanon and other Arab countries; the third is very popular among middle and low income groups of Iran.

Table 1 shows the proportion of different ingredients in each dish; data on the composition of the final diets are presented in Table 2. The dishes used were:

1. "Mujadarah": This is a name given to a dish prepared from lentils (Lens esculenta) and rice. After the lentils and rice are cooked together, olive oil, table salt and fried onions are added to the mixture.

2. "Ful": This dish is usually prepared by adding lemon juice, olive oil and salt to cooked broad beans (Vicia faba).

3. "Yakhni": The term in Persian means any legume (peas or beans) cooked with poor quality mutton and served ground with onions. The special kind of "Yakhni" used in this study was one made from chick peas (Cicer arietinum) and meat. All these dishes are normally served with generous amounts of bread.

The recipes for "Mujadarah" and "Ful" were those described by G.N. Alrayes (6). The average amount of bread normally eaten at a meal in which each of these dishes is served was calculated from results obtained in several trials conducted by the author. The recipe for "Yakhni" and the amount of bread eaten with it were derived from information gathered from several Persian families.

"Mujadarah"

Red lentils were cooked until soft in a double jacketted steam kettle; rice was then added and cooking continued for 15-20 minutes.

The cooked material was dried in a forced draft oven at 70°C for two days. Both the dried "Mujadarah" and the dried bread were ground in an Alpine mill and thoroughly mixed together in a Hobart mixer; corn oil and salt were added during mixing. In the preparation of this diet, corn oil was substituted for olive oil and the fried onions were omitted.

"Ful"

Dried broad beans were soaked in water overnight after which the water was decanted and the beans cooked until soft. After adding lemon juice and salt to the cooked beans, the mixture was dried at 70°C. The method of preparation of the final diet was the same as described for "Mujadarah". Garlic and other spices were excluded.

"Yakhni"

In the preparation of this diet, beef was used instead of mutton and Arabic bread replaced Persian bread; onions were excluded. The chick peas were soaked in 1-2 percent salt water overnight. The meat was cut into small pieces and boiled until soft. At this time the chick peas were added to the meat and boiling continued for one hour. The mixture was then ground in a meat mincer and dried. The final diet was prepared in the same manner as the other two. The prepared diets were analyzed for proximate composition, methionine, cystine, threonine, lysine and tryptophane.

Each diet was supplemented with 20 and 30 percent cottonseed cake of Sudanese origin, and fed to weanling rats. In addition to the groups of animals receiving the supplemented diets, a group of control

animals was maintained on each of the basal diets. The gain in weight and food consumption of each rat was recorded weekly for a period of four weeks. At the end of the experiment the animals were sacrificed with chloroform, and the livers removed and weighed.

Table 1. Percentage of different ingredients in the Middle Eastern dishes[1] used in the supplementation experiment

Dish	Lentils	Rice	Broad beans	Lemon juice	Salt	Chick peas	Meat
	%	%	%	%	%	%	%
"Mujadarah"	87	17	-	-	-	-	-
"Ful"	-	-	82.0	16.4	1.6	-	-
"Yakhni"	-	-	-	-	-	50	50

1. All values were calculated on an air dry basis.

Table 2. Proportion of different ingredients in the basal diets used in the supplementation experiment.

Basal diet	Dried bread	Dried "Muj."	Dried "Yak."	Dried "Ful"	Corn oil	Salt
	%	%	%	%	%	%
"Mujadarah"	42.8	46.8	-	-	9.7	0.7
"Ful"	59.3	-	-	28.5	12.2	-
"Yakhni"	66.0	-	32.0	-	-	2.0

RESULTS

Five different Middle Eastern cottonseed meals were analyzed for proximate composition, gossypol content and for methionine (met), cystine (cys), lysine (lys), threonine (thr) and tryptophane (try). The protein quality was evaluated using two different bioassay procedures: namely, protein efficiency ratio (PER) and net protein utilization (NPU). In addition, the effect on the growth of rats fed three typical Middle Eastern (M.E.) dishes supplemented with two levels of Sudanese cottonseed meal was studied. The results obtained have been reduced to tabular form and are presented in Tables 3 - 12 inclusively.

Figure 1 shows the growth curves for rats on PER assay diets; growth curves from the supplementation experiment are shown in Figures 2, 3 and 4.

Table 3. Proximate composition of five cottonseed meals

Origin of sample	Moisture	Ether extract	Crude fiber	Crude protein	Ash	N.F.E.[1]
	%	%	%	%	%	%
Syria (undecorticated).	6.4	5.2	24.7	22.9	5.6	35.2
Egypt	10.8	4.5	25.5	23.8	4.7	30.7
Sudan	5.3	4.9	24.9	22.6	5.5	36.8
Syria (decorticated).	6.0	5.1	9.2	40.3	6.0	33.4
Iran	6.2	3.4	11.0	39.9	5.1	34.4

1. Nitrogen free extract

Table 4. Gossypol content of five cottonseed meals

Origin of sample	Free	Total	Bound[1]	Crude protein	Free	Total	Bound
	%	%	%	%	gm. per 100 gm. crude protein		
Syria (undecorticated)	0.071	0.655	0.584	22.9	0.31	2.86	2.55
Egypt	0.170	0.884	0.714	23.8	0.71	3.71	3.00
Sudan	0.054	0.799	0.745	22.6	0.23	3.53	3.30
Syria (decorticated)	0.124	0.859	0.735	40.3	0.31	2.13	1.82
Iran	0.026	0.785	0.759	39.9	0.07	1.97	1.90

1. Total gossypol minus free gossypol.

Table 5. Amino acid composition of cottonseed meals

Origin of sample	Thr	Lys	Try	Met	Cys	Met + Cys
	gm. amino acid per 16 gm. nitrogen					
Syria (undecorticated)	3.112	5.717	0.698	1.890	2.231	4.121
Egypt	2.389	6.404	0.737	2.173	2.043	4.216
Sudan	2.782	6.577	0.787	2.209	2.019	4.229
Syria (decorticated)	2.930	4.962	0.729	1.972	2.056	4.029
Iran	2.739	5.252	0.739	2.039	2.105	4.144
	percent of egg values					
Syria (undecorticated)	72	103	47	69	110	86
Egypt	55	116	50	79	100	87
Sudan	64	119	53	80	99	88
Syria (decorticated)	68	89	49	72	101	84
Iran	63	94	50	74	104	86

Table 6. Portion of rat requirement for various essential amino acids contributed by cottonseed meals in 10 percent protein diets[1]

Origin of sample	Thr	Lys	Try	Met	Met + Cys
	%	%	%	%	%
Syria (undecorticated)	61	63	64	39	84
Egypt	47	71	64	45	86
Sudan	55	73	72	45	86
Syria (decorticated)	57	55	66	40	82
Iran	53	58	67	41	84

1. The values were calculated by dividing the amino acid content of cottonseed meals (gm. per 100 gm. meal) by the requirement of rats (gm. per 100 gm. diet) using data of Rao et al. (68).

Table 7. Average food consumption and weight gains of rats fed diets containing 10 percent protein from cottonseed meal[1]

Source of protein in diet	No. of rats	Food consumption	Gain in weight
		gm.	gm.
Casein[2]	10	320	96
Syrian C.S.M.[3] (undecorticated)	10	261	27
Egyptian C.S.M.	2[4]	170	0.5
Sudanese C.S.M.	9	364	66
Syrian C.S.M. (decorticated)	10	198	11
Iranian C.S.M.	10	201	20

1. 28-day experiment.
2. Control diet.
3. Cottonseed meal.
4. Only two animals survived.

Table 8. Protein efficiency ratio (PER) and net protein utilization (NPU) values of cottonseed meals

Source of protein in diet[1]	PER(Corrected)[2,3]	NPU[4,5]
Casein	2.50	——[7]
Syrian C.S.M. (undecorticated)	0.84 ± 0.42[6]	32.2 ± 7.1
Egyptian C.S.M.	-0.08 ± 0.66	28.0 ± 6.8
Sudanese C.S.M.	1.48 ± 0.12	40.5 ± 7.9
Syrian C.S.M. (decorticated)	0.44 ± 0.40	32.5 ± 8.3
Iranian C.S.M.	0.82 ± 0.16	30.7 ± 8.9

1. All diets contained 10 percent protein.
2. 28-day assay.
3. L.S.D. at 1% level = 0.14.
4. 10-day assay.
5. L.S.D. at 1% level = 9.1.
6. Standard deviation.
7. Not determined.

Table 9. Proximate composition of the three Middle Eastern diets used in the supplementation study[1,2]

Diet	Moisture	Ether extract	Crude fiber	Crude protein	Ash	N.F.E.
	%	%	%	%	%	%
"Ful" (basal)	5.2	13.4	6.1	13.8	2.8	58.7
+ 20% C.S.M.	5.3	11.7	9.9	15.5	3.3	54.3
+ 30% C.S.M.	5.2	10.9	11.8	16.4	3.6	52.1
"Mujadarah" (basal)	6.1	10.6	4.1	15.2	2.7	61.3
+ 20% C.S.M.	6.0	9.5	8.3	16.7	3.3	56.2
+ 30% C.S.M.	5.9	8.9	10.4	17.4	3.5	53.9
"Yakhni" (basal)	8.5	3.3	2.2	18.5	4.4	63.1
+ 20% C.S.M.	7.8	3.6	6.8	19.3	4.6	57.9
+ 30% C.S.M.	7.5	3.8	9.0	19.7	4.7	55.3

1. Sudanese cottonseed meal used for supplementation.
2. Proximate composition of supplemented diets was calculated using values previously obtained for the cottonseed meal and basal diets.

Table 10. Amino acid composition of the three Middle Eastern diets used in the supplementation study[1,2]

Diet	Thr	Lys	Try	Met	Cys	Met + Cys
	gm. per 16 gm. nitrogen					
"Ful" (basal	0.38	0.86	0.12	0.17	0.11	0.28
+ 20% C.S.M.	0.36	0.82	0.11	0.18	0.13	0.31
+ 30% C.S.M.	0.35	0.80	0.11	0.18	0.14	0.32
"Mujadarah" (basal)	0.57	1.02	0.12	0.19	0.10	0.29
+ 20% C.S.M.	0.51	0.95	0.11	0.20	0.12	0.32
+ 30% C.S.M.	0.48	0.91	0.11	0.20	0.13	0.33
"Yakhni" (basal)	0.68	1.56	0.18	0.33	0.17	0.50
+ 20% C.S.M.	0.60	1.38	0.16	0.31	0.18	0.49
+ 30% C.S.M.	0.56	1.30	0.15	0.30	0.18	0.48

1. Sudanese cottonseed meal used for supplementation.
2. Amino acid composition of supplemented diets was calculated using values previously obtained for cottonseed meals and basal diets.

Table 11. Portion of rat requirement for various essential amino acids contributed by the three Middle Eastern diets used in the supplementation experiment[1,2]

Diet	Met	Met + Cys	Thr	Lys	Try
	%	%	%	%	%
"Ful" (basal)	35	57	75	96	109
+ 20% C.S.M.	36	61	71	91	100
+ 30% C.S.M.	37	65	69	89	100
"Mujadarah" (basal)	39	59	112	113	109
+ 20% C.S.M.	41	65	100	105	100
+ 30% C.S.M.	41	67	94	101	100
"Yakhni" (basal)	67	102	133	173	164
+ 20% C.S.M.	63	100	118	153	145
+ 30% C.S.M.	61	98	110	144	136

1. Based on data of Rao et al. (68).
2. Sudanese cottonseed meal used for supplementation.

Table 12. Average weight gain and food consumption of weanling rats fed three Middle Eastern diets supplemented with Sudanese cottonseed meal

Diet	No. of animals	Weight gain	Food consumption	Assay period
		gm.	gm.	days
"Ful" (basal)	8	47.2[1]	244.1	28
+ 20% C.S.M.	7	63.6	341.1	28
+ 30% C.S.M.	7	61.3	333.0	28
"Mujadarah" (basal)	7	47.1[2]	256.0	27
+ 20% C.S.M.	8	65.6	305.7	27
+ 30% C.S.M.	6	73.7	341.6	27
"Yakhni" (basal)	6	67.5[3]	264.5	21
+ 20% C.S.M.	8	65.4	263.7	21
+ 30% C.S.M.	7	80.8	316.0	21

1. L.S.D. at 5% level = 12.9.
2. L.S.D. at 5% level = 13.9.
3. L.S.D. at 5% level = 13.9.

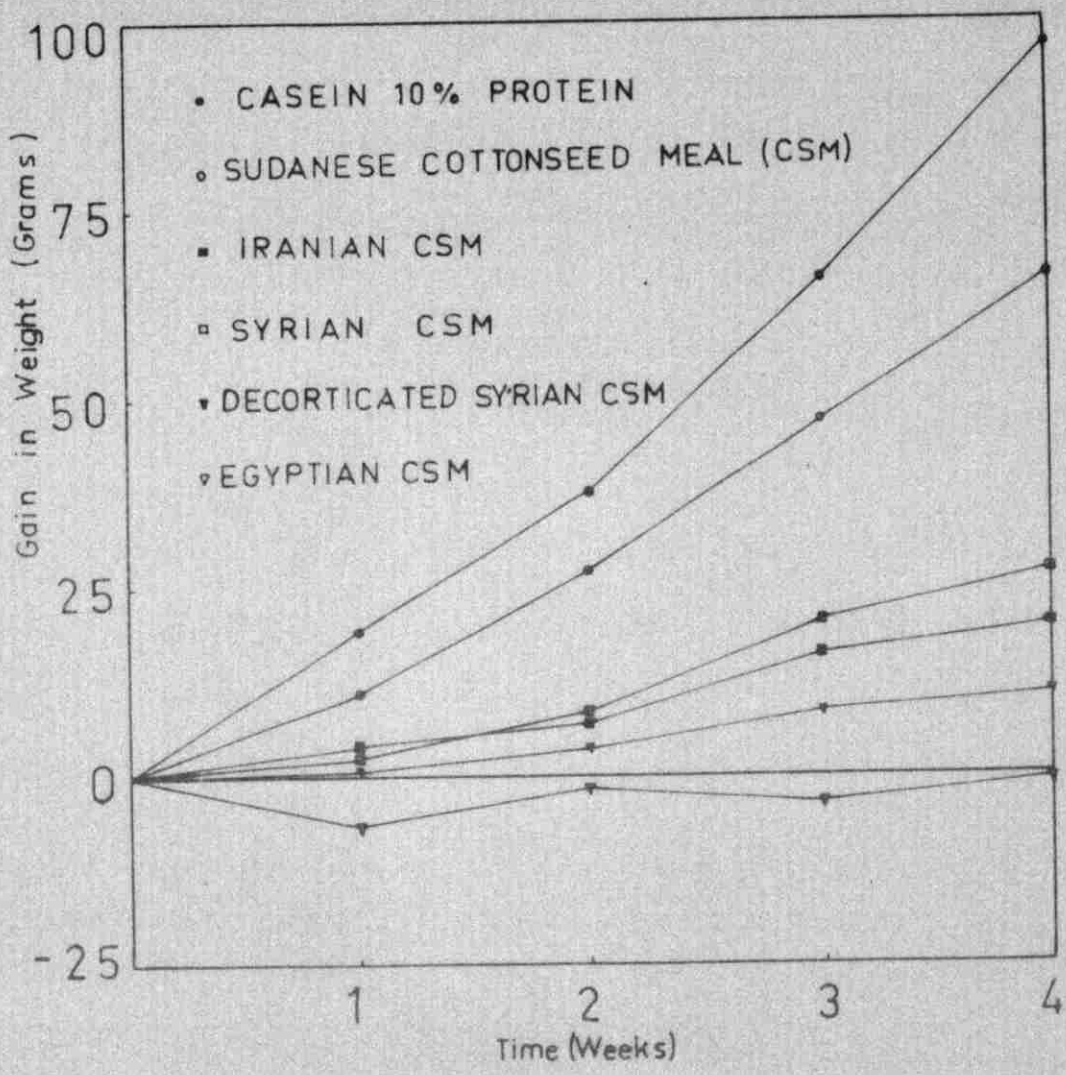

Figure 1. Growth curves comparing weight gains of rats fed diets containing protein from casein or from various cottonseed meals

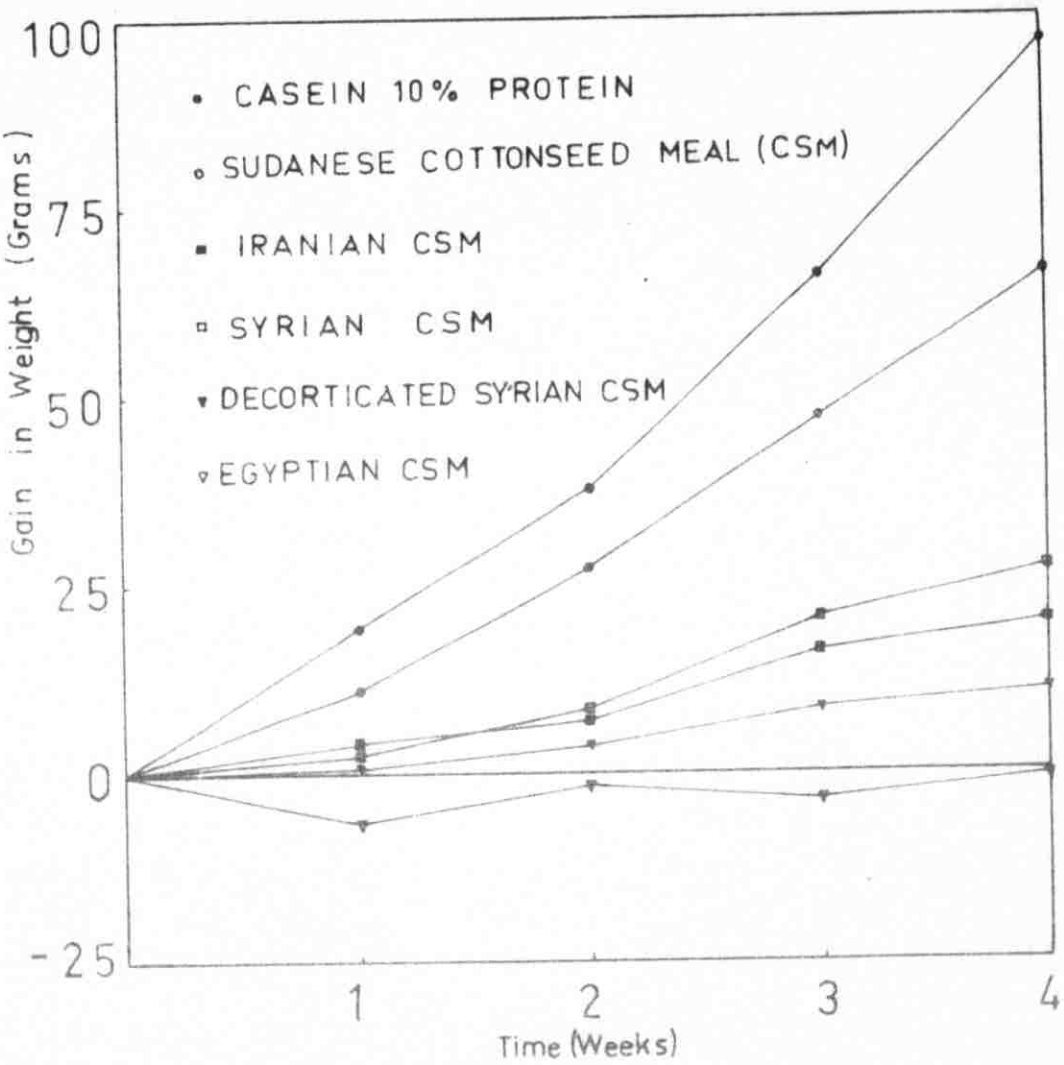

Figure 1. Growth curves comparing weight gains of rats fed diets containing protein from casein or from various cottonseed meals

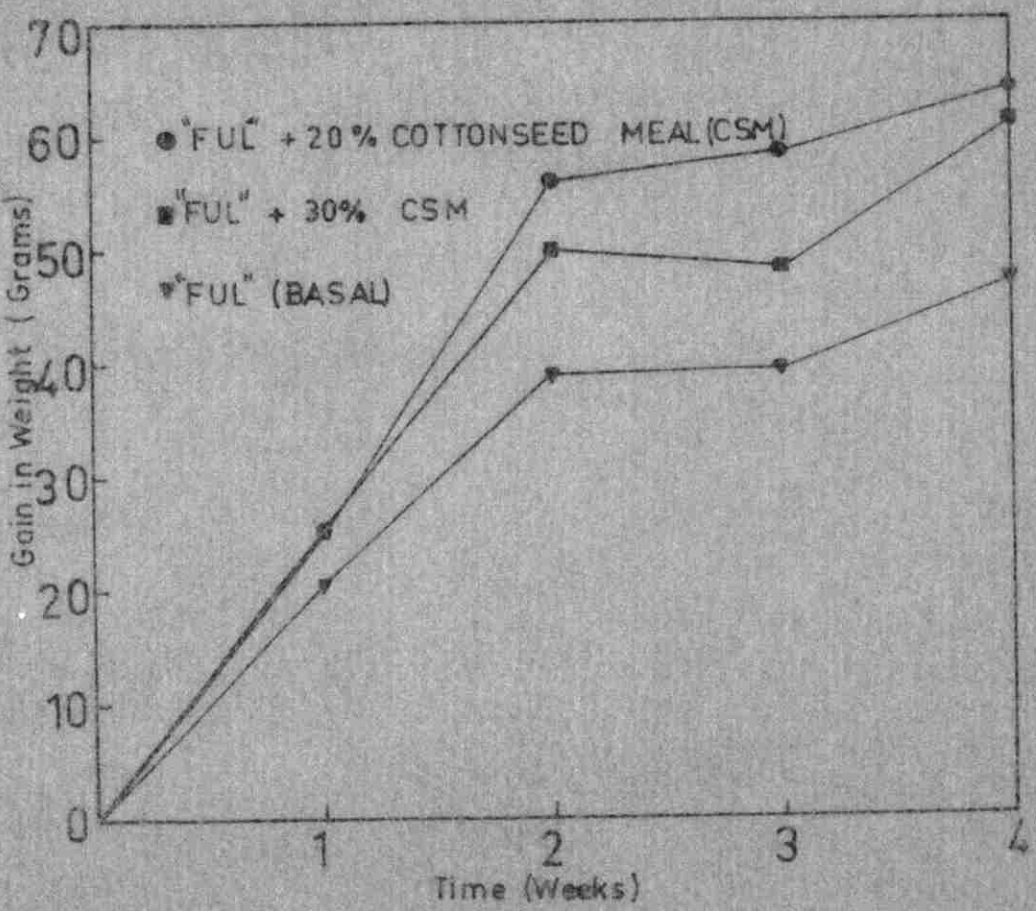

Figure 2. Four-week growth curves of rats fed the basal and supplemented "Ful" diets

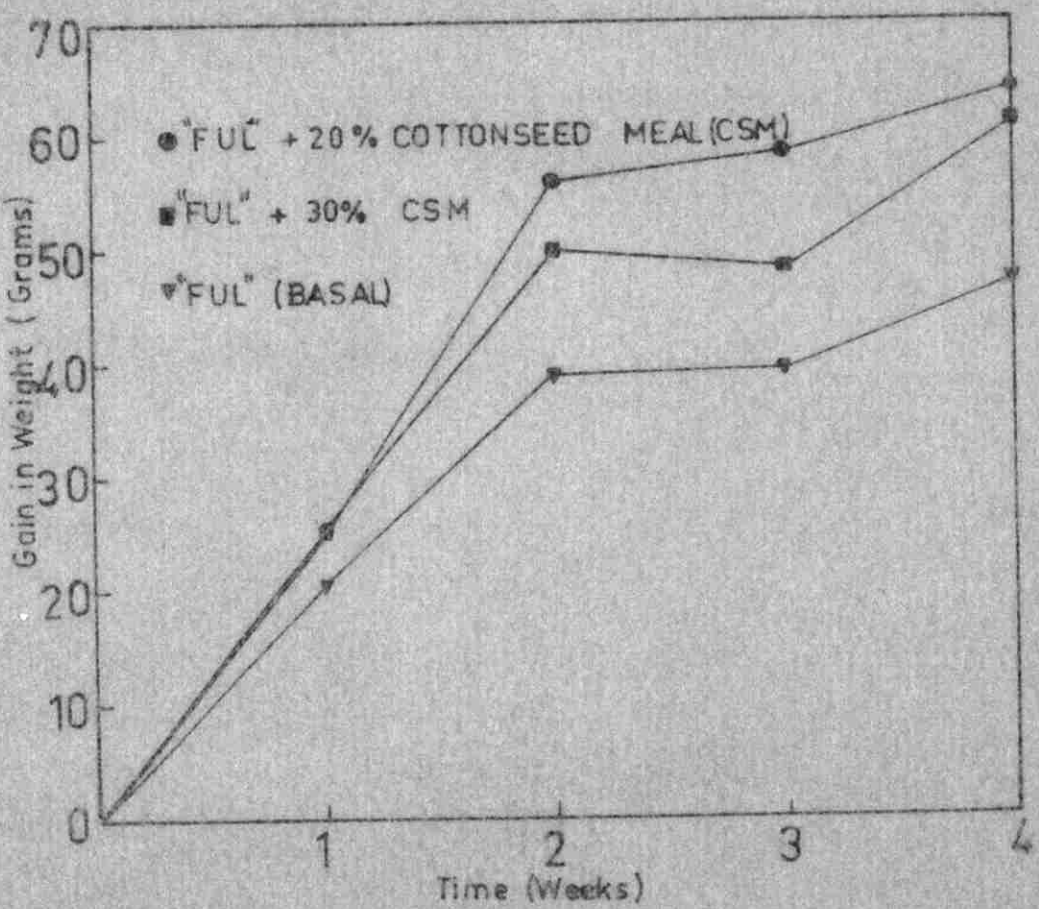

Figure 2. Four-week growth curves of rats fed the basal and supplemented "Ful" diets

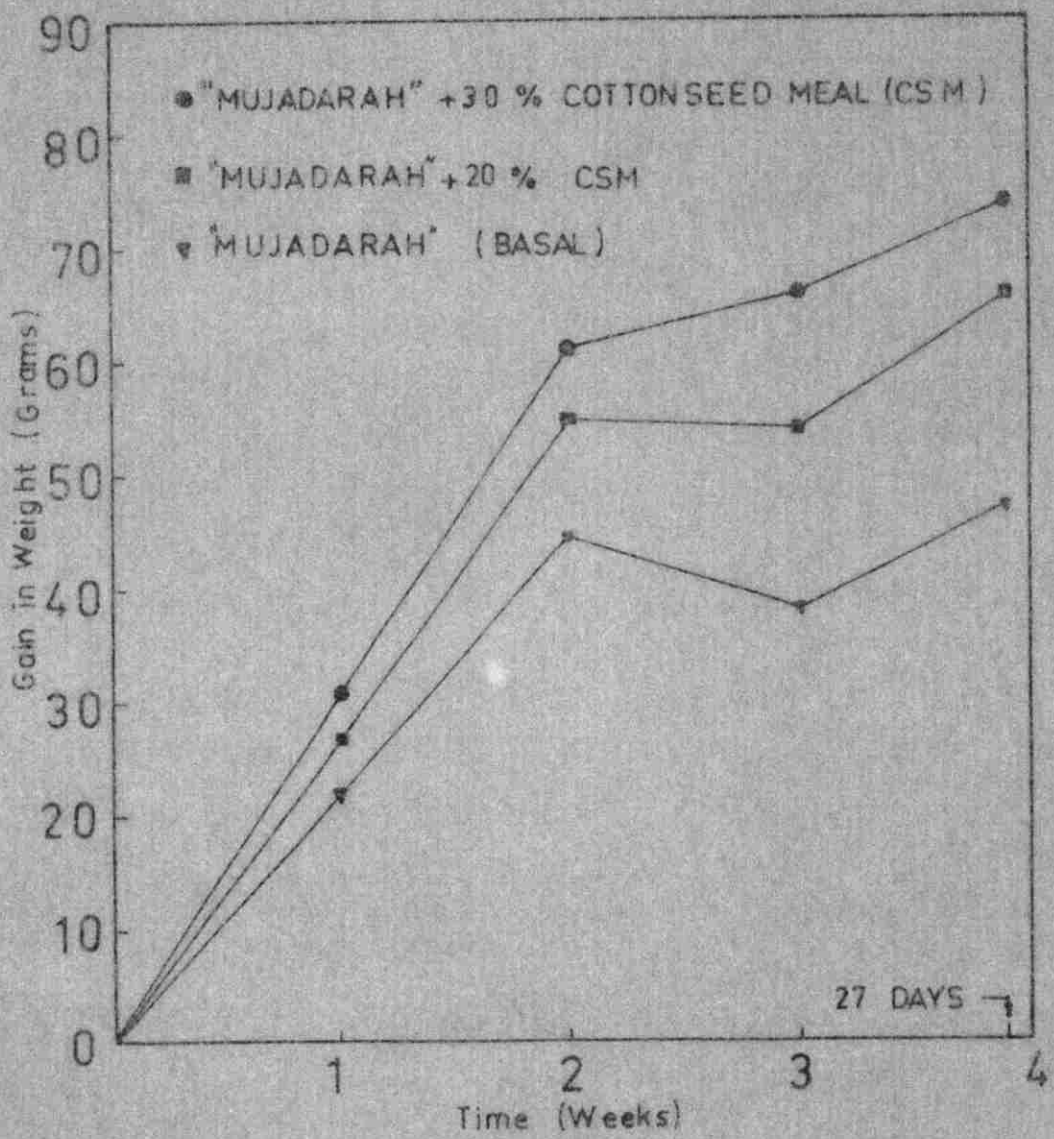

Figure 3. Growth curves of rats fed the basal and supplemented "Mujadarah" diets

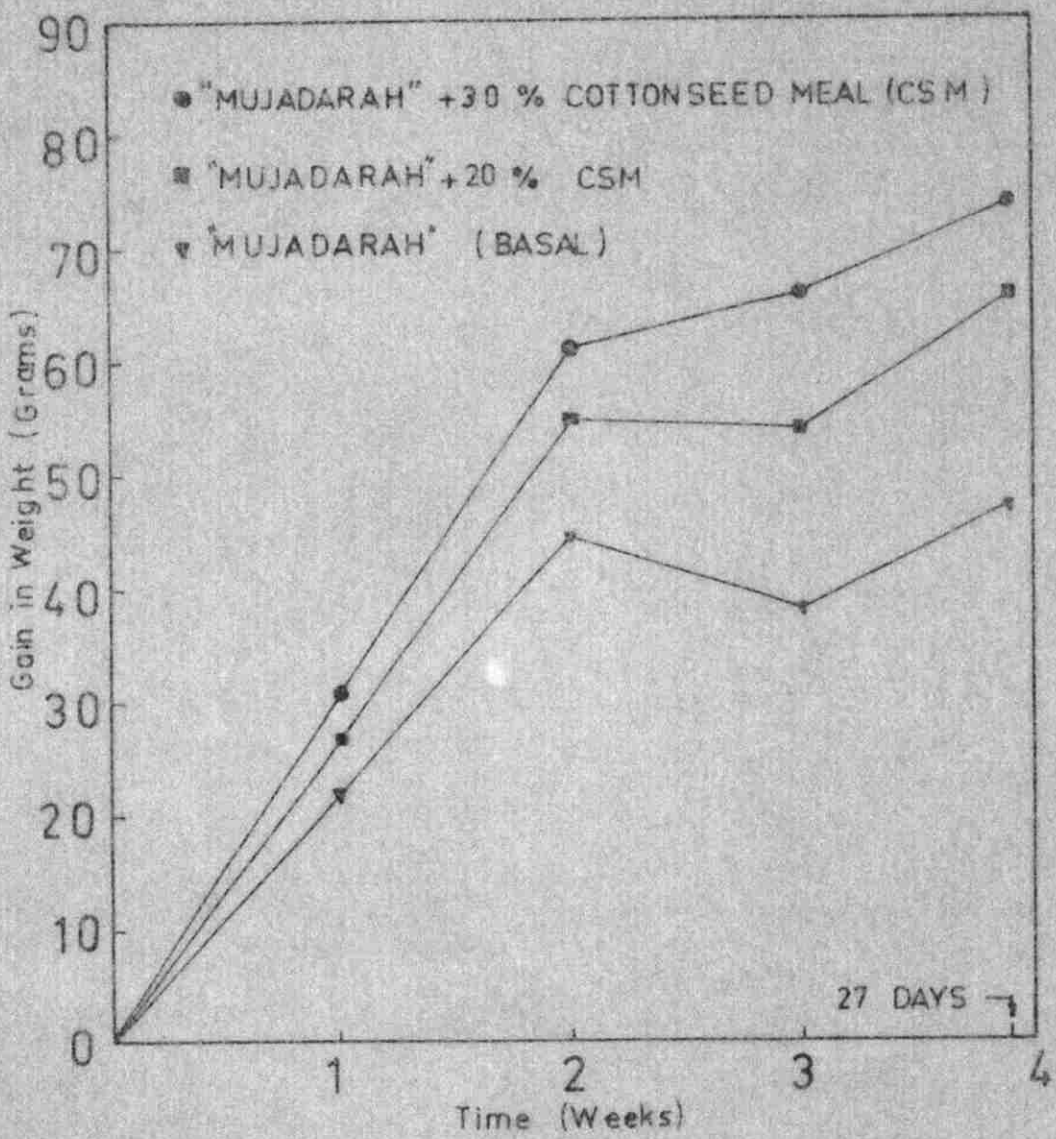

Figure 3. Growth curves of rats fed the basal and supplemented "Mujadarah" diets

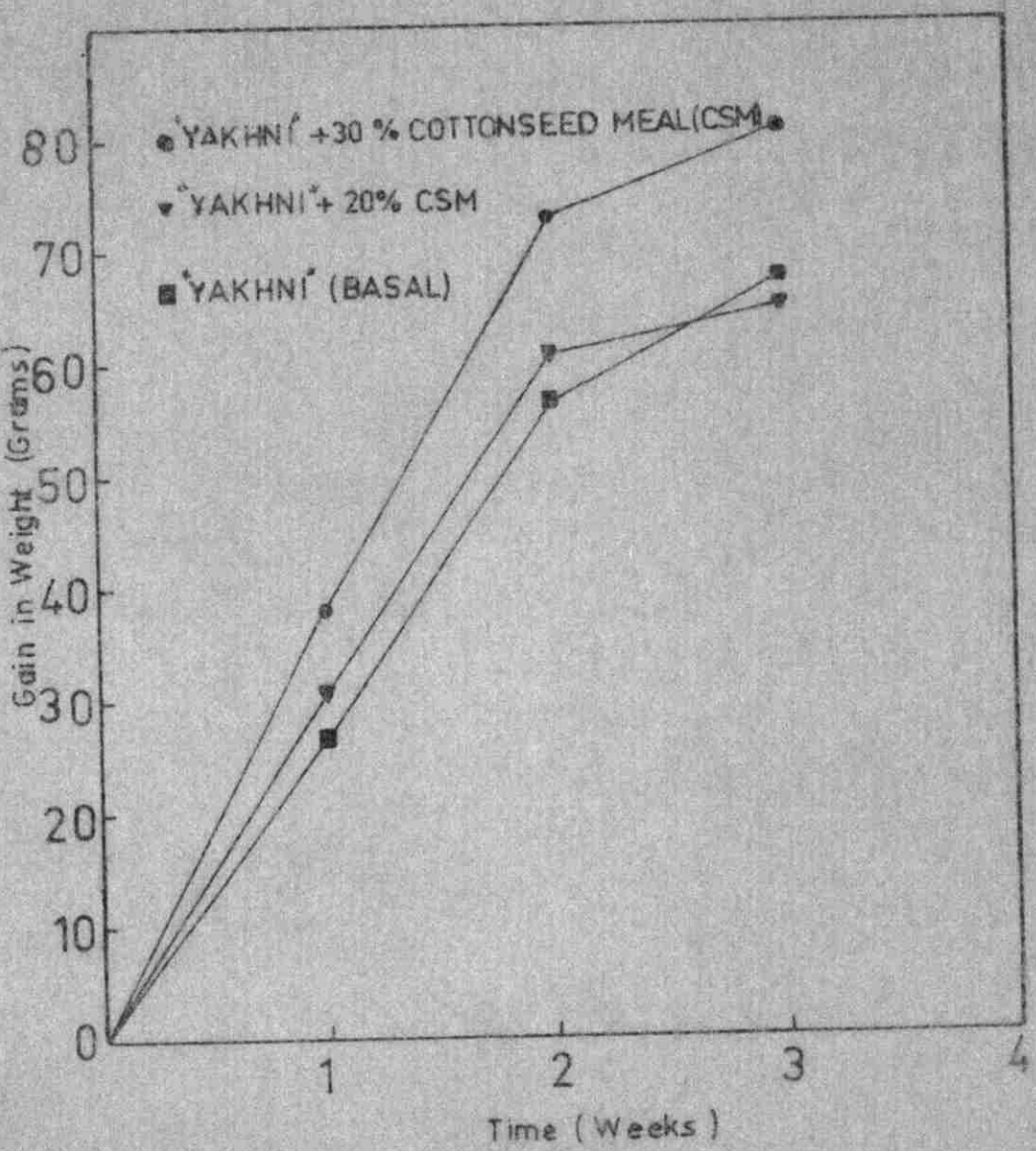

Figure 4. Three-week growth curves of rats fed the basal and supplemented "Yakhni" diets

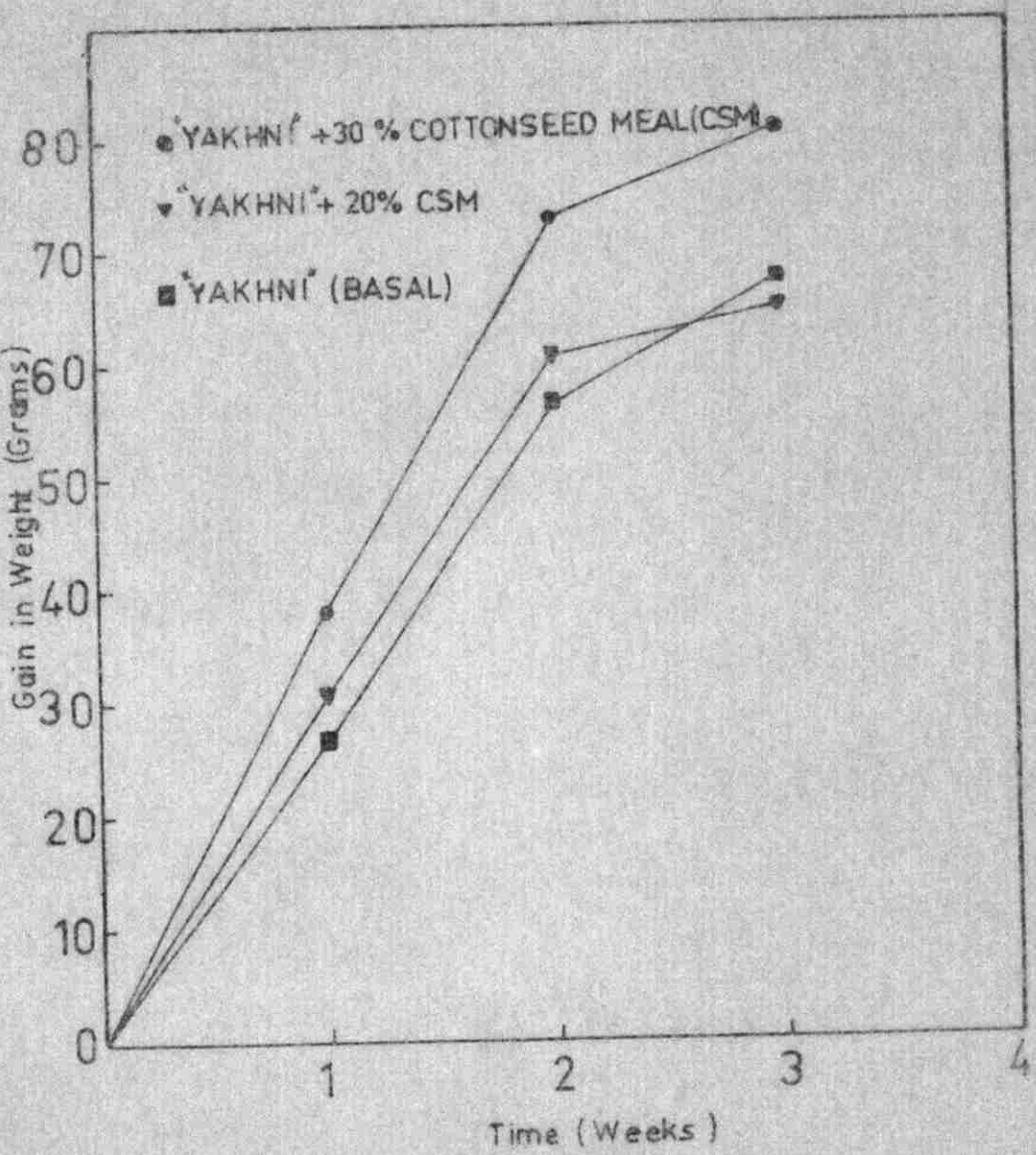

Figure 4. Three-week growth curves of rats fed the basal and supplemented "Yakhni" diets

DISCUSSION

Proximate Composition of Cottonseed Meals

The data presented in Table 3 show that the meals were of similar proximate composition except for crude fiber and crude protein. The Egyptian, the Sudanese and one of the Syrian meals were high in fiber because the seed coats had not been removed in the process of oil extraction (undecorticated meals). The protein content of these three high fiber meals was approximately half that of the decorticated Syrian and Iranian meals.

Gossypol Content

The results presented in Table 4 show the free, bound and total gossypol content of the meals expressed in two ways: (1) per 100 gm. of the air dry meal, and (2) percent protein. Clark (21) has discussed the need for the expression of gossypol content on an adjusted basis of protein for the comparison of different samples, irrespective of the total protein content. According to Clark, the gossypol content of a meal is dependent upon the protein level, and as this level increases upon dehulling, the gossypol content also increases. From the data in Table 4, it is apparent that Egyptian meal was highest in free gossypol (0.71 gm. F.G. percent protein) and the Iranian meal was the lowest (0.07 gm. F.G. percent protein). The Sudanese and the Egyptian meals had the highest bound gossypol (3.30 and 3.00 gm. B.G. percent protein respectively), whereas the decorticated Syrian meal had the lowest value (1.82). It is known that most of the gossypol in the cottonseed is in the free form, and that high temperatures encountered

during oil extraction result in the binding of free gossypol to lysine molecules of the protein. In the Sudanese and Egyptian meals, high processing temperatures may have accounted for the high content of bound gossypol.

Amino Acid Composition

Since plant foods, in general, are limiting in methionine, methionine + cystine, threonine, lysine and tryptophane, only these amino acids were assayed; the results are presented in Table 5. When these values are compared with those for egg (Table 5), it is apparent that the meals are most limiting in tryptophane. However, for the purpose of the present work, it was thought beneficial to calculate the most limiting amino acid on the basis of the requirement of the growing rat (Table 6). The data in Table 6 show that, on this basis, methionine, and not tryptophane, is the most limiting individual amino acid. This disagreement can be explained by the fact that the rat's requirement for tryptophane is low relative to that for methionine. Although it is clearly evident (Table 6) that methionine was most limiting, the fact that the meals were high in cystine requires another conclusion. The rat can synthesize methionine from cystine and, when the cystine + methionine level is considered, methionine is no longer the most limiting. Therefore, according to the data in Table 6, the most limiting amino acid, based on the rat requirement was threonine. There was a possibility that lysine was actually the most limiting amino acid in these meals since, as previously discussed, it may have become unavailable during processing. However, no attempt was made to determine the "available" lysine of these meals.

The only way to have established the actual order of limiting amino acids would have been to conduct an experiment using diets supplemented with the respective amino acids.

Animal Assays

The 4-week growth curves for the PER experiment are shown in Figure 1; weight gains and food consumption data are presented in Table 7. Animals fed a diet containing 10 percent protein from the Sudanese meal gained the most, followed by those fed undecorticated Syrian, Iranian, decorticated Syrian and Egyptian meals.

The growth depressing effect of free gossypol became apparent in the rats fed the Egyptian meal which contained a large amount of this pigment (0.71 gm. F.G. percent protein). Most of the rats on this diet lost weight, and after the nineth day of the experiment, the animals began to die; only two of the original 10 animals survived the 28-day assay period. Unusual darkening of the livers of these animals was observed upon post mortem examination.

Food consumption values followed the same pattern as weight gains (Table 7), with the animals on the Sudanese cottonseed meal diet having the highest, and those on Egyptian meal the lowest food consumption.

The gain in weight of the animals fed Iranian meal cannot be explained on the basis of the gossypol content. Although this meal had much less free and bound gossypol (Table 4) than the Sudanese and the undecorticated Syrian meals, the rats had lower weight gains than those of the other two groups. These lower gains may be explained on the basis of the lower levels of certain more limiting amino acids, such

as threonine, as compared to the same amino acid values for Sudanese and undecorticated Syrian meals (Table 5). The highest gain in weight of animals on Sudanese meal was probably related to the low content of free gossypol (0.23 gm. F.G. percent protein) and also to the higher levels of limiting amino acids (Tables 5 and 6).

The protein efficiency ratio (PER) and net protein utilization (NPU) values for the different cottonseed meals are presented in Table 8. The data show that the Sudanese meal had the highest PER (1.48), whereas the Egyptian meal had the lowest (-0.08). There was a highly significant difference between the PER values of each meal and that of casein. Furthermore, there were significant differences (L.S.D. at 1 percent level = 0.14) among the PER values of the different samples, with the exception of Iranian and undecorticated Syrian meals. These latter two meals had similar PER values (0.82 and 0.84 respectively).

A non-significant negative correlation ($r = -0.80$; $P = 0.1$) was found between the PER values and the free gossypol content of the meals. The difference between the PER values of the Sudanese and the Egyptian meals may be discussed on the basis of their free gossypol content (Sudanese meal, PER = 1.48, F.G. percent protein = 0.23 gm; Egyptian meal, PER = -0.08, F.G. percent protein = 0.71 gm.). In the interpretation of the PER values of other meals, however, the amino acid composition as well as gossypol content should be considered. The undecorticated Syrian meal had a higher PER than the decorticated Syrian meal, but both of these meals had the same free gossypol value (0.31). However, the former was higher in bound gossypol (2.55 gm. B.G. percent protein) than the latter (1.82 gm.); thus, the difference in the PER

values of these meals cannot be explained on the basis of gossypol level alone. The lower threonine content of the decorticated Syrian meal (2.930 gm. per 16 gm. N) compared to that of the undecorticated meal (3.112) may have been related to the lower PER value of the former. By the same reasoning, the difference between the PER value of the Iranian and Sudanese meal may be related to the difference in the lysine content of the two meals (Table 6).

Although there were statistically significant differences among certain of the meals, and these differences may be partially explained as in the above discussion, a PER value of less than one indicates a very poor quality protein. It should be emphasized that differences between a PER of 0.44 (decorticated Syrian) and 0.84 (undecorticated Syrian) or 0.82 (Iranian) are of no real practical importance.

The differences among the average liver weights (per 100 gm. body weight) of the groups of animals from the PER assay were not significant.

There was a highly significant difference between the NPU values of the Sudanese meal and the other four meals, but the differences among the NPU values of the latter four meals were not significant (L.S.D. at 1 percent level = 9.1).

The two bioassay procedures (PER and NPU) gave practically the same order of ranking of samples according to their protein quality, and a significant positive correlation ($r = 0.89$, $p = 0.05$) was obtained between the NPU and the PER values of the different meals. This is in agreement with the results of Bender (10) who obtained good

correlation between the NPU and PER values of a wide variety of proteins.

It is apparent from the PER and NPU data that the Sudanese cottonseed meal was by far the most promising of all the meals for use as a protein source.

Supplementation Experiment

The cottonseed meals were found to be relatively high in total sulfur amino acids and, in 10 percent protein diets, they had the potential of supplying about 85 percent of the requirement of growing rats for these amino acids (Table 6). Since the Sudanese meal had a relatively low level of gossypol and the highest protein quality (PER = 1.48, NPU = 40.5), it was decided to study the effect of supplementing 3 typical Middle Eastern (M.E.) dishes with this meal.

In Table 9 are presented the proximate composition of the diets containing the M.E. dishes before and after supplementation with 20 and 30 percent Sudanese cottonseed meal. The composition of the supplemented dishes was calculated using values obtained for each dish and for the cottonseed meal.

The amino acid composition (threonine, lysine, tryptophane, methionine and cystine) of the diets (Table 10) is expressed in gm. amino acid per 100 gm. of the final diet rather than in gm. per 16 gm. nitrogen. This method of expression was necessary for the interpretation of the results from the feeding experiment since, in the assay, the protein content of the different diets after supplementation was not the same (Table 9).

The data in Table 11 show that, based on the rat requirement, all the final diets were most limiting in methionine followed by

methionine + cystine in "Mujadarah" and "Ful". "Yakhni" alone had a good pattern of essential amino acids because of its meat content. As a result of the supplementation of "Ful" with 20 and 30 percent cottonseed meal, there was an increase in the amount of methionine + cystine; the level of the other amino acids decreased (Tables 10 and 11). A similar result was obtained upon supplementation of "Mujadarah". After supplementation of "Yakhni" at both the 20 and 30 percent level, the amount of all the amino acids decreased (Tables 10 and 11).

In Table 12 are presented data on food consumption and average weight gains of rats fed the basal diets alone and supplemented. The assay period was 28 days for rats on the "Ful" diet but, due to the insufficient supply of Sudanese cottonseed meal, the experiments with "Mujadarah" and "Yakhni" were stopped after 27 and 21 days respectively. The growth curves for each diet are presented in Figures 2, 3 and 4.

There was a significant difference between the weight gains of the rats on the control diets of "Ful" and "Mujadarah" and the same diets supplemented with 20 and 30 percent cottonseed meal (L.S.D. at 5 percent level = 12.9 for "Ful" and 13.9 for "Mujadarah"). However, no significant difference was obtained between the 20 and 30 percent supplementation levels. The difference in average weight gains between the groups on "Yakhni" alone and "Yakhni" supplemented with 20 percent cottonseed meal was not significant. In fact, supplementation at this level reduced gains slightly. Supplementation of the "Yakhni" diet with 30 percent cottonseed meal, however, resulted in a significant increase in weight gain compared to the control diet (Table 12) (L.S.D. at 5 percent level = 13.9).

The increase in growth of rats on "Ful" supplemented with cottonseed meal (Figure 2) was due, most probably, to the increase in the amount of sulfur amino acids (Table 10 and 11). Although the addition of 30 percent cottonseed meal increased, by 4 percent, the sulfur amino acid level obtained by 20 percent supplementation (Table 11), gains and food consumption were essentially the same at both levels. No clear explanation can be offered for this observation.

In the case of the "Mujadarah" diet, rat growth was increased by adding 20 percent cottonseed meal; a further increase was observed after raising the supplementation level in the diet to 30 percent (Figure 3). This further increase can be attributed to the greater consumption of the latter diet. In addition, there was a significant positive correlation between weight gains and the methionine + cystine content of the diets ($r = 0.998$; $p = 0.05$).

Because of the meat in "Yakhni", the essential amino acid pattern was far better than that of the Sudanese cottonseed meal (compare Tables 11 and 6). Supplementation of this diet with increasing levels of the meal, therefore, resulted in a "dilution" of the essential amino acids (Table 10). The decrease in these amino acids was accompanied by an increase in the crude protein content. Although there was no significant difference between the growth of rats on "Yakhni" and the same diet supplemented with 20 percent cottonseed meal, there was a significant increase in growth on the 30 percent supplemented diet. This increase cannot be explained on the basis of amino acid composition; however, it may be attributed to the increased food consumption (316 gm. compared to 264 gm.). Perhaps the 30 percent

supplemented diet was more palatable.

There was a leveling off in the growth curves of all groups during the third week of the assay (Figures 2, 3 and 4). This was possibly due to the flooding of the animal room which undoubtedly resulted in changes in temperature and humidity.

The differences among the average liver weights (per 100 gm. body weight) of the groups of animals on diets with and without cottonseed meal were not significant.

It can be concluded that Sudanese cottonseed meal can improve the protein quality of certain high legume diets.

SUMMARY AND CONCLUSIONS

Five Middle Eastern cottonseed meal samples (two from Syria and one each from the Sudan, Egypt and Iran) were analyzed for proximate composition, gossypol content, and for methionine, cystine, lysine, tryptophane and threonine. The protein quality of each sample was evaluated using two different bioassay procedures: namely, protein efficiency ratio (PER) and net protein utilization (NPU). In addition, the effect of supplementing three typical Middle Eastern dishes with 20 and 30 percent Sudanese cottonseed meal was studied.

The Egyptian, the Sudanese and one of the Syrian meals were high in fiber and low in protein compared to the other two meals. The Egyptian meal had the highest free gossypol content (0.71 gm. F.G. percent protein) and the Iranian meal the lowest (0.07). The Sudanese and the Egyptian meals had the highest bound gossypol (3.30 and 3.00 gm. B.G. percent protein respectively).

According to the requirement of the growing rat for essential amino acids, threonine was the most limiting amino acid in four of the meals; lysine was most limiting in one of the Syrian samples.

The protein efficiency ratio (PER) and net protein utilization (NPU) values of the Sudanese sample were the highest (1.48 and 40.5 respectively); the other meals had PER values of less than 1.0, indicating very poor protein quality.

A non-significant negative correlation was obtained between the PER values and the free gossypol content of the meals ($r = -0.80$, $P = 0.1$). There was a significant correlation between the NPU and PER values ($r = 0.89$, $P = 0.05$).

The growth of rats on two high legume Middle Eastern diets ("Ful" and "Mujadarah") supplemented with 20 percent Sudanese cottonseed meal was better than growth on the basal diets alone. This increased growth was due to the greater consumption of the supplemented diets. Supplementation of these two dishes with 30 percent cottonseed meal did not result in a further increase of weight gains. Rat growth on the meat-containing M.E. diet ("Yakhni") did not increase after supplementation with 20 percent cottonseed meal; a significant increase was obtained with 30 percent supplementation.

From the results of this study, the following conclusions were drawn:

1. The Sudanese cottonseed meal was by far the most promising of the meals as a protein source.
2. The measurement of protein quality appeared to be affected by the presence of gossypol, although its effect was not consistant with all meals.
3. Protein efficiency ratio (PER) and net protein utilization (NPU) gave essentially the same results for the ranking of these meals according to protein quality.
4. The nutritive quality of high legume diets can be improved by the addition of cottonseed meal.

LITERATURE CITED

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