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Dietary Supplementation of Tarhana with Soya Bean Flour and Fish Protein Concentrate

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To the Graduate Council:

I am submitting herewith a thesis written by Tahire O.K. Merdol entitled "Dietary Supplementation of Tarhana with Soya Bean Flour and Fish Protein Concentrate." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Jane R. Savage, Major Professor

We have read this thesis and recommend its acceptance:

Mary N. Traylor, Cyrus Mayshark

Accepted for the Council:
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(Original signatures are on file with official student records.)

October 15, 1968

To the Graduate Council:

I am submitting herewith a thesis written by Tahire Oya Koçtürk Merdol entitled "Dietary Supplementation of Tarhana with Soya Bean Flour and Fish Protein Concentrate." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Jane R. Savage
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We have read this thesis and recommend its acceptance:

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Cyrus Mayshark

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DIETARY SUPPLEMENTATION OF TARHANA WITH SOYA BEAN FLOUR AND FISH PROTEIN CONCENTRATE

A Thesis
Presented to
the Graduate Council of
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In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Tahire Oya Koçtürk Merdol

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T. O. K. M.

ABSTRACT

Protein malnutrition is a problem in a major portion of the world where good quality protein sources are in short supply and expensive. Extensive studies have been carried out in order to improve the protein quality of certain existing foods which may be more abundant in a given community.

In this study the protein quality of a Turkish soup, tarhana, was determined by measuring its Net Protein Utilization (NPU). Diets containing tarhana were fed to rats at a level which gave 10 percent protein. The effect of supplementing tarhana with either soya bean flour or fish protein concentrate on the NPU was also investigated. The resulting NPU values were compared with the NPU of a 10 percent casein diet which served as a control.

In determining the NPU values, two methods of estimating total body nitrogen were employed. These were calculation from total body water utilizing a regression equation computed for the rats of the Wistar strain and determination by chemical analysis of the carcass. Both of these methods gave similar results.

It was found that the NPU of tarhana was significantly inferior to that of a casein diet, but could be improved to compare favorably with casein when soya bean flour is incorporated at a 20 percent level

during the preparation of tarhana. The addition of fish protein concentrate at a 3 percent level also tended to improve the protein quality of tarhana, but in view of the skin rash and poor growth that was observed in rats fed the tarhana with fish protein concentrate diet, more research may be necessary before this product could be prescribed as a supplement to tarhana.

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CHAPTER I

INTRODUCTION

In a large portion of the world, the most pressing public health problem is still that of attaining a desirable nutritional level for the populations. This is particularly true in terms of protein; excluding some very deprived areas, the problem is one of securing foods of "good" protein quality rather than one of sufficient quantity (1,2). For many countries, increasing the gross quality of the food yield involves the adaptation of modern techniques to methods of food production, processing, and distribution, before any improvement which will influence every individual in the community is realized (3). To accomplish these adaptations of technology will require considerable time, money, and the efforts of technically trained people from many fields. An approach which might not require as great an investment in terms of time and money would be to utilize the available foods of the country to a better advantage than is currently being done. Educational programs designed to illustrate how the existing food-processing techniques could be changed in order to improve the quality of the diet would be one example.

Each community has its own popular food product which is frequently and commonly prepared and consumed in every home. Supplementation of such a product or products could be a way of improving the quality of the diet without changing the existing food habits.

Tarhana is a very popular food product in Turkey, the author's home country. It is a food mixture often prepared in the home by mixing yogurt and wheat flour together and allowing lactic acid fermentation to occur for several days. The mixture is then dried and powdered for storage. Tarhana could be reconstituted by the addition of water or broth to the dried mixture, and served as a soup or porridge during the winter months. The popularity of this food, the relative simplicity of processing, coupled with indefinite keeping quality, especially if non-fat yogurt is used, and the presence of milk solids which supplement the wheat protein in the mixture, have led several workers to believe that this product might be an excellent low cost food that could be supplemented so that the quality of the protein would be improved (4,5).

In this study an attempt was made to ascertain the protein quality of tarhana as compared to a standard casein mixture, and to determine whether the supplementation of tarhana with soya bean flour or fish protein concentrate would cause a significant improvement in the protein quality. In addition, two methods for the shortened determination of protein quality in diets were compared. These two methods were estimation of body nitrogen by chemical analysis and by employing a regression equation relating body water to body nitrogen.

CHAPTER II

REVIEW OF LITERATURE

I. FOOD SUPPLEMENTATION

In this thesis, the term "food supplementation" will be used exclusively to indicate the process of adding essential amino acids (not vitamins or minerals), in some form to an originally deficient food product in order to increase its biological value.

The practicability of supplementing locally accessible food in many developing countries of the world to improve the quality of the protein in the diet until better methods of food production, processing and distribution especially of animal sources of protein are available is well recognized. Foods of plant origin are most commonly subjected to supplementation because of their low biological value. Since the people in most countries frequently derive a large percentage of their calories from one type of grain the need of securing a well-balanced protein intake from cereals and grains is of vital importance.

The proteins in most cereal grains are usually deficient in one or more essential amino acids. Zein, a protein in corn, is deficient in tryptophan and lysine; wheat gluten is deficient in lysine, and the protein of rice lacks adequate amounts of lysine and threonine. The biological value of zein was significantly improved when it was supplemented in a stepwise fashion with lysine, tryptophan, and isoleucine (6).

Several studies showed that the addition of lysine to wheat gluten increased its biological value (6,7). Howe *et al*. (2) demonstrated that amino acid supplementation involving only lysine, tryptophan, and threonine invariably increased the quality of the proteins of staple cereals so that the growth rate of rats was comparable with that of rats fed standard casein diets, and concluded that proper amino acid supplementation of cereal-based diets may be helpful in preventing protein deficiencies.

The use of purified amino acids to supplement the poor-quality protein in vegetables and cereals is at the present time not considered practical because of the high cost (8). Another approach to the problem is the supplementation of a food containing a protein of low biological value with small amounts of other foods which contain good-quality protein (9). Numerous research studies indicate that supplementation through such food combinations is possible, practical, and generally acceptible (9,10,11). Autret and Van Veen (12) have given a short account of the possible infant formula combinations based on vegetable mixtures used by the Food and Agriculture Organization (FAO), and the United Nations Children's Fund (UNICEF). The Institute of Nutrition for Central America and Panama (INCAP) has developed a good quality vegetable mixture known as Incaparina. It is a mixture containing cornmeal, cottonseed flour, and sorghum flour which has been shown to have a high biological value (13).

There is sufficient evidence to indicate that Turkey is one of the countries where the consumption of animal protein has not yet reached a satisfactory level (14). Table 1 shows the per capita food supplies available in several countries. The people of Turkey consume a high level of cereals and, like most Middle Eastern countries, the major portion of the caloric intake is furnished primarily by wheat. Only 37 g. of meat are available per person per day; the amounts of fish, milk, and eggs are also low.

In view of these data and the fact that nutrition education programs in meal planning are not at a satisfactory level in Turkey (14), it could be assumed that most probably the diets are generally low in good quality protein.

II. TARHANA

Tarhana is a staple food in every Turkish home. It has been a food customarily eaten throughout centuries. This food stuff was carried from Central Asia, where it is still used under the same name, by Mongoloid nomads to Turkey and other countries such as Hungary and Finland as early as A.D. 450. Its use spread to Middle Eastern and North African countries during the Ottoman reign. Today, tarhana is known in all of these countries; in Arab countries it is called "kisk," in Hungary, "tarhana," and in Finland, "talkuna."

The preparation of this food stuff is based on lactic acid fermentation, initiated by the presence of yogurt or buttermilk. Each country and region has its own practice of using the type of cereal

TABLE 1

PER CAPUT FOOD SUPPLIES AVAILABLE FOR HUMAN CONSUMPTION
IN SELECTED COUNTRIES (15)

Country	Period	Cereals	Vegetables	Fruit	Meat	Eggs	Fish	Milk
		g/day	g/day	g/day	g/day	g/day	g/day	g/day
Iran	1960	394	22	101	44	5	2	176
Jordan	1960-62	368	319	315	33	5	2	99
Syria	1960-62	432	153	435	38	4	-	146
Turkey	1960-61	611	288	340	37	5	6	193
Yugoslavia	1960-62	519	151	128	78	9	2	358
Canada	1960-62	182	204	211	213	42	15	663
United States	1960-62	181	270	248	262	52	13	674

they prefer; wheat flour and bulgur are used in the Middle East, Hungarians use barley, and the Finns use a mixture of pulses.

In Turkey tarhana is prepared by mixing equal parts of yogurt or buttermilk with wheat flour or bulgur. A variety of cooked vegetables and herbs, differing with regional taste preferences, are added and the mixture is allowed to ferment from one to five days. If the product is prepared at home, it will be sun-dried. Later it is pounded and milled to a coarse, flour-like consistency and stored in cloth bags for use during the winter months. It is usually served as a main course accompanied with eggs, vegetables, or bread according to the wealth of the family (16).

The biological value of the protein in tarhana is not known. One might postulate that probably the standard tarhana mixture would not have a very high biological value because of the relatively large amount of wheat flour present. It is known that wheat protein is deficient in several amino acids, primarily lysine (1). It is questionable whether this deficiency is completely overcome by the milk solids in the mixture.

III. POSSIBLE SUPPLEMENTS FOR TARHANA

Previous work done by this researcher at the Nutrition Department of the University of Tennessee indicated that the standard mixture of tarhana was not adequate to promote optimal growth in weanling rats. On a theoretical basis the supplementation of a standard tarhana mixture with soya bean protein at a 15 percent level should provide an improved assortment of amino acids and thus a higher quality protein than tarhana

alone. However, the protein efficiency ratios (PER) of rats fed either a standard tarhana diet or a tarhana supplemented with soya protein diet were lower than the PER of rats fed an 18 percent casein diet. The reason why the tarhana-soya protein mixture had a low PER value was not determined. It is possible that an antitryptic factor was present in the crude soya protein extract, which decreased the digestibility of the proteins.

It was decided to use soya bean flour in future studies rather than soya protein extract mainly because of the questionable nature of amino acid availability, as well as the fact that the extraction of the soya bean protein is not feasible in Turkey and its production would probably be costly.

Soya bean flour is high in protein content and has a good biological value. It has been successfully used to supplement other vegetable proteins. Sure (17) obtained a high protein food with a mixture consisting of defatted soya bean flour, skim milk powder, and whole wheat flour plus vegetable shortening supplemented with vitamins and minerals; a basic mixture which strongly resembles that of tarhana. Block *et al.* (18) found that the protein quality of soya bean flour was slightly increased by the addition of sulphur-containing amino acids which are relatively abundant in milk protein. Autret and Van Veen (12) mention the possibility of using soya beans as supplements in infant formulas. Patwardhan (19) also gives an account of their use as protein supplements.

Skim milk powder is an excellent source of good-quality protein and has successfully been used in the production of yogurt. Skim milk powder has been supplied to Turkey through various international organizations (14) and also is being produced on a small scale in the country.

Fish protein concentrates have been introduced as potential protein supplements recently (20). Fish "flour," fish protein concentrate (FPC), marine protein concentrate are essentially the names of the same product, resulting from the removal of the water, oil, and bones of fish (21). In some cases, even the characteristic odor of fish has been removed. The VioBin Corporation in Illinois has been manufacturing FPC on a large scale for the last eleven years by the method described by Levin and Finn (22). Autret and Van Veen (12) in a review article on the efforts of the FAO to supplement the diets of infants and children in various parts of the world, indicate that foods supplemented with as much as 10 percent FPC were well accepted. Sure (23) found that the addition of small amounts of fish flour to cereal diets fed growing rats markedly increased the protein efficiency ratio. Morrison and Campbell (24) compared the PER of diets fed to weanling rats containing 7, 10, and 15 percent protein supplied by either fish protein or casein. After feeding the various diets to the rats for four weeks, they found that the PER for diets containing the 7 and 10 percent fish flour were significantly higher than comparable casein diets. No differences were observed at the 15 percent level. In further studies by the same workers, the addition of 10 percent fish flour to bread made with or without 4.2 percent milk solids increased the PER 82 and 198 percent, respectively.

Jansen et al. (25) fed rats white bread diets supplemented with various combinations of fish flour, lysine, and threonine. They found that the growth rate of rats fed diets containing the fish flour supplement were superior to that of rats fed a control casein diet and also that the PER of the fish flour supplemented diet was higher than the casein diet.

Turkey, a country surrounded by four seas, could increase its utilization of fish with relative ease. There have been attempts by the governmental "Meat and Fish Organization" to manufacture fish flour (14) and some success has been obtained. However, lack of knowledge as to the ways fish flour can be incorporated into the diet and the advantages to be gained by its use has limited its acceptance by the general public. It is thought that if proper educational programs are used to demonstrate the benefits of fish flour in animal and human nutrition to the general population it may gain more acceptance than it has had in the past.

The use of wheat flour in tarhana could not be avoided altogether because apart from yogurt, it is the major constituent and the staple grain in Turkey. Omission of wheat may or may not cause a significant change in the characteristic taste of tarhana but at any rate it might present a problem in gaining the acceptance of the local people.

All the products mentioned above could be used in the preparation of tarhana, whose basic composition is given in Table 2..

IV. MEASUREMENT OF PROTEIN QUALITY

The protein quality of foods is generally determined either by analyzing the protein for its amino acid composition or by the

TABLE 2

AVERAGE COMPOSITIONS OF TARHANA SAMPLES FROM FOUR DIFFERENT REGIONS OF TURKEY (16)

Region	pH	Water	Ca	Fe	Protein	Carbohydrate	Fat
		%	%	%	%	%	%
South-East	4.4	9.48	6.30	0.11	13.78	64.46	5.87
Central	4.5	8.71	6.25	0.13	10.78	61.44	12.69
Aegean	4.4	8.08	10.10	0.32	15.17	60.01	6.32
Black Sea	4.4	9.60	9.48	0.07	12.17	65.94	3.64
Average	4.4	8.96	8.03	0.15	13.12	64.44	7.13

utilization of a biological assay technique. The latter method involves either the measurement of growth or nitrogen balance in experimental animals when a diet of unknown protein quality is fed under standardized conditions.

Mitchell (26) and Mitchell and Carman (27) in early studies on this subject proposed the basic methodology for determining the biological value of proteins. Since then many studies have been conducted and several other relationships have been established which can serve as indices for protein quality (28).

Net Protein Utilization (NPU) is one of these suggested methods in which the protein quality is measured as a function of the biological value times the digestibility of a protein.

The biological value of a protein is defined as the proportion of the absorbed nitrogen that is retained by the body, while digestibility is the proportion of the nitrogen intake that is absorbed. The conventional formula which expresses NPU is:

$$NPU = \frac{I - (F - F_k) - (U - U_k)}{I - (F - F_k)} \times \frac{I - (F - F_k)}{I} \qquad (1)$$

where I = nitrogen intake, F = total fecal nitrogen, F_k = total endogenous fecal nitrogen, U = total urinary nitrogen, and U_k = total endogenous urinary nitrogen.

NPU is mainly a measurement of the change of total body nitrogen during the experimental period divided by the total nitrogen intake during this period, but allowance is made for maintenance values by

subtracting the obligatory endogenous fecal and urinary nitrogen losses of the body from the total nitrogen losses. This is the main difference between NPU and PER; in determining PER maintenance values are ignored. Taking the maintenance values into account is important, from the standpoint of measuring the exact amount of nitrogen retained, since this gives a more accurate indication of the protein quality.

Platt and Miller (29) and Miller and Payne (30) have pointed out that NPU depends not only on the amino acid composition of the diet but also on the protein-calorie ratio, the adequacy of the caloric intake, and the level of vitamins and minerals in the diet. In studies to determine the NPU of diets as ordinarily eaten by man, the food is fed in amounts that would be eaten under normal conditions and the NPU value obtained is termed NPU operative (NPU_{op}). When animals or humans are fed diets in which the protein intake is just below maintenance levels and NPU is determined, it is referred to as NPU standardized (NPU_{st}). The latter value is useful when the protein quality of several foods are to be compared. Miller and Payne (31) indicate that the best protein intakes for the determination of NPU_{st} vary between 8 and 15 percent, according to the nature of the protein.

As has been previously mentioned, NPU is a function of the biological value times the digestibility of the protein under investigation. Study of the formula (1) indicates that such a determination involves tedious and time-consuming efforts in collecting the urine and feces of the animals, measuring the food intakes, and analyzing for fecal and urinary nitrogen of rats fed both the test protein diet and

a nonprotein diet. Bender and Miller (32) have pointed out the complicated nature of the method and have suggested a simplification of the conventional formula (1):

$$NPU = \frac{I - (F - F_k) - (U - U_k)}{I} \quad . \qquad (2)$$

When allowance is made for the nitrogen intake of animals on a nonprotein diet, a symbol I_k is assigned. If the value I_k/I is subtracted from both sides of equation (2) one obtains:

$$NPU - \frac{I_k}{I} = \frac{(I - F - U) - (I_k - F_k - U_k)}{I} \qquad (3)$$

where (I - F - U) = gain in body nitrogen by the animal fed a test diet, or final body nitrogen minus initial body nitrogen, and $(I_k - F_k - U_k)$ = loss in body nitrogen of the animals fed a nonprotein diet, or final body nitrogen minus initial body nitrogen. Substituting these values in the equation (3) would give:

$$NPU - \frac{I_k}{I} = \frac{(B - B_o) - (B_k - B_o)}{I} \qquad (4)$$

where B = final body nitrogen of animal on test diet, B_o = initial body nitrogen of animal at beginning of study, and B_k = body nitrogen of animal fed a nonprotein diet. Simplification of the equation (4) gives:

$$NPU = \frac{B - B_k + I_k}{I} \quad . \qquad (5)$$

This equation was adopted and used throughout the present study for the calculation of NPU in tarhana mixtures. Using equation (5), it is thus necessary to determine only the total nitrogen intake of the rats and the body nitrogen of rats fed the test protein diet and the nonprotein diet. In an attempt to further shorten the determination of NPU Miller and Bender (33) investigated a relationship first mentioned by Moulton (34). He suggested that a constant proportionality exists between the body constituents of mammals and a decrease in the relative water content and an increase in body nitrogen occurs until the end of the growth period. It was therefore concluded by Miller and Bender that the nitrogen to water ratio is a constant and thus body nitrogen could be calculated from body water content at any given period of growth.

In a preliminary investigation, Bender and Miller (35) used 147 rats, aged 35 to 57 days. The animals were sacrificed after being fed various test diets for ten days and the carcasses were analyzed both for body nitrogen and water. There was a high correlation between the N/H_2O ratio expressed as $100 \times \%N/\%H_2O$ and age. The regression equation approached a straight line and was $y = 2.92 + 0.024x$, where y equals the N/H_2O ratio and x equals the age of rat in days. A subsequent investigation by the same workers (33) employing rats within the age range of 0-503 days showed that the criteria for linearity was best satisfied at approximately 100 days. The regression equation was $\log(4.8 - y) = 0.437 - 0.0123x$. The equations were calculated from data obtained using rats of a black-and-white hooded colony, thus it was questionable whether the values obtained would be applicable to animals of different strains

and species. Other investigators (36,37,38) have determined regression equations relating age to the N/H_2O ratio which were different than those given by Miller and Bender for their black-and-white hooded colony (Table 3).

The use of such a ratio for the estimation of body nitrogen shortens the time and work involved in the determination of NPU, since only the measurement of total body water and nitrogen intake are required. The N/H_2O ratio can be calculated for the group of rats used in the study and this value may be used for the determination of NPU. But several workers (37,38,39) have indicated that the error of this method is higher than the error introduced when the chemical analysis method is used. The former method therefore requires a higher number of experimental animals. It has also been indicated that the sensitivity of the method may depend on the limiting amino acid of the test diet. Henry and Toot-hill (37) observed that when methionine alone or methionine with cystine were the limiting amino acids in a diet, the NPU values obtained through the use of the body water technique were consistently lower than those obtained using the chemical analysis method. Another source of error might be related to the fat content of the animal, since the water and nitrogen content of the tissues are more constant when measured on a fat-free basis (40).

In this study, the body nitrogen of the animals was determined using both of the methods mentioned by Bender and Miller (32,33,35); that is, body nitrogen was determined by chemical analysis and by

TABLE 3

PUBLISHED REGRESSION EQUATIONS RELATING AGE AND N/H_2O RATIO (38)

Investigators	Age Range (days)	No. of Rats	Strain	Regression Equation[a]
Dreyer (1957)	32-49	300	Wistar	$y = 0.015x + 3.39$ (male) $y = 0.015x + 3.46$ (female) $y = 0.015x + 3.43$ (both sexes)
Bender & Miller (1953)	33-57	147	Black & white hooded	$y = 0.024x + 2.92$ (both sexes)
Miller & Bender (1955)	0-503	197	Black & white hooded	$y = 4.83 - \text{antilog}_{10}(0.4304 - 0.0115x)$ (both sexes)
Henry & Toothill (1962)	36-42	--	Hooded Norwegian	$y = 0.034x + 2.63$ (male) $y = 0.034x + 2.71$ (female)
Pellett (1967)	30-40	766	Sprague-Dawley	$y = 0.027x + 2.86$ (male)

[a]x = Age in days, $y = (100 \times N\%)/(H_2O\%)$.

calculation from total body water. From these data, the NPU of standard tarhana and tarhana supplemented with either soya bean flour or FPC were calculated and compared with the NPU of a casein diet.

CHAPTER III

EXPERIMENTAL PROCEDURE

I. PREPARATION OF TARHANA MIXTURES

The tarhana mixtures were prepared at the laboratories of the Food Technology Department, College of Agriculture of the University of Tennessee, Knoxville. The materials used in the mixtures were purchased locally.[1] The Fish Protein Concentrate[2] was ordered from the manufacturer because the product is not available on the market.

The tarhana mixtures consisted only of their basic components and no yeast or taste-giving foods were added because these differ according to regional preferances. The composition of each of the mixtures is given in Table 4. The quantity of each supplement was predetermined; soya bean flour was added at a 20 percent level and FPC was added at a 3 percent level at the expense of the wheat flour.

An attempt was made to prepare all the mixtures under standardized conditions. For this purpose the change in pH, fermentation time and temperature, drying time and temperature, and percent water lost were determined (Table 5).

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[1]"Gingham Girl" self-rising flour, enriched and bleached; "Fearn" Soya Bean Flour, full fat; "USDA" skim milk powder; "Daisy Brand" Yogurt culture.

[2]"VioBin" Fish-Protein Concentrate, deodorized, 90.7 percent protein.

TABLE 4

COMPOSITION OF TARHANA MIXTURES

Mixture	Yogurt	Wheat Flour	Soya-Bean Flour	Fish Protein Concentrate
	g%	g%	g%	g%
Tarhana	50	50	0	0
Tarhana with Soya Bean Flour	50	30	20	0
Tarhana with FPC	50	47	0	3

TABLE 5

STANDARDIZED DETERMINATIONS IN THE PREPARATION
OF TARHANA MIXTURES

Mixture	Initial pH	Fermentation Period	Final pH	Drying Temperature	Drying Period	Water Lost
		hr.		°C	hr.	%
Tarhana	4.5	60	4.6	70	54	48
Tarhana with Soya Bean Flour	4.5	60	4.6	70	48	34
Tarhana with FPC	4.5	60	4.6	70	52	42

Approximately ten liters of reconstituted skim milk were prepared; skim milk powder was mixed with water in the specified amount (three parts water to one part skim milk powder) and brought to a boil. The milk was allowed to cool to approximately 35°C. One liter of the reconstituted milk was transferred to a separate container and a tablespoon of the commercially purchased yogurt culture was added. The milk with the yogurt culture was sealed and allowed to coagulate at room temperature for 24 hours to produce the initial yogurt culture. The remainder of the milk was refrigerated.

Yogurt was prepared the next day using the reconstituted milk and the initial yogurt culture. The refrigerated milk mixture was reboiled and cooled to approximately 35°C, and was thoroughly mixed with the initial yogurt culture. The milk and yogurt mixture was divided into four portions of two liters each. Each two liter portion was placed in a jar which was sealed and allowed to stand for 24 hours at room temperature until the milk coagulated to form yogurt. Then the jars of yogurt were refrigerated.

Yogurt was mixed with the dry components in predetermined quantities as indicated in Table 4 and placed in the fermentation equipment supplied by the Food Technology Department. The fermentation time was standardized at 60 hours, or until the mixture gave a pH reading of 4.6 on a Beckman pH meter.

The fermented mixtures were dried in an electric oven, at approximately 70°C for 48 to 54 hours. The percent loss in water was determined (Table 5).

After drying, each mixture was ground in an electric mill to a thin, flour-like consistency and the nitrogen content was determined by the Kjeldahl method (41). The nitrogen and protein content of the tarhana mixtures is given in Table 6.

II. COMPOSITION OF DIETS

A total of five different diets were prepared for use in the study. These were a nonprotein diet, a 10 percent casein diet, and three test diets containing either tarhana, tarhana with soya bean flour, or tarhana with FPC. The composition of the nonprotein basal diet in percent was as follows: fat, 15; glucose, 15; salt mix,[3] 5; vitamin mix,[3] 5; and cornstarch, 60. The casein and tarhana mixtures were incorporated into the nonprotein basal diet to provide a diet containing approximately 10 percent protein at the expense of the cornstarch. In the case of the standard tarhana diet 15 percent of glucose, 3 percent of the salt mix, and 5 percent of the cooking fat was sacrificed in addition to the cornstarch so that sufficient amounts of tarhana to give the required protein concentration could be added. The respective composition of each diet is given in Table 7.

All diets were analyzed for nitrogen by Kjeldahl determinations and the protein content of the diet was calculated using the conventional conversion factor of 6.25 g. protein per g. of nitrogen. The nitrogen and protein content of the diets are given in Table 8.

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[3]Salt and vitamin mixtures: Nutritional Biochemicals Corporation.

TABLE 6

NITROGEN AND PROTEIN CONTENT OF TARHANA MIXTURES

Mixture	Percent Nitrogen	Percent Protein
		% N x 6.25
Tarhana	1.92	12.00
Tarhana with Soya Bean Flour	3.49	21.81
Tarhana with FPC	2.26	14.12

TABLE 7

COMPOSITION OF DIETS

Ingredients	Diets[a]				
	I	II	III	IV	V
	g %	g %	g %	g %	g %
Cooking fat	15	15	10	15	15
Cornstarch	60	50	0	15	1.6
Glucose	15	15	0	15	15
Vitamin mix	5	5	5	5	5
Salt mix	5	5	2	5	5
Casein	0	10	0	0	0
Standard tarhana	0	0	83	0	0
Tarhana with Soya Bean Flour	0	0	0	45	0
Tarhana with FPC	0	0	0	0	58.4

[a]Diets:
I. Nonprotein diet.
II. Casein diet.
III. Tarhana diet.
IV. Tarhana with soya bean flour diet.
V. Tarhana with FPC diet.

TABLE 8

NITROGEN AND PROTEIN CONTENT OF DIETS

Diet	Percent Nitrogen	Percent Protein
		% N_2 x 6.25
Nonprotein	0.28	1.75
Casein	1.49	9.31
Tarhana	1.68	10.50
Tarhana with Soya Bean Flour	1.70	10.62
Tarhana with FPC	1.62	10.13

III. GENERAL PLAN

Both male and female albino rats of the Wistar[4] strain were weaned at 21 days, and fed ad libitum a stock diet consisting of 64.5 percent whole wheat flour, 32.3 percent whole milk powder, 1.3 percent salt, and 1.9 percent yeast for one week. The animals were weighed at the beginning and end of this one-week period. The rats were 28 days old when the actual study began. A total of seven litters were available from which the animals to be used were chosen. Exceptionally large or small animals in each litter were discarded; the animals which were used in the study weighed an average of 46 ± 4 g. at the beginning of the experimental period. Sex was not a factor considered in the distribution of the animals in the various diets.

Two trials of the experiment were performed. Animals from four different litters were used in each of the two trials. Rats from three litters were assigned to each of the diets in both trials. Mean weights of animals fed each diet did not differ by more than 5 g. in the first trial and 3 g. in the second trial; a range of 7 g. in all, at the beginning of the experiment. The distribution pattern of animals from the various litters in the two experimental trials is shown in Table 9.

The experimental period was ten days during which time the animals were housed in individual cages and fed the various assigned experimental diets and water ad libitum. Food intake was recorded

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[4]"Wistar Strain"; Nutrition Department, University of Tennessee.

TABLE 9

BREAKDOWN OF LITTERS WITHIN EXPERIMENTAL TRIALS

Litter	No. of Animals	Trial I Diet[a]					Trial II				
		I	II	III	IV	V	I	II	III	IV	V
A	4	1	1	1	1	0	0	0	0	0	0
C	5	1	1	1	1	1	0	0	0	0	0
G	10	1	1	1	1	1	1	1	1	1	1
H	5	0	0	0	0	0	1	1	1	1	1
I	10	1	1	1	1	1	1	1	1	1	1
K	10	1	1	1	1	1	1	1	1	1	1
L	5	0	0	0	0	0	1	1	1	1	1

[a]Diets: I. Nonprotein.
II. Casein.
III. Tarhana.
IV. Tarhana with soya bean flour.
V. Tarhana with FPC.

every fifth day. Samples of the experimental diets were dried to constant weight at approximately 65°C for nearly three days, and the loss in water, percent total solids, and nitrogen content was determined. These values were used with the measured food intake of each animal to determine the true food and nitrogen intakes for each rat for the entire ten-day period.

At the end of the experiment, the animals were weighed and killed with chloroform. The carcasses were analyzed for total body water and body nitrogen.

IV. TOTAL BODY WATER ANALYSIS

Incisions were made into the body and skull cavities and the carcasses were dried to constant weight in an electric oven at approximately 100°C. Constant weight was obtained in about a week. The body water content for each rat was determined as the difference in the weight of the carcass before and after drying.

V. TOTAL BODY NITROGEN ANALYSIS

Total body nitrogen of each rat was determined by the Kjeldahl method. The whole dried carcass was weighed and placed into a 2000 ml. Erlenmeyer flask. The digestion was accomplished by heating the carcass with 10 ml. concentrated sulphuric acid per g. of dry body weight, plus an additional 25 ml. concentrated sulphuric acid and 20 g. catalyst (2 g. Hengar crystals, 2 g. $CuSO_4 \cdot 5\ H_2O$, and 16 g. Na_2SO_4). Care was taken to keep the digestion mixture below 170°C during the first two

hours of the digestion because of the tendency of the digestion mixture to froth. The mixture was then boiled vigorously for an additional hour until a clear green color was obtained. The digested mixture was cooled to room temperature, transferred quantitatively to a 250 ml. volumetric flask and diluted to the mark. A 25 ml. sample of the diluted digestion mixture was analyzed for nitrogen by using the Kjeldahl method.

Body nitrogen was determined for each animal using two different methods; by chemical analysis of dried carcass as described above and by calculating body nitrogen from a regression equation relating total body water to total body nitrogen.

VI. NET PROTEIN UTILIZATION

An attempt was made to follow the methods suggested by Miller and Bender (33) as closely as possible in order to determine the NPU. The NPU of the casein and tarhana containing diets fed each animal was determined by using the formula:

$$NPU = \frac{B - (B_k - I_k)}{I} \times 100 \quad .$$

The average total body nitrogen (B_k) and average total nitrogen intake (I_k) of animals on the nonprotein diet was subtracted from the total body nitrogen values (B) of each animal on the test diets and the difference was divided by the nitrogen intake (I) of that animal. The result was multiplied by 100 in order to express NPU as a whole number.

VII. STATISTICAL ANALYSIS

At the end of the experimental period, the data on weight gain and nitrogen intake were recorded and total body water, body nitrogen and the NPU were calculated for each animal. These data were then statistically analyzed.

The parameters used for statistical analysis were calculation of the mean and the standard deviation and error of the mean. The Student's t test was applied in order to determine the significance of the differences between the mean values obtained in trial I and II as well as the various diets.

Pearson's Correlation Coefficient Test was used to determine the relationship between body nitrogen and water, and a regression equation of the type "y = a + bx" was calculated from this relationship.

CHAPTER IV

RESULTS AND DISCUSSION

The mean weight gains and the mean nitrogen intakes of the rats fed the various diets during the ten-day experimental period are given in Tables 10 and 11, respectively.

A comparison of the mean weight gains of rats fed the comparable diets in trials I and II showed no significant differences for the two trials except for the rats fed the tarhana with soya bean flour diet. The animals fed this diet in the first trial gained between 31 and 33 g. per ten days, whereas in the second trial the weight gains ranged between 11 and 29 g. per ten days. Thus, the mean weight gain of animals in the second trial was significantly lower ($P < 0.05$) than that of the animals in the first trial. The mean nitrogen intake of the animals in the second trial was lower, but the difference was not significant, so that it can not wholly account for the difference in the weight gains. Although the difference between the mean weight gains of the rats fed the casein diet in the two trials is not significant, a 15 g. per ten day weight gain of rats fed the casein diet is somewhat low. This can be attributed in part to the fact that one animal lost 5 g. during the course of the experiment, thus giving a low mean weight gain.

In trial I, the weight gains of rats fed the tarhana with soya bean flour diet was significantly better than that of the rats fed either

TABLE 10

MEAN WEIGHT GAINS OF RATS

Diet	Trial I		Trial II	
	No. of Animals	Gain in Weight	No. of Animals	Gain in Weight
		g/10 days		g/10 days
Nonprotein	3	-6 ± 1.8[a]	4	-7 ± 2.0
Casein	4	22 ± 5.3	5	15 ± 5.1
Tarhana	4	17 ± 2.9	5	16 ± 2.3
Tarhana with soya bean flour	4	31 ± 0.5[b,c]	5	22 ± 3.3[d]
Tarhana with FPC	3	19 ± 3.5	4	22 ± 2.6[d]

[a]Mean ± S.E.

[b]The growth of the rats fed the tarhana with soya bean flour diet was significantly superior to the growth of rats fed tarhana ($p < 0.02$) and tarhana with FPC ($p < 0.02$) diets in trial I.

[c]The growth of rats fed the tarhana with soya bean flour diet in trial I was significantly different than the growth of the animals fed the same diet in trial II ($p < 0.05$).

[d]The growth of rats fed the tarhana with soya bean flour and the tarhana with FPC diets in trial II was significantly better ($p < 0.10$) than the growth of rats fed the casein and tarhana diets in trial II.

TABLE 11

MEAN NITROGEN INTAKES OF RATS DURING THE EXPERIMENT

Diet	g. Nitrogen Intake/10 days	
	Trial I	Trial II
Nonprotein	0.13 ± 0.01[a]	0.14 ± 0.00
Casein	1.19 ± 0.13	1.01 ± 0.08
Tarhana	1.10 ± 0.07	1.12 ± 0.10
Tarhana with soya bean flour	1.29 ± 0.08	1.22 ± 0.14
Tarhana with FPC	1.06 ± 0.02	1.25 ± 0.04

[a]Mean ± S.E.

the tarhana ($P < 0.02$) or the tarhana with FPC ($P < 0.02$) diets. There were no significant differences between the growth rate of rats fed the casein diet and the other diets in trial I. The weight gain of rats fed the tarhana and casein diets in the second trial was also significantly inferior to that of the rats fed the tarhana with soys bean flour ($P < 0.10$) and tarhana with FPC ($P < 0.10$) diets. The failure in getting uniform weight gains in all animals could be attributed in part to the possible effect of the sex differences between the animals fed a particular diet. Miller and Bender (33) indicated that sex differences gave negligible variations in NPU determinations. Dreyer (36) found that sex differences affected the N/H_2O ratio of the animals, presumably also affecting their weight gain. There were no significant differences between the mean nitrogen intakes among the rats fed the various diets in the two trial groups.

The data of some of the rats originally included in the study do not appear in the results due to experimental errors made at the beginning of the study. The data obtained from one rat fed the tarhana with FPC diet in the second trial are also excluded because this animal developed a skin rash and lost weight during the ten-day experimental period, although his food intake was quite high. The NPU calculated from data obtained with this rat gave a negative value. Two other rats fed the same diet also developed a skin rash, but their data is included in the results. These animals will be discussed further in the chapter.

The exclusion of the data of some of the animals from the results unfortunately reduced the size of the population, which could also

account for the variation in Table 10, page 33. It could be concluded, however, from the study of the table on weight gains that the supplementation of tarhana tended to improve growth in the experimental animals. This was particularly true for the tarhana with soya bean flour diet.

The total body nitrogen values obtained by chemical analysis, together with the total body water values are shown in Table 12. There were no statistically significant differences between the mean body nitrogen values or the total body water values of the animals fed comparable diets in trials I and II. Thus, the data obtained for all rats fed comparable diets in trials I and II are averaged.

The relationship between the chemically analyzed total body nitrogen and the total body water of the animals is presented in Figure 1. The values showed a highly significant ($P < 0.01$) linear relationship with a correlation coefficient of $r = 0.97$. This result agrees with the findings of other workers (34,37,38).

A regression equation for the relationship between total body water and chemically analyzed total body nitrogen was calculated and found to be $y = 0.12 + 0.037x$, where y = total body nitrogen in grams and x = total body water in grams. This equation is applicable to 38-day-old rats from the Wistar strain that were used in the present study. Although this equation relates body nitrogen to body water, the equation is somewhat different from those reported in other studies (Table 3, page 17), because it omits age as a variable. It will be recalled that in the equations given by other workers, y equals $\%N/\%H_2O \times 100$, and x equals age in days. Dreyer (36) calculated an equation of this type

TABLE 12

MEAN TOTAL BODY WATER AND MEAN TOTAL BODY NITROGEN OBTAINED BY CALCULATION FROM TOTAL BODY WATER AND BY CHEMICAL ANALYSIS

Diets	No. of Animals	Total Body Nitrogen		Total Body Water
		Chemical Analysis	Calculation from Body Water	
		g.	g.	g.
Nonprotein	7	1.04 ± 0.02[a]	1.05 ± 0.02	25.3 ± 0.7
Casein	9	1.68 ± 0.08	1.64 ± 0.07	41.6 ± 2.0
Tarhana	9	1.51 ± 0.03	1.54 ± 0.03	38.4 ± 0.8
Tarhana with soya bean flour	9	1.83 ± 0.05	1.81 ± 0.05	45.9 ± 1.4
Tarhana with FPC	7	1.67 ± 0.07	1.71 ± 0.06	42.4 ± 2.0

[a]Mean ± S.E.

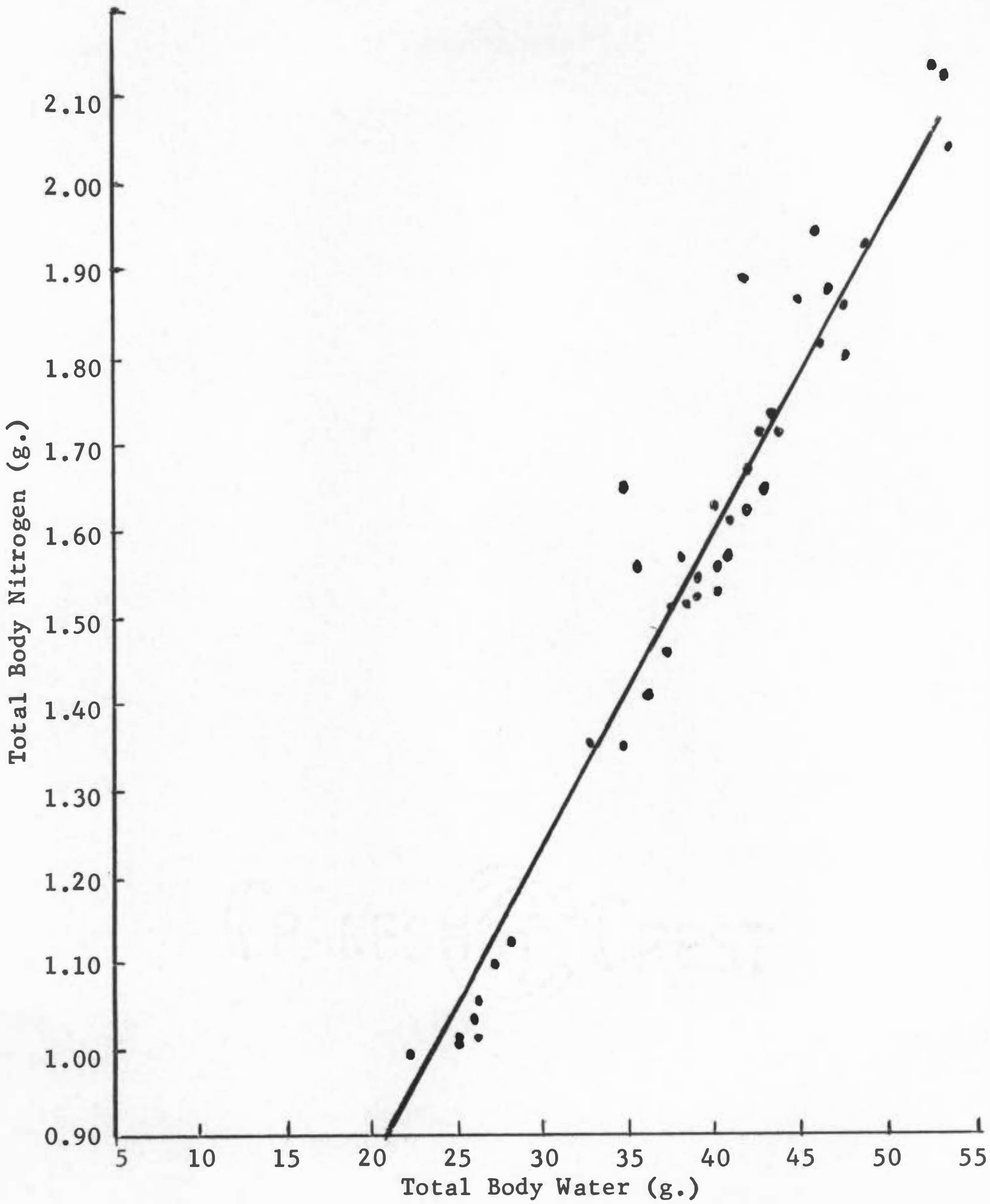

FIGURE 1

RELATIONSHIP BETWEEN TOTAL BODY NITROGEN
AND TOTAL BODY WATER

for rats of the Wistar strain in his laboratories and reported it as $y = 3.4331 + 0.0149x$, where y equals $\%N/\%H_2O \times 100$, and x equals age in days. If Dreyer's equation is solved for 38-day-old rats, y equals 3.9993. This value is comparable to the $\%N/\%H_2O \times 100$ calculated using the mean body nitrogen and body water values of all animals in the present study. The mean $\%N/\%H_2O \times 100$ ratio was calculated to be 3.9981. For the purpose of stating this similarity more accurately, a Student's t-test was used to compare the mean body nitrogen values calculated from Dreyer's equation and the mean body nitrogen calculated from the regression equation computed from the data obtained in the present study. In both calculations, the mean body water (Table 12, page 37) obtained in the present study were used. The result ($P < 0.01$) confirmed that the difference is quite negligible and that both of the regression equations would give similar results with 38-day-old Wistar rats, but the equation computed by the researcher was used in this study.

The NPU of the diets was calculated from the body nitrogen values obtained by calculation from body water and also from body nitrogen obtained by chemical analysis (Tables 13 and 14). The NPU values of comparable diets in trials I and II, regardless of the method of calculation, were in agreement.

The difference between the NPU of the casein diet between trials appears large but it was not significant. Henry and Toothill (37) reported an NPU value of 71 for a 10 percent casein diet; Tagle and Donoso (42) give a value of 70.7 for a similar diet. An NPU value of 69.5 was obtained for casein in an initial trial experiment by this

TABLE 13

COMPARISON OF NET PROTEIN UTILIZATION VALUES FOR TRIAL I AND II BASED ON CALCULATION OF TOTAL BODY NITROGEN FROM TOTAL BODY WATER

Diet	Net Protein Utilization	
	Trial I	Trial II
Casein	56.5 ± 5.5[a]	72.0 ± 5.4[b]
Tarhana	54.2 ± 6.4	59.8 ± 2.2[b]
Tarhana with soya bean flour	71.7 ± 1.2	73.9 ± 2.4[b]
Tarhana with FPC	62.8 ± 3.7	71.4 ± 4.9[b]

[a]Mean ± S.E.

[b]No significant difference was observed between these values and the corresponding Net Protein Utilization values of trial I.

TABLE 14

COMPARISON OF NET PROTEIN UTILIZATION VALUES FOR TRIAL I AND II BASED ON TOTAL BODY NITROGEN DETERMINED BY CHEMICAL ANALYSIS

Diet	Net Protein Utilization	
	Trial I	Trial II
Casein	61.9 ± 7.3[a]	78.9 ± 5.3[b]
Tarhana	51.3 ± 5.6	58.1 ± 3.0[b]
Tarhana with soya bean protein	76.8 ± 4.6	73.4 ± 1.8[b]
Tarhana with FPC	56.3 ± 7.6	70.8 ±10.5[b]

[a]Mean ± S.E.

[b]No significant difference was observed between these values and the corresponding Net Protein Utilization values of trial I.

researcher. In view of these findings, it would seem that the NPU value obtained for casein in the initial trial is low, although it is difficult to determine the reason for such a result.

The tarhana with FPC diet also gave a lower, but not significantly different, NPU value in the first trial than in the second trial. Two rats, one in each trial, developed a skin rash which resembled seborrhea and gained very little weight in spite of a nearly normal food intake. The data of these rats were not excluded since the calculated NPU values were low, but positive. It is questionable whether the use of these NPU values in the calculation of the mean NPU gives a true indication of the protein quality of the tarhana with FPC diet. When the data of these two rats were excluded, the mean NPU values obtained by the use of the body water technique of the two trials were 63.1 and 74.9, respectively.

The mean NPU values of comparable experimental diets in trials I and II showed no significant differences, thus the NPU values obtained for the two trials for each method of calculation were averaged. The results are given in Table 15. A Student's t-test indicated that there were no significant differences between the NPU values obtained for each diet by the two different methods of calculation. Henry and Toothill (37) have indicated that the error in the method of calculating NPU by the body water technique is higher than that of the chemical analysis method, but this was not observed in this study.

The results of the comparison of the NPU of the various diets with one another is presented in Table 16. The NPU values obtained from body nitrogen data by chemical analysis were used in order to make

TABLE 15

SIGNIFICANCE OF DIFFERENCES BETWEEN THE NET PROTEIN UTILIZATION VALUES OBTAINED FOR EACH DIET USING TWO DIFFERENT METHODS OF CALCULATION

Diet	Net Protein Utilization		t-value of Difference
	Nitrogen by Body Water	Nitrogen by Chemical Analysis	
Casein	65.1 ± 4.5[a]	71.3 ± 5.1	1.4020[b]
Tarhana	57.3 ± 3.0	55.1 ± 3.0	-0.4842[b]
Tarhana with soya bean flour	72.6 ± 1.4	74.9 ± 2.2	0.8511[b]
Tarhana with FPC	67.7 ± 3.4	64.6 ± 4.9	0.6041[b]

[a]Mean of both trials ± S.E.

[b]t-value was not significant at respective degrees of freedom.

TABLE 16

SIGNIFICANCE OF DIFFERENCES IN MEAN NET PROTEIN UTILIZATION VALUES OF THE EXPERIMENTAL DIETS BASED ON TOTAL BODY NITROGEN OBTAINED BY CHEMICAL ANALYSIS

Diet	Comparison Diet	Significance of Difference
Casein	Tarhana	S ($P < 0.01$)[a]
Casein	Tarhana with soya bean flour	N.S.
Casein	Tarhana with FPC	N.S.
Tarhana	Tarhana with soya bean flour	S ($P < 0.01$)[a]
Tarhana	Tarhana with FPC	S ($P < 0.02$)[a]
Tarhana with FPC	Tarhana with soya bean flour	N.S.

[a]The tarhana diet was consistently inferior to the diets it was compared with in all three instances.

comparisons between the NPU values of the various diets. The NPU calculated in this manner was selected because it had been indicated to yield a smaller error than the NPU calculated from body water data. Another reason for choosing these NPU values was the fact that the NPU value of casein obtained by this method was in close agreement to literature values (42). The diets containing casein, tarhana with soya bean flour and tarhana with FPC were equal to one another in protein quality, and tarhana was consistently inferior to all three of them.

From Table 15, it could therefore be stated that tarhana has an NPU value between 55.1 and 57.3. It is possible to improve the protein quality of tarhana with other protein sources. Soya bean flour, when utilized as a supplement at a 20 percent level in the preparation of tarhana, will improve the protein quality and make it comparable with a casein diet. Tarhana may also be similarly supplemented with FPC at a 3 percent level, but in view of the skin rash that developed in three of the eight animals which were used in determining the NPU of the tarhana with FPC diet, more research would certainly be necessary before FPC is incorporated as a supplement to tarhana for human consumption.

In agreement with others it was shown that there is a significant linear relationship between body water and body nitrogen during growth. The regression equation which was computed during the present study for the 38-day-old Wistar rats, could be used for the purpose of determining the body nitrogen of these animals after their total body water is determined, in future studies on the NPU of proteins. Thus the time involved in NPU determined would be considered reduced.

CHAPTER V

SUMMARY

The effect of supplementing a local Turkish soup called tarhana with soya bean flour or Fish Protein Concentrate on Net Protein Utilization (NPU) was investigated.

Three mixtures of tarhana were prepared under standardized conditions. These were a standard tarhana mixture and tarhana supplemented with either 20 percent soya bean flour or 3 percent fish protein concentrate at the expense of the wheat flour. These mixtures were incorporated into a nonprotein basal diet at levels adjusted to give 10 percent protein and each diet was fed to two groups of rats for ten days. The results obtained were compared with those obtained from rats fed a 10 percent casein diet which served as a control. A nonprotein diet was also fed to two groups of rats in order to determine the endogenous nitrogen necessary for the computation of NPU.

NPU was determined by employing two different methods for the estimation of body nitrogen. The first method consisted of a chemical analysis of the total carcass for nitrogen. The second method involved carcass analysis for total body water. A regression equation relating total body water to total body nitrogen was computed for the 38-day-old rats of the Wistar strain used in this study. The nitrogen content of carcass was then calculated from the total body water using the regression equation. Both methods gave similar results in terms of total body

nitrogen and NPU values. A decided advantage of the latter method using the regression equation over the chemical analysis is the reduction in the amount of analytical work required for the determination of NPU.

The data show that tarhana has an NPU value between 55.1 and 57.3, which is significantly inferior to the NPU of a casein diet which yields values between 65.1 and 71.3. It is possible, however, to improve the protein quality of tarhana and bring it to a level which compares very favorably with the NPU of casein diets by the addition of soya bean flour during preparation at a 20 percent level. FPC may also serve as a supplement, but more research may be necessary to this end.

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