THE EFFECT OF BAKING ON THE PROTEIN QUALITY OF ARABIC BREAD WITH AND WITHOUT AMINO ACID SUPPLEMENTATION

By

ABOLGHASSEM DJAZAYERY

A THESIS

Submitted to the

AMERICAN UNIVERSITY OF BEIRUT

In partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE IN
AGRICULTURE

August 1966

THE EFFECT OF BAKING ON THE PROTEIN QUALITY OF ARABIC BREAD WITH AND WITHOUT AMINO ACID SUPPLEMENTATION

By

ABOLGHASSEM DJAZAYERY

Approved:

Morteza Maleki

Morteza Maleki: Assistant Professor of Food Technology. In Charge of Major.

Nabil Wakid

Nabil Wakid: Assistant Professor of Biochemistry.

Samir M. Badawi

Samir Badawi: Assistant Professor of Animal Production.

Walter W. Worzella

W.W. Worzella: Professor and Chairman of Graduate Committee.

Date Thesis is presented: August 2, 1966.

PROTEIN QUALITY OF ARABIC BREAD

DJAZAYERY

ACKNOWLEDGEMENTS

The author wishes to extend his thanks to Dr. Morteza Maleki for his guidance, advice and suggestions during the course of this study. He is also indebted to Dr. James W. Cowan for his advice and encouragement.

AN ABSTRACT OF THE THESIS OF

Abolghassem Djazayery for M.S. in Food Technology and Nutrition

Title: The effect of baking on the protein quality of Arabic bread with and without amino acid supplementation.

Bread is one of the major constituents of the Middle Eastern diet, providing large percentages of the daily protein and calorie intake of an average person. Studies on European-type bread have shown that baking usually decreases the protein quality of bread, mainly due to losses in lysine and threonine. It has also been shown that supplementation of flour with lysine and threonine results in improvement in the protein quality of bread.

In the present work the effect of supplementation of flour with lysine, lysine-threonine, lysine-methionine and lysine-threonine-methionine on the protein quality of white Arabic bread was studied. In order to find if there was a correlation between the quality of protein and the quantity of supplemented amino acids, total amino acids in all flours and breads were determined microbiologically and available lysine in unsupplemented and lysine-supplemented flours and breads was measured by the DNFB procedure. In addition, the effect of baking on the protein quality of nonsupplemented and supplemented breads was investigated. For the preparation of bread, the dough was made in the laboratory and baked in a local bakery. The protein quality of mixtures of bread and common dietary items was also estimated by calculation of NDpCal% and amino acid scores.

The results showed that addition of 0.30 percent l-lysine to flour resulted in considerable improvement in protein quality. Supplementation of lysine-fortified flour with 0.62 percent dl-threonine caused further improvement in protein quality, whereas methionine had no effect. Baking did not change the protein quality of lysine-or lysine-and threonine-supplemented breads, although threonine content decreased in the latter. However, the protein quality of bread supplemented with lysine, threonine and methionine was decreased by baking.

For protein evaluation PER, NPR, weight gain and feed consumption were used.

Computor calculations showed that in bread and in some mixtures of bread and plant protein foodstuffs lysine was limiting and NDpCal percent was low. In most mixtures of bread and animal protein foodstuffs sulfur amino acids were limiting, and NDpCal percent was relatively high.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Cereals are among the principal staple foods in the Middle East. In Lebanon it has been reported that they provide over 55 percent of the daily protein and calorie intake of an average person (50, p.3). Wheat bread is by far the most common form of cereal product consumed in the area. However, wheat protein is of poor quality because of being low in lysine, threonine and possibly one or more other essential amino acids. Furthermore, there may be further damage to the quality of bread protein due to heat and other conditions of baking processes.

There are four major types of bread in Lebanon: (i) European type bread; (ii) cylindrically-shaped French bread (Khubz franji); (iii) mountain bread (Khubz markouk); (iv) white and brown Arabic bread (Khubz arabi).

The Arabic bread is by far the most common type of bread used in Lebanon; the brown type is consumed mostly in villages and the white in towns. The baking conditions of Arabic bread are unique in that the bread is baked at a very high temperature of about 400-500^{o}C for a short time of about one minute.

The effect of baking conditions on the protein quality of European bread has been studied rather extensively, but there is no published work on the nutritional

quality of Arabic bread of Lebanon or of other countries of the Middle East. The entire processes of dough preparation and baking considered as baking, the present work was initiated to investigate the order of limiting amino acids and the effect of baking on the protein quality of the white Arabic bread with and without supplementation with the amino acids lysine, threonine, and methionine. Also the supplementary effect of common food items consumed with bread was evaluated with the use of a computor.

II. REVIEW OF LITERATURE

The consumption of bread in Lebanon as well as in other countries of the Middle East is considerably higher than that of many other areas of the world (50, p.3). Recent surveys conducted in Lebanon have revealed that due to a rise in the standard of living the bread consumption is rapidly increasing (50, p.3). The surveys showed that 47 percent of the energy and 48 percent of the protein intake of nonrefugee civilians come from bread (40, p.63). The wheat flour is the only kind of flour used for bread-making in Lebanon.

Amino Acids and Protein Quality

Osborne and Mendel (47) were the first workers who, after many experiments on rats, showed that the nutritive value of different proteins depends on their indispensible amino acid content. This was later confirmed by other workers (44, 22, p.13).

Since the beginning of the century many workers have shown that lysine and threonine content of cereals and cereal products, such as bread, is relatively low (39, 54). There is general agreement that the first limiting amino acid in wheat flour and bread is lysine (31, 41). However, there is disagreement among different workers regarding

the second and third limiting amino acids in wheat.

Ericson (18) reported that besides lysine the only other limiting amino acid is threonine, while Bender (5) believes there is also a third limiting amino acid, and that is methionine. Rosenberg et al. (56) could not get any beneficial effect from addition of threonine to bread diets supplemented with lysine. Sure (60) confirmed the findings of Ericson (18) and of Bender (5) regarding the second limiting amino acid, but his results of animal experiments suggested that valine is the third limiting amino acid in wheat flour. In another experiment, the same worker (59) reported valine to be the second and threonine to be the third limiting amino acid in whole wheat flour. King et al. (41) reported the order of limiting amino acids in wheat protein to be lysine, tryptophane, methionine, isoleucine, valine and threonine. Therefore, there is no agreement among workers on the second and third limiting amino acids in flour and bread.

The quality of a protein depends not only on the quantity of its essential amino acids, but also on the percent availability of each of these essential amino acids. Often, processing, such as baking, causes nonavailability of heat-labile amino acids, e.g., lysine, threonine and methionine (31, 53). The changes that take place and make these amino acids unavailable are not well established. In the case of lysine, one explanation is that it reacts with

reducing sugars, forming complex compounds (10); thus the result is a reduction in its biological availability. The extent of the loss depends on the duration of baking and the intensity of heat (13, 55). However, Clegg and Davis (14) reported that there was no significant reduction in the available lysine of bread due to baking. Clarke and Kennedy (13) observed very little loss of available lysine in microwave-baked bread, whereas they reported that the loss in oven-baked bread was in the order of 25-30 percent. Ericson et al. (19) observed a significant loss of threonine, but not of lysine during baking of European bread. In contrast, McDermott and Pace (42) found no significant difference between the amino acid composition of flour and bread. Not much work has been done on the effect of baking on the availability of methionine in bread. Hepburn et al. (33) observed a 10 percent increase in the methionine content of flour during baking of bread. These contradictory findings may be due to differences in the baking method used.

Amino Acid Supplementation

Many workers have attempted to improve the protein quality of bread by supplementing flour with limiting amino acids. Osborne and Mendel (47), for the first time in 1914, supplemented gliadin (a part of cereal protein) with lysine, which proved to support a better growth of rats than did

the unsupplemented gliadin. Later, Hutchinson *et al*. (38), Rerat and Jacquot (54) and several other workers (21, 41) improved the protein quality of flour and bread by addition of lysine. Other investigators have supplemented bread and flour with lysine-rich proteins, such as fish, milk and soy flour (31, 57). Ericson *et al*. (20) observed that the supplementary effect of the added foods was directly proportional to their lysine content. Flodin (26) and Feldberg (25) claimed that the addition of lysine to the wheat bread gave a product with a biological value very close to that of animal protein. Ericson (18) and Ericson *et al*. (19) supplemented wheat flour with lysine and threonine and got improvement in the protein quality, although baking caused a decrease in the protein quality of the corresponding bread. The losses of lysine and threonine were 5-10 percent and 25-30 percent, respectively (19). Rosenberg and Rohdenberg (55) reported a 30 percent loss of added lysine due to baking. Deshpande *et al*. (17) and Hundley (37) have used algae as a good source of lysine and threonine for bread supplementation. It has been demonstrated by Hutchinson *et al*. (39) that the lysine-threonine interrelationship depends, to some extent, on the nitrogen content of diet.

The effect of methionine supplementation on the protein quality of flour and bread has not been studied to any great extent. This is probably due to the fact that

diets consisting almost entirely of cereals are unlikely to be deficient in methionine (36). Ericson (18) observed no improvement in the quality of bread by adding methionine to bread already supplemented with lysine and threonine.

Estimation of Total Amino Acids

Block and Weiss (7, pp. 32-130) have described the chemical, microbiological and chromatographical methods for estimation of different amino acids in food substances. More recently the microbiological and column chromatographic methods have been reviewed by NAS-NRC[1] committee on protein malnutrition (22, pp. 3-5).

Estimation of Available Amino Acids

The available amino acids are estimated by either chemical or biological methods; some of these methods have been reviewed by Campbell (9, pp. 48-50). The most common procedure for the determination of available lysine is the 2,4-dinitrofluorobenzene (DNFB) method originally developed by Carpenter and Ellinger (11) and later modified by Carpenter (10). This technique has been used successfully for the measurement of available lysine in animal proteins.

---

1. National Academy of Science-National Research Council.

The estimation of available lysine in plant materials, such as cereals with a high level of carbohydrates, presents special problems due to the presence and formation of interfering pigments as a result of baking and Maillard type reactions. For these reasons, several chromatographic techniques have been developed in order to overcome some of these difficulties by removing the interfering pigments (3, 52). However, no satisfactory procedure has yet been developed for the estimation of available lysine in plant materials. Some bioassay techniques have also been developed, which have not been used extensively (32, 49).

Ford (28) developed a microbiological technique for the determination of available methionine in foods, using Streptococcus zymogenes NCDO 592. Other workers (34) have used biological methods, using rats as the experimental animals.

Protein Evaluation

Frost (30, pp. 225-274) and Allison (1, 2, pp.97, 115) reviewed extensively the methodology of protein evaluation. More recently Campbell (9) and NAS-NRC committee on protein malnutrition (22) have published critical reviews on the subject.

Protein efficiency ratio (PER): Osborne et al. (48) in 1919 introduced the concept of PER; they defined it as

weight gain in g of experimental animals per g protein consumed. These workers, and later Barnes and Basshardt (4), determined PER at different levels of protein intake and assumed the maximum value obtained to be the PER of the test diet. However, Campbell (9) and other investigators recommended using a 10 percent protein diet and ad libitum feeding. Chapman et al. (12) and Derse (16) recommended using weanling rats for the PER assay, because the age of rats has a significant effect on the PER value of a protein.

As early as 1926, Hoagland and Sinder (35) demonstrated that the sex of rats may affect the PER value. The female rats usually do not gain weight as rapidly as males. For this reason and because of the fact that variation within groups of females is larger than in males, Morrison and Campbell (45) have recommended the use of male rats for PER assay. The same authors found that the strain of rats also has an effect on the PER value.

Friedman and Kline (29) recommended the use of a casein diet as a reference diet. Chapman et al. (12) arbitrarily assigned a PER value of 2.5 for casein. The same authors and some other workers (45, 61) recommended a 4-week assay period, because longer periods caused a decrease in the PER value.

The PER method is the most widely used method of protein evaluation mainly because of its simplicity

(9, p.20). However, it has been criticized by Mitchel (44) and Bender and Doell (6) on the basis that: (i) it varies with the food intake and the protein level of the diet; (ii) gain in body weight may vary in composition for different protein sources; (iii) the method makes no allowance for body maintenance and (iv) it is not altogether suitable for the evaluation of poor quality proteins.

Net protein retention (NPR): Bender and Doell (6) suggested the use of NPR, which they defined as the weight loss of a non-protein group plus the weight gain of the test group divided by the protein consumed by the experimental animals (6). According to Bender and Doell (6) NPR value is independent of food intake. In general PER and NPR are closely correlated except probably in the case of some processed foods which may lower the food intake appreciably (22, p.24).

There are other biological methods for the estimation of the protein quality of foods. However, since the laboratory conditions where this study was made were set up for PER, the latter was used. NPR was also run to see how the two methods correlate.

From the foregoing review of literature one can see that all the studies on the effect of baking and amino acid supplementation on the protein quality of bread have been done on European type bread and there is no mention of

other types of bread, such as Arabic bread. Furthermore, there are disagreements among workers on the effect of baking on the protein quality of bread and the order of limiting amino acids. There are many differences in formula and baking conditions of Arabic bread and European type bread. Apart from the differences in formula and fermentation processes the baking time and temperature are markedly different in these two types of bread.

Due to the differences in baking conditions between European and Arabic breads different results may be expected from the effect of baking and amino acid supplementation on Arabic bread. Therefore, this study was undertaken to clarify some of these differences and to establish the order of limiting amino acids in Arabic bread.

III. MATERIALS AND METHODS

The column chromatographic technique developed by Rao *et al*. (52) as well as the colorimetric procedure of Bruno and Carpenter (8) were tried to measure available lysine in experimental diets. It was found that the latter method gave more reproducible results; therefore, this method was used to measure available lysine throughout this work. Attempts were also made to measure available methionine by the microbiological method described by Ford (28), who used *Stroptoccus zymogenes* NCDO 592. However, since this organism was not available, *Leuconostoc mesenteroides* P-60 (which also requires methionine for its growth) was used. Assays using different experimental conditions during the course of study showed that the latter organism was not suitable for this purpose. Therefore, only total methionine was measured and is reported in this work.

Preparation of bread: The formula used for baking experimental breads was as follows:

Flour (65% extraction)	100 g
Water	40-50 g
Salt	2 g
Yeast (compressed)	0.5 g

The flour used was from the same batch throughout this study. The ingredients were mixed in a Crypto electrical mixer

(Model No. EB12, Crypto Ltd., London) for 12-15 minutes, and the resulting dough was allowed to ferment at room temperature for 1.5-2.0 hours. The rest of the operations, i.e., rolling, rounding and baking were done in a local bakery as described by Pelshenke (50, pp. 10-22); the baking temperature was 400-500°C and the baking time was about one minute. The bread samples were dried overnight at 70°C, ground and used for the preparation of diets.

Determination of available lysine: The available lysine was determined by the 2,4-dinitrofluorobenzene (DNFB) method of Bruno and Carpenter (8) with minor modifications. A sample of the food material containing approximately 50 mg of nitrogen was suspended in 8 ml of 10 percent (w/v) sodium bicarbonate in a 100 ml round-bottomed flask. A solution of 0.3 ml 100% DNFB in 12 ml ethanol was added, and the flask was gently agitated in a mechanical shaker for 2 hours. To the blank used for setting the spectrophotometer to zero, only 12 ml of ethanol were added, the rest of the procedure being exactly like that for the sample. It has been shown that two hours reaction time is enough for the complete reaction between DNFB and the free epsilon amino group of lysine (15). The ethanol was evaporated in a boiling water bath till there was no effevescence. Twenty-four ml of 8.1 N HCl were added (by stages to reduce frothing) and the flask was refluxed for 16 hours. In order to reduce the subsequent work of extraction, the contents were refrigerated overnight and filtered. Two g of sodium sulfide

($Na_2S.9H_2O$) were added to the filtrate and the volume was made up to 200 ml with distilled water. The flasks were then allowed to stand at room temperature for 5 minutes.

Twenty ml of the hydrolyzate were transferred into a separatory funnel and extracted twice with 50 ml portions of ether, then the acquous layer was transferred into a 100 ml volumetric flask and the remaining ether was evaporated in a water bath. The residue in the flask was made alkaline with 2N NaOH (after titration of a dummy aliquot with phenolphthalein as the indicator), 20 ml of a carbonate-buffer (pH 9)[1] added, and the volume was made up to 100 ml with distilled water. Aliquots of 10 ml were pipetted into the separatory funnels used for the ether extraction mentioned above, 0.05 ml of methoxycarbonyl chloride added and the mixture shaken for 10 minutes. Finally, three-fourth of a ml of concentrated HCl was added and a second ether extraction was done, using two portions of 10 ml of ether. The ether extract was collected into a 50 ml Erlenmeyer flask, the ether evaporated and the residue dissolved in 10 ml of 1N HCl by heating. After cooling, the optical density of the solution was read at 435 mu. The concentration of epsilon-dinitrophenyllysine-hydrochloride (DNP-lysine-HCl) was extrapolated from a standard curve prepared by plotting the optical densities

---

1. 8 percent $NaHCO_3$ + sufficient quantity of Na_2CO_3 to obtain a pH of 9.

of known solutions of DNP-lysine-HCl against its concentration (Figure 1).

The amount of available lysine as mg lysine per g nitrogen was calculated as follows:

$$\text{Mg lysine/g N} = \frac{\text{Mcg DNP-lysine-HCl/ml (from curve)}}{\text{M.W. of DNP-lysine-HCL X Wt. of sample (g) X \%N}} \times \frac{\text{M.W. of lysine X 100}}{\text{M.W. of DNP-lysine-HCl}}$$

Since $\frac{\text{M.W. of lysine X 100}}{\text{M.W. of DNP-lysine-HCl}} = 41.71$,

$$\text{therefore, mg lysine/g N} = 41.71 \times \frac{\text{Mcg DNP-lysine-HCl/ml}}{\text{Wt. of sample (g) X \%N}}$$

Determination of total amino acids: The amino acids lysine, threonine and methionine were assayed by the microbiological methods described by Block and Weiss (7) with minor modifications. As recommended by these authors, the following test organisms were used: (i) Streptococcus faecalis, ATCC 8043 (American Type Culture Collection, Washington D.C.) for threonine, and (ii) Leuconostoc mesenteroides P-60, ATCC 8042 for lysine and methionine. The lyophilized cultures of the organisms were revived as described by Sakr (58, p.14). Both test organisms were kept as stab cultures by monthly transfer to a medium having the following composition:

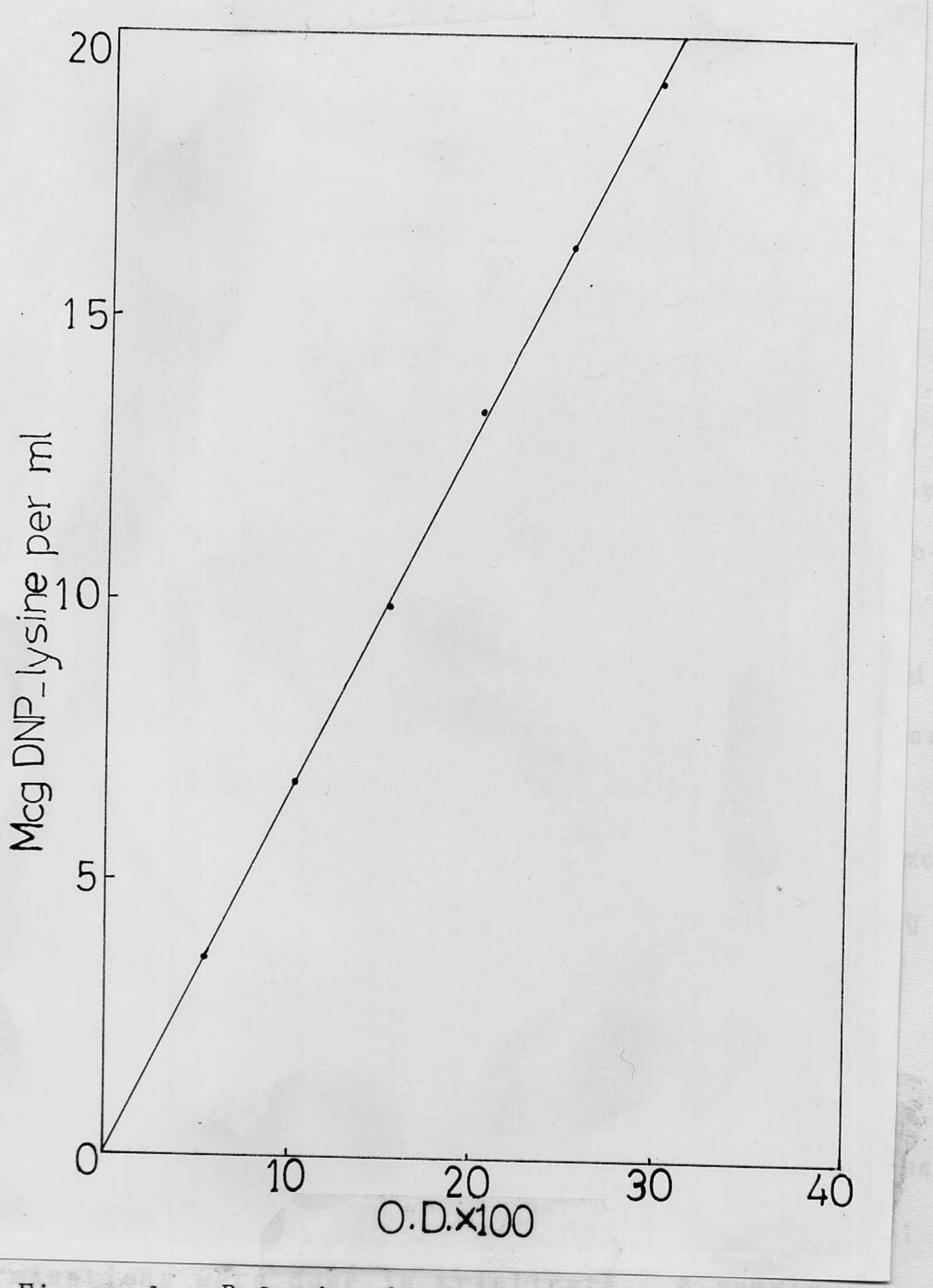

Figure 1. Relation between concentration and optical density of standard solutions of DNP-lysine.

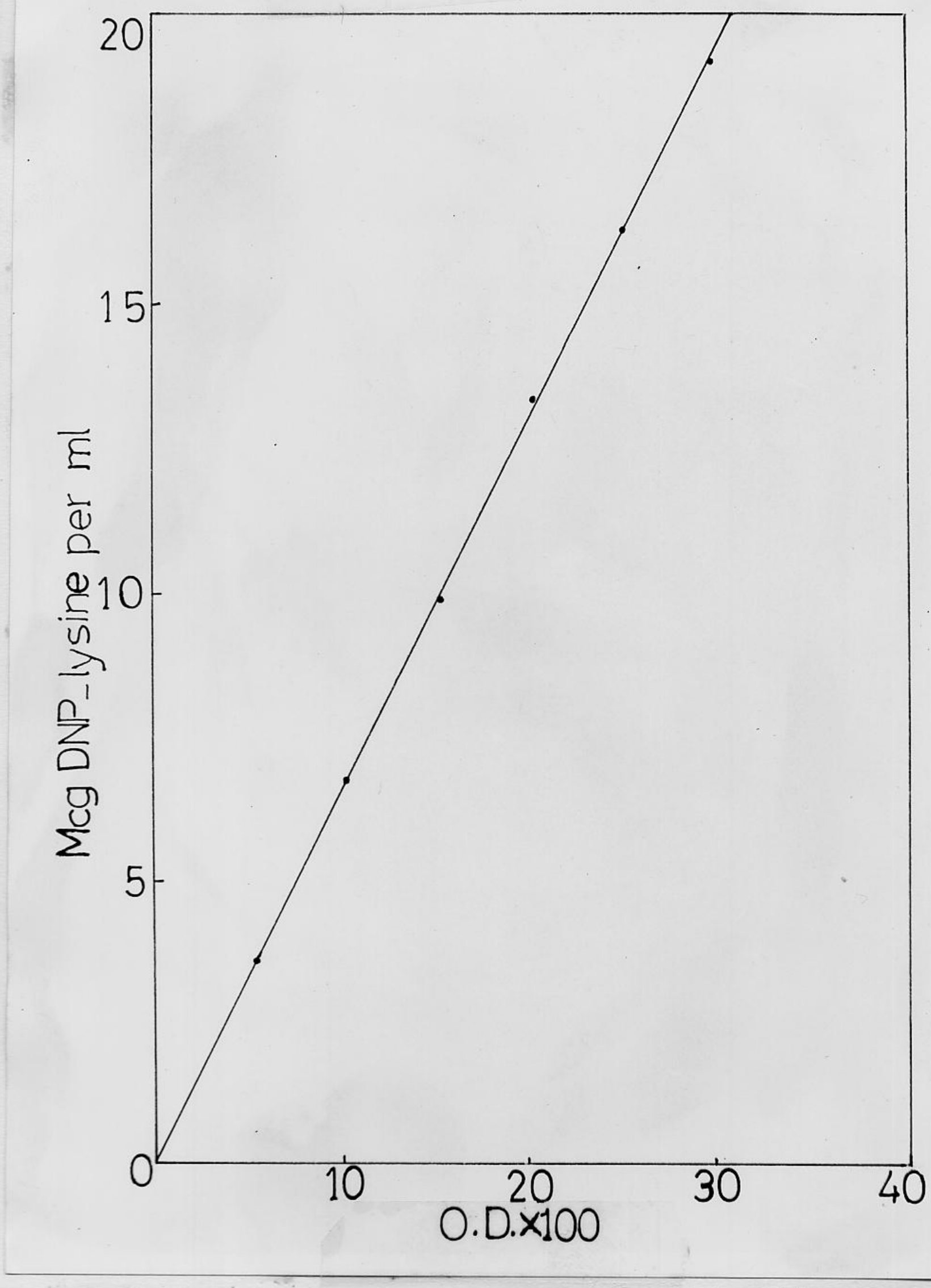

Figure 1. Relation between concentration and optical density of standard solutions of DNP-lysine.

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

The basal assay media were purchased in dry form from Difco Laboratories (Detroit 1, Michigan); these were prepared and sterilized according to the manufacturer's specifications.

Culture tubes containing the sterilized broth (above medium without agar) were inoculated with the proper micro-organisms from the stab cultures and grown at 37°C for 10-12 hours. The cells were removed by centrifugation and suspended in 10 ml saline solution under sterile conditions. To establish growth curves for the organisms, standard amino acid solutions were made with 20 mcg l-lysine[1], 20 mcg dl-threonine[1] (equivalent to 10 mcg l-threonine), or 5 mcg l-methionine[1], per ml, prepared according to the methods of Association of Official Agricultural Chemists.

The procedure followed for preparation of samples of diets for all three amino acids was the same, except that the microorganisms used was specified for each amino acid; all determinations were done in triplicate. A sample containing about 0.5 g protein was refluxed with 60 ml of

---

1. Obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

2N HCl for 8 hours. Most of the HCl was removed by evaporation on a hot plate. Sixty ml of distilled water were added to the residue and the pH was adjusted to 4.0 to precipitate the humins. After refrigerating the hydrolyzate for two hours, it was filtered through Whatman No. 1 filter paper. The pH of the filtrate was adjusted to 6.8 by addition of 1N NaOH, and the volume was made up to 250 ml with distilled water.

Serial amounts of diluted hydrolyzate (0.3, 0.6 and 1.0 ml) were placed in sterile test tubes. If the concentration of amino acids was higher than that of the standard solutions of amino acids, necessary dilutions were made so that the concentration in the hydrolyzate would be approximately as that of the standard solutions. For quantitative comparisons of the experimental samples, every time different levels of standard solutions ranging from 0-1 ml were run. The volume in all tubes was made up to 1 ml with distilled water; the tubes were then randomly placed in test tube racks, covered with aluminium foil and autoclaved for 15 minutes at 250^{o}F and 15 psi.

A sufficient volume of the proper basal medium previously sterilized for 10 minutes at 250^{o}F and 15 psi was inoculated with one drop of the saline suspension of the appropriate organism per 5 ml of medium. Each of the assay and standard tubes was inoculated with 1 ml of the above inoculum and incubated at 37^{o}C for 72 hours. The

lactic acid produced in each tube was titrated with 1N NaOH solution, using a 0.004 percent bromothymol blue solution as the indicator.

For each amino acid a standard curve was established by plotting the volume of base used for titrating the amount of lactic acid in standard tubes against the concentration of the amino acid. The concentration of each amino acid in the tubes containing the unknown samples was read off from the respective standard curve. The amino acid content of diets was calculated as follows:

$$\text{Mg amino acid/g N} = \frac{\text{Mcg amino acid/ml X dilution factor}}{\text{N content of sample X 1000}}$$

Animal experiments: The protein evaluation methods used in this study were protein efficiency ratio (PER) and net protein retention (NPR). In both cases, groups of 10 weanling albino rats were used. For NPR determination, in addition to the test groups, a control group was maintained on an unmodified basal ration (protein-free). A group was kept on a casein diet to serve as the reference group for both PER and NPR. The strain of the rats used was Sprague-Dawley[1]. After arrival by air, the animals were kept on a stock diet[2] for 2-3 days for recovery. They were housed

---

1. Obtained from Animal Suppliers (London), Ltd.

2. Obtained from Vitansi Feed Company, Beirut.

individually in mesh-bottomed cages in an air conditioned laboratory maintained at 70±2°F and at a relative humidity of about 60 percent, and assigned to the experimental diets according to a randomized block design. Food and water were provided ad libitum, and weekly records of food consumption and body weight were kept for PER determination. Also at 10 days, the food consumption and body weight of the experimental groups and body weight of control group were recorded for NPR determination.

The experimental diets were prepared by incorporating a sufficient amount of the test materials into the basal diet at the expense of corn starch to provide 10 percent protein. The composition of the basal diet is shown in the following table:

Table 1. Basal diet used for the preparation of experimental diets.

Ingredients	Percent
Corn starch	83
Corn oil	10
Alphacel (Non-nutritive cellulose)[1]	2
Mineral mixture (USP XIV)[1]	4
Vitamin mixture[1]	1

1. Obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. For composition see Appendix p.56

The reference diet consisted of the basal ration with casein at the level of 10 percent protein.

PER and NPR were determined according to the standardized methods suggested by Campbell (9); the assay period was four weeks for PER and ten days for NPR. They were calculated as follows:

$$\text{PER} = \frac{\text{Weight gain of test group (g)}}{\text{Protein consumed by test group (g)}}$$

$$\text{NPR} = \frac{\text{Weight gain of test group (g)} + \text{Weight loss of control group (g)}}{\text{Protein consumed by test group (g)}}$$

The PER values were corrected by using the following formula:

$$\text{Corrected PER} = \frac{2.5^{1}}{\text{Determined PER of casein}} \times \text{PER of test diet.}$$

The results of the experiments were analyzed statistically by the use of a computor (IBM 1620, Model No. II) specially programmed for this purpose.

Three experiments were carried out: experiment I to determine the optimum level of lysine supplementation to flour and bread. The test diets consisted of un-supplemented flour, flours supplemented with 0.30, 0.50 and 0.70 percent l-lysine (l-lysine-HCl), and ground breads made from these flours.

Experiment II was run to determine the second and

---

1. Assumed value for PER of casein (12).

third limiting amino acids in flour and bread. The test diets used were the following supplemented flours and the corresponding breads: (i) flour + 0.30 percent l-lysine; (ii) flour + 0.30 percent l-lysine + 0.28 percent l-methionine; (iii) flour + 0.30 percent l-lysine + 0.62 percent dl-threonine; (iv) flour + 0.30 percent l-lysine + 0.28 percent l-methionine + 0.62 percent dl-threonine.

The third experiment was carried out to determine the possible optimum level of methionine supplementation to flour and bread. The test diets consisted of flours supplemented with lysine and threonine (as in experiment II), to which were added 0.15, 0.28, and 0.35 percent l-methionine, and breads made from these flours.

To investigate the supplementary effect of some common dietary food items consumed with bread, mixtures of bread and such food items were formulated (Table 2). The protein quality of these mixtures was estimated by calculating amino acid scores and the net dietary protein calories percent (NDp Cal percent) by the use of a computor (IBM 1620, Model No. II). Only the scores of lysine and total sulfur amino acids (methionine + cystine) in each mixture were calculated, because it has been shown that in most mixed diets the most limiting amino acid is either lysine or sulfur amino acids (9, p.41). The scoring method used was that of FAO (23, p.30), according to which the amount of each essential amino acid in a

Table 2. Mixtures of bread and other dietary food items commonly consumed in the Middle East.

Mixture No.	Food item	% in mixture
1.	Bread	100
2.	Bread	75
	Concentrated yoghurt	25
3.	Bread	70
	Concentrated yoghurt	25
	Olive oil	5
4.	Bread	75
	Cheese	25
5.	Bread	70
	Cheese	15
	Tea	5
	Sugar	10
6.	Bread	40
	Grapes	60
7.	Bread	40
	Grapes	40
	Cheese	20
8.	Bread	75
	Walnuts	25
9.	Bread	70
	Walnuts	18
	Cheese	12
10.	Bread	65
	Eggs	35
11.	Bread	65
	Eggs	25
	Vegetable oil	10
12. "Falafel" sandwich	Bread	40
	Crushed sesame	12
	Tomatoes	8
	Broadbeans	13
	Chickpeas	6
	Onions	8
	Garlic	1
	Salt	1
	Flour	1
	Vegetable oil	10
13. "Shawarmah" sandwich	Bread	45
	Crushed sesame	8
	Tomatoes	8
	Beef	35
	Vegetable oil	4

protein is expressed as percent of that in FAO provisional pattern. The formula used for the calculation of amino acid score is;

$$\text{Amino acid score} = \frac{\text{Mg of amino acid in sample/gN}}{\text{Amino acid pattern of FAO}} \times 100$$

The lysine and total sulfur amino acids content of food-stuffs used for programming the computor were mostly those found in the U.S.D.A. Home Economics Research Report No. 4 (46).

The NDp Cal percent, which is an index of both quality and quantity of a protein, was calculated according to the following formula (43) :

$$\text{NDp Cal percent} = P \times S \left(\frac{54-P}{54-400/S}\right)$$

Where P = protein calories as percent of total calories, and

S = score of total sulfur amino acids.

The nitrogen contents of food items were taken from Food Composition Tables for Use in the Middle East (27) and Medical Research Council Special Report Series No. 302 (51).

IV. RESULTS AND DISCUSSION

Three experiments were conducted to determine the order of limiting amino acids and the optimum level of lysine and methionine supplementation to bread. In addition, the effect of baking on the protein quality of non-supplemented, as well as supplemented breads was investigated. For the evaluation of the protein quality, the following parameters were used: PER, NPR, growth and feed consumption in rats and the chemical determination of available lysine. It was observed that PER values were much more consistant than NPR; however, in each experiment a statistically significant correlation was found between values obtained with the two methods. The r values ($P = 0.01$) were as follows: experiment I, 0.37; experiment II, 0.53 and experiment III, 0.47. In general, the PER values agreed more closely with the available lysine content of diets than did NPR. For this reason, in order to avoid confusion, the discussion of the results will be based mainly on PER; NPR values will be given for comparison. The results of computor evaluation of mixtures of bread and some common dietary items are also reported.

Experiment I

Lysine supplementation: This experiment was performed to determine the optimum level of lysine supplementation to flour and bread as well as its improving effect on NPR and PER. Also the effect of baking on lysine retention and the protein quality of bread was assessed.

The data presented in Table 3 report the levels of lysine supplementation, NPR and PER of non-supplemented flours and their corresponding breads. The PER of non-supplemented flour was 0.57, which was decreased to 0.40 by baking. These PER values are very low and insignificant from a nutritional point of view, because any protein food with a PER value below 1 is considered of little nutritional value. Addition of 0.30 percent lysine caused a statistically significant increase in PER value of flour from 0.57 to 1.40 and of bread from 0.40 to 1.50. These findings are confirmed by those of Sabiston and Kennedy (57) who obtained a significant increase in PER value of flour upon lysine supplementation. In the present work, no further improvement was observed upon addition of higher levels of lysine; similar results were reported by other workers (21, 39) who found for the optimum rat growth the level of lysine to be added to flour is 0.20-0.30 percent.

Figure 2 shows the comparison between the PER values in Table 3 and the values for available lysine of flour and bread samples with different levels of lysine

Table 3. Protein quality of flour and bread supplemented with different levels of lysine.

Diet	Lysine added (mg/gN of) flour	PER Mean ± S.E.	NPR Mean ± S.E.
Flour			
+0.0% Lysine	0	0.57 ± 0.05	2.77 ± 0.19
+0.3% "	150	1.40 ± 0.11[x]	2.29 ± 0.18
+0.5% "	250	1.38 ± 0.19[x]	2.14 ± 0.13
+0.7% "	350	1.38 ± 0.15[x]	2.53 ± 0.18
Bread			
+0.0% Lysine	0	0.40 ± 0.06	2.81 ± 0.17
+0.3% "	150	1.50 ± 0.14[xx]	3.37 ± 0.28
+0.5% "	250	1.45 ± 0.13[xx]	2.84 ± 0.20
+0.7% "	350	1.58 ± 0.29[xx]	3.27 ± 0.18

x. Significant at 1% level (compared with flour + 0.0% lysine).

xx. Significant at 1% level (compared with bread + 0.0% lysine).

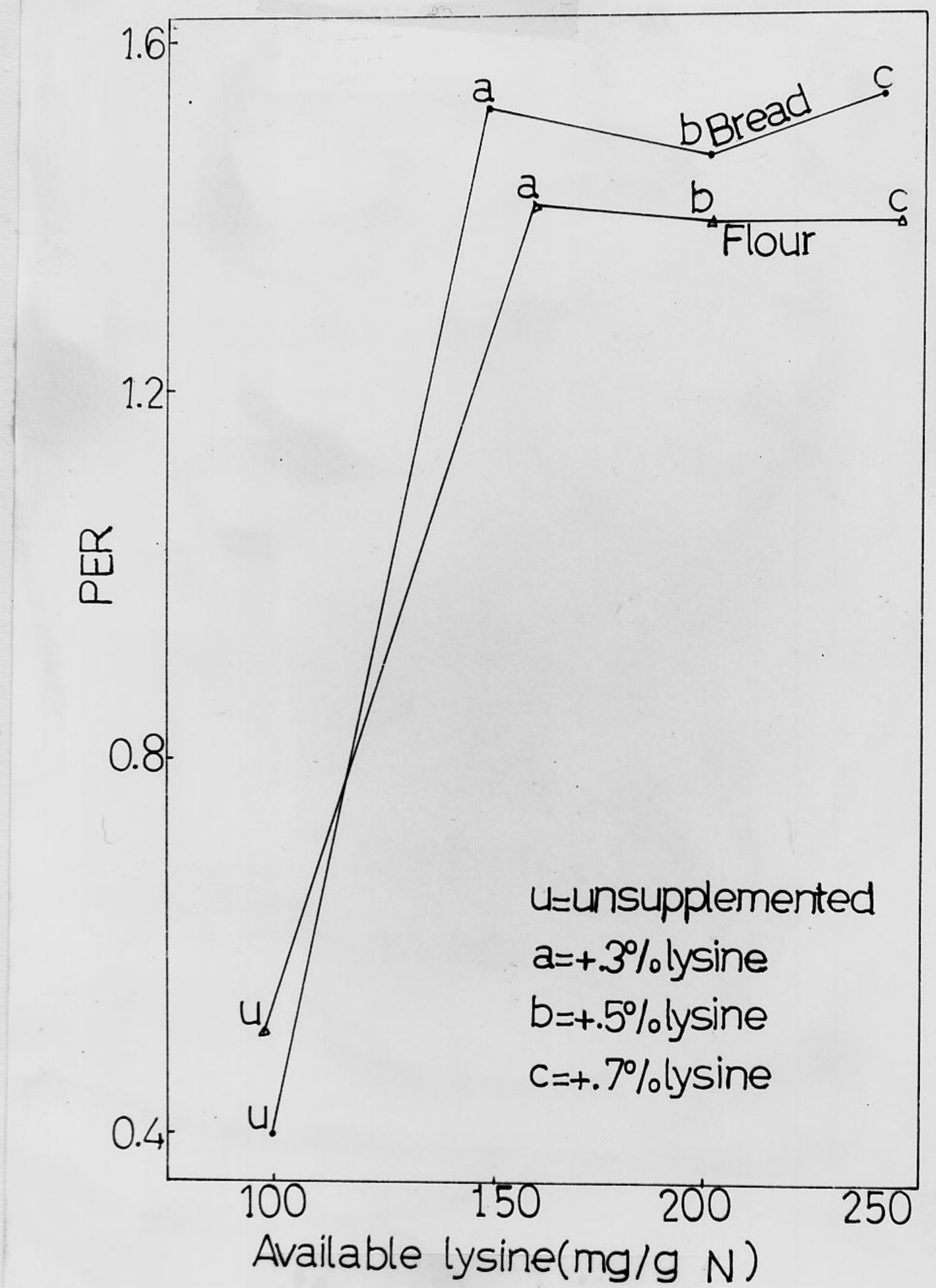

Figure 2. Relation between available lysine content and PER of unsupplemented or supplemented flours and breads.

supplementation shown in Table 4. It was found that, although increase in lysine supplementation above 0.30 percent of the flour weight increased the available lysine content, like before, no significant change in PER value was observed.

In Table 4 are shown the feed consumption and weight gain of experimental animals over a 4-week test period; also presented are the values for total lysine, available lysine and percent recovery of total lysine and of added lysine as available. The feed consumption of rats on unsupplemented flour and bread was 168 g and 159 g, respectively; the corresponding figures for weight gain were 8.4 g and 6.1 g. When 0.30 percent lysine was added to flour, the average feed consumption in the case of both flour and bread increased to 214 g, while the average weight gain increased from 8.4 g for bread and 6.1 g for flour to 27 g for both. Other workers have reported an increased weight gain due to lysine supplementation (38,54). Levels higher than 0.30 percent lysine in general caused a slight but statistically insignificant decrease in feed consumption and weight gain (Table 4).

As indicated in Table 4, the method used for the determination of total lysine could measure only 56-71 percent of the theoretical values. The recovery of added lysine as available lysine with the method used in this experiment was only about 40 percent in the case of flour

Table 4. Total and available lysine of diets and the feed consumption and weight gain of rats fed lysine-supplemented flours and breads.

Diet	Lysine added (mg/gN of flour)	Feed consumption (g/4 weeks)	Weight gain (g/4 weeks)	Total lysine (mg/gN)			Available Lysine (mg/gN)	Recovery of available lysine (%)[1]
				Calculated	Determined	% Recovery[1]		
Flour								
+0.0% Lysine	0	168	8.4	-	118	-	98	-
+0.3% "	150	214	26.8	268	224	71	160	41
+0.5% "	250	211	26.5	368	284	66	202	42
+0.7% "	350	192	23.9	468	351	66	240	41
Bread								
+0.0% Lysine	0	159	6.1	-	118	-	100	-
+0.3% "	150	214	27.5	268	202	56	150	33
+0.5% "	250	194	23.5	368	283	66	198	39
+0.7% "	350	186	25.0	468	350	66	231	37

1. Percent Recovery = $\frac{\text{Lysine (mg/gN) - Original lysine content (Mg/gN)}}{\text{Lysine added (mg/gN)}} \times 100$

and slightly lower (33-39 percent), but not significantly so, in the case of bread. These observations agree with the data of Clegg and Davis (14) who observed no significant reduction in the available lysine content of flour due to baking.

The growth curves in Figure 3 are for rats for unsupplemented flour and bread, flour and bread supplemented with 0.30 percent lysine (optimum level), and casein. As compared with unsupplemented flour and bread, the addition of 0.30 percent lysine increased the growth rate of rats considerably. The rate of growth of rats on the experimental diets was always less than that of rats on the reference casein diet.

The results of this experiment indicate that: (i) addition of 0.30 percent lysine to flour caused an appreciable improvement in the protein quality of bread; (ii) baking had no effect on the protein quality of bread; and (iii) the microbiological method of Block and Weiss (7) shows 56-71 percent recovery of the calculated amount of total lysine, while the DNFB procedure of Bruno and Carpenter (8) reveals only about 40 percent availability of added lysine in the case of supplemented flours and breads.

Experiment II

Threonine and methionine supplementation: In this experiment lysine-supplemented flour and bread were further

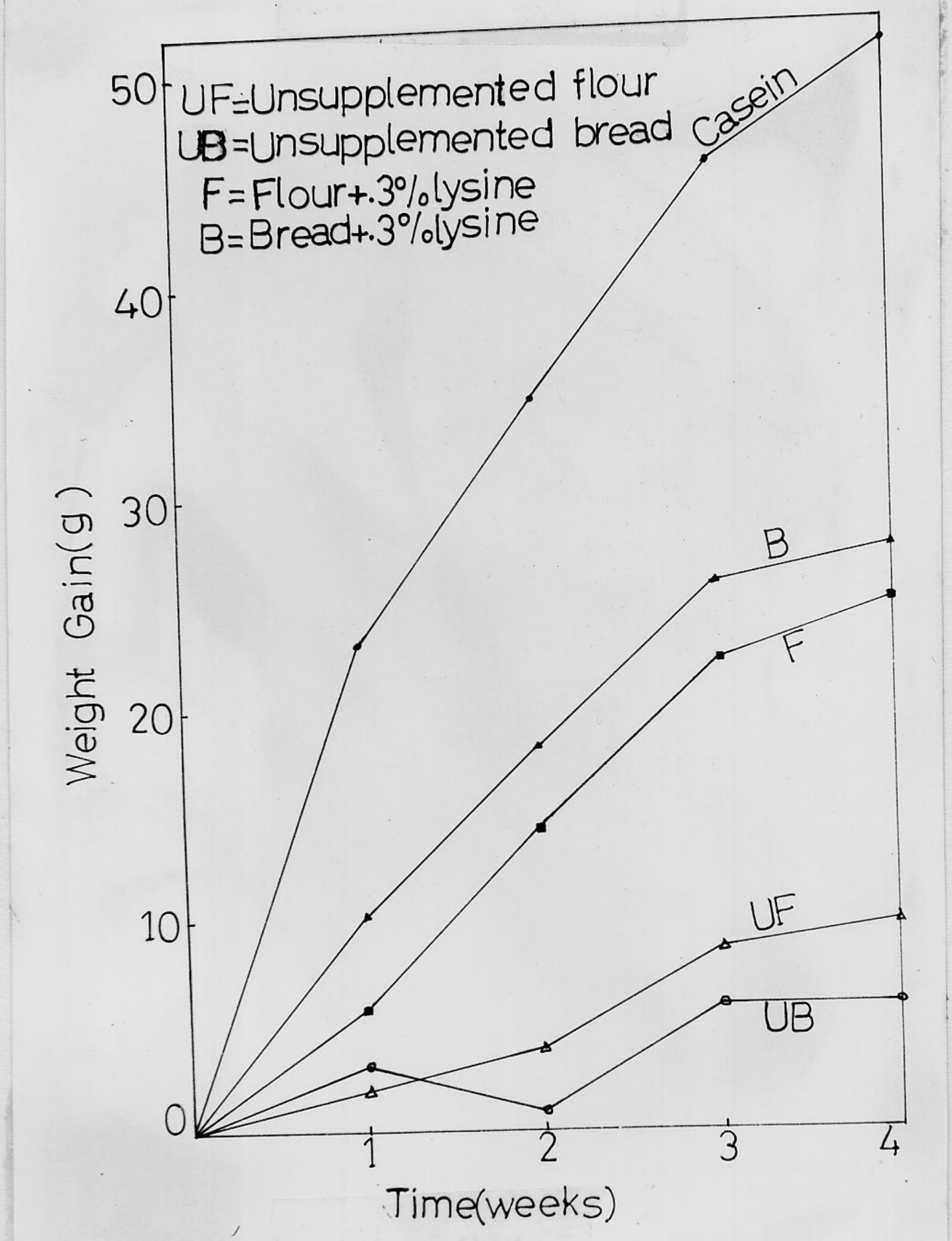

Figure 3. Growth rates of rats fed different experimental diets compared with that of those fed casein.

supplemented with threonine and methionine to determine the order of their limitation in flour and bread. The effect of baking on the protein quality of bread was also studied. Table 5 and Figure 4 show the results obtained from this experiment. These results show that the addition of threonine alone to lysine-supplemented flour caused increase in PER of flour from 1.05 to 2.16, and of bread from 1.51 to 2.30. When methionine was added to flour already supplemented with lysine or lysine and threonine, no further significant change was observed in PER of the flour or bread. These results suggest that threonine is the second limiting amino acid in flour and bread and methinone has no effect among the amino acids studied. Bender (5) also found threonine to be the second limiting amino acid, and Ericson (18) obtained no effect by addition of methionine to flour and bread supplemented with lysine and threonine.

The data in Table 6 report the average feed consumption and weight gain of rats fed different experimental diets over a 4-week test period; presented are also threonine and methionine contents of the diets. The weight gain of rats on lysine-supplemented flour and bread was 22.5 g and 29.7 g respectively; addition of threonine increased the weight gain of both groups to about 60.0 g. Similarly, the feed consumption increased from 221.7 g for lysine-fortified flour to 310.9 g and from 224.6 g to

Table 5. Protein quality of lysine-supplemented flours and breads fortified with threonine and methionine.

Diets	PER Mean ± S.E.	NPR Mean ± S.E.
Flour + 0.30%L[1]	1.05 ± 0.11	2.83 ± 0.16
+ 0.30%L + 0.62%T[2]	2.16 ± 0.10[x]	3.51 ± 0.24
+ 0.30%L + 0.28%M[3]	1.11 ± 0.23	2.70 ± 0.29
+ 0.30%L + 0.28%M + 0.62%T	2.44 ± 0.07[x]	3.76 ± 0.11
Bread + 0.30%L[1]	1.51 ± 0.12	3.05 ± 0.17
+ 0.30%L + 0.62%T[2]	2.30 ± 0.12[xx]	4.03 ± 0.22
+ 0.30%L + 0.28%M[3]	1.33 ± 0.09	3.31 ± 0.25
+ 0.30%L + 0.28%M + 0.62%T	2.06 ± 0.08[xx]	3.48 ± 0.15

1. l-Lysine

2. dl-Threonine

3. l-Methionine

x. Significant at 1% level (compared with flour + 0.30% lysine.

xx. Significant at 1% level (compared with bread + 0.30% L).

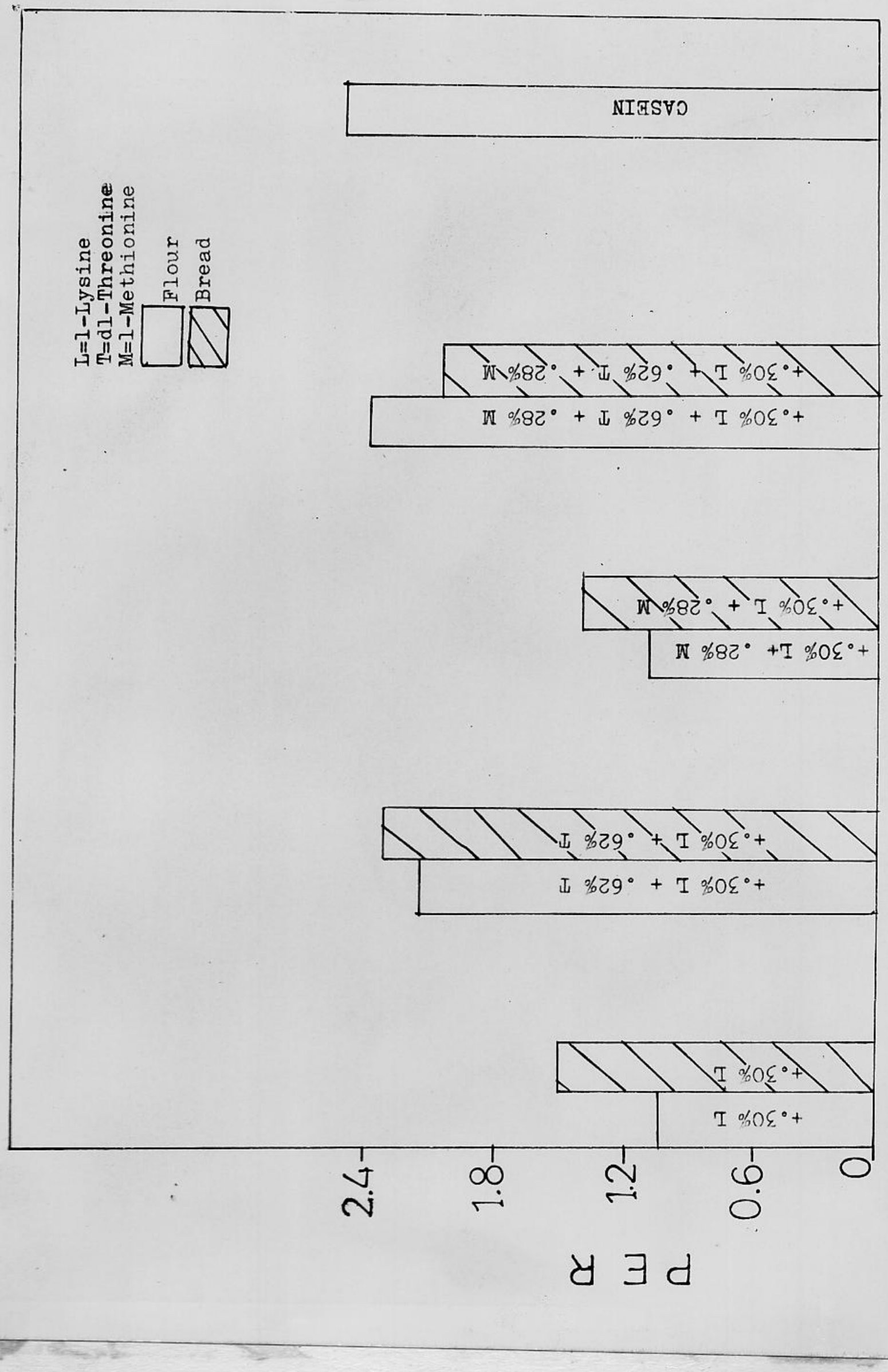

Figure 4. The effect of supplementation of lysine-fortified flour and bread with threonine and methionine on their PER.

Table 6. Methionine and threonine content of lysine-supplemented flours and breads fortified with threonine and methionine and the feed consumption and weight gain of rats fed these test diets.

Diets	Feed Consumption (g/4 weeks)	Weight gain (g/4 weeks)	Total methionine (mg/gN) Calculated	Determined	% Recovery	Total threonine (mg/gN) Calculated	Determined	% Recovery
Flour								
+ 0.30% L[1]	221.7	22.5	-	84.82	-	-	141.0	-
+ 0.62% T[2]	310.9	62.3				298.0	280.3	90
+ 0.28% M[3]	204.2	17.7	219.6	213.6	95			
+ 0.62% T+0.28%M	332.1	76.2	219.6	220.0	98			
Bread								
+0.30% L	224.6	29.7	-	80.53	-	-	141.0	-
+0.62% T	285.3	60.1				298.0	238.2	63
+0.28% M	236.1	29.4	219.6	200.4	91			
+0.62% T+0.28%M	287.9	57.5	219.6	191.6	85			

1. l-Lysine
2. dl-Threonine
3. l-Methionine

285.3 g for the bread made from the same flour. Other workers (17, 18) have reported similar findings. However, Rosenberg et al. (56) obtained no beneficial effect by the addition of threonine to lysine-supplemented bread. Addition of methionine to flour already supplemented with lysine caused a slight, but insignificant, decrease in the feed consumption of the rats, while there was no change in the consumption of the corresponding bread. The feed consumption of rats fed flour supplemented with all three amino acids was lower compared with that of rats fed lysine-and-threonine-supplemented flour. The decrease was from 76.2 g to 62.3 g. However, the feed consumption on the corresponding breads, although lower than that of flours, was about the same.

As indicated in Table 6, the recovery of total methionine was quite high. Although the recovery of total threonine in flour supplemented with lysine and threonine was about 90 percent, in the case of bread made from this flour the recovery was only 63 percent. This decrease might be an indication of the binding of threonine due to the baking processes. These results are in agreement with those of Ericson et al. (19) who reported a 20-40 percent loss of threonine during baking.

On the basis of the results of this experiment the following conclusions may be drawn: (i) among the amino acids studied in relation to the protein quality of flour

and bread threonine is the second limiting, while methionine has no effect and (ii) the protein quality of lysine-supplemented bread fortified with threonine or methionine was not affected by baking, while the protein of bread supplemented with lysine, threonine and methionine was seriously damaged during baking. This difference in the protein quality of flour and bread may possibly be explained on the basis of amino acid imbalance. According to the data in Table 6, threonine is heat-labile and methionine heat-resistant. Therefore, while different amino acids are in a balanced state in flour, due to partial destruction or binding of threonine in the process of baking bread an imbalance is created, which may result in the lowering of the protein quality of bread. In other words, as threonine is destroyed, methionine becomes in excess and acts as a cause of imbalance and poorer quality of protein. Other workers (30, p.270, 62) have reported a toxicity effect for methionine when it is present in excess.

Experiment III

<u>Different levels of methionine supplementation:</u> This experiment was conducted to determine the level of methionine to be added to flour and bread that may result in a better growth of rats. Also, the effect of baking on bread supplemented with lysine, threonine and different

levels of methionine was studied.

Table 7 shows the PER and NPR of lysine-and threonine-supplemented flours and breads fortified with 0.15, 0.28 or 0.35 percent methionine. In the case of flours, the PER values were all about 2.0; there was no statistically significant difference among the diets. The same trend was observed in breads baked from these flours, although the PER values were lower (1.53-1.88) than those for the flours. These observations indicate that the flour supplemented with 0.15 percent methionine supported as good rat growth as did those fortified with the higher levels of this amino acid used in this experiment. According to Table 7, baking lowered the protein quality in the breads supplemented with the lowest and highest (0.15 and 0.35 percent) levels of methionine, while the decrease in the case of supplementation with the intermediate level (0.28 percent) was less and statistically insignificant.

The average feed consumption and weight gain of rats over a 4-week assay period are shown in Table 8 and Figure 5; the methionine content of the experimental diets is also presented in Table 8. The feed consumption of rats fed different flours ranged from about 304 g to about 317 g, and in the case of breads from 285 g to 302 g, none of the differences being statistically significant. Wider differences were observed among weight gains of the experimental animals fed different test diets. The weight gain of rats

Table 7. Protein quality of lysine-and threonine-supplemented flours and breads fortified with different levels of methionine.

Diets	PER Mean ± S.E.	NPR Mean ± S.E.
Flour + L[1] + T[2]		
+ 0.15% methionine	1.99 ± 0.11	3.25 ± 0.11
+ 0.28% "	2.03 ± 0.05	3.14 ± 0.18
+ 0.35% "	2.16 ± 0.09	3.10 ± 0.10
Bread + L[1] + T[2]		
+ 0.15% methionine	1.53 ± 0.17[x]	3.10 ± 0.09
+ 0.28% "	1.88 ± 0.11	3.21 ± 0.21
+ 0.35% "	1.74 ± 0.06[xx]	3.02 ± 0.13

1. 0.30% l-lysine

2. 0.62% dl-threonine

x. Significant at 5% level (compared with flour + L + T + 0.15 percent methionine).

xx. Significant at 1% level (compared with flour + L + T + 0.35 percent methionine).

Table 8. Feed consumption and weight gain of rats fed lysine-and threonine-supplemented flours and breads fortified with different levels of methionine and methionine content of these diets.

Diets	Feed consumption (g/4 weeks)	Weight gain (g/4 weeks)	Total methionine (mg/gN)		
			Calculated	Determined	% Recovery
Flour + L[1] + T[2]			-	82.0	
+0.15% methionine	304.2	72.6	156.7	150.1	91
+0.28% "	312.2	64.5	221.4	209.5	91
+0.35% "	317.4	81.8	256.2	241.3	91
Bread + L + T			-	80.8	
+0.15% methionine	294.3	58.6	156.7	145.9	93
+0.28% "	302.2	61.2	221.4	210.9	95
+0.35% "	285.1	55.8	256.2	240.5	94

1. 0.30% l-lysine

2. 0.62% dl-threonine

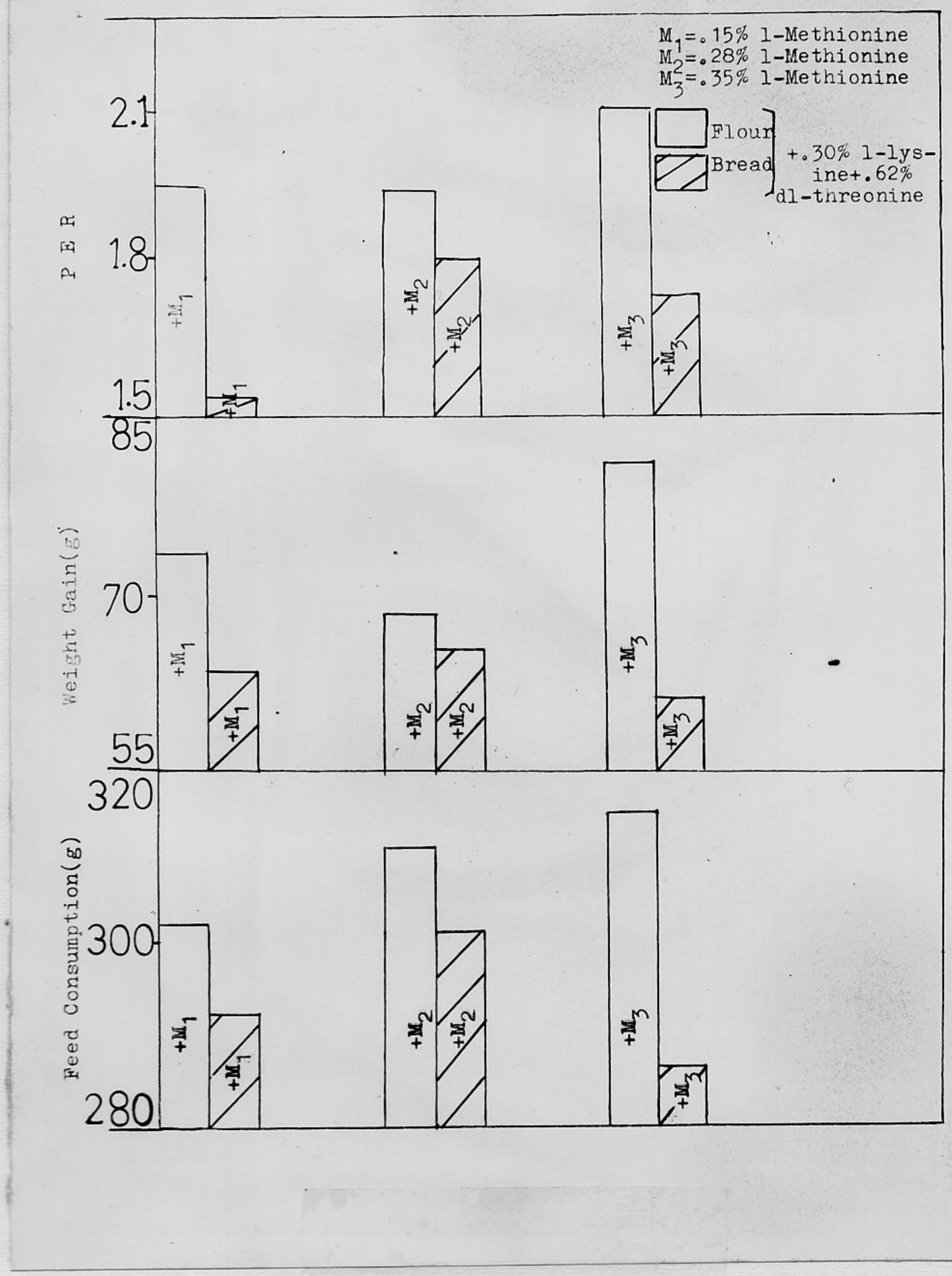

Figure 5. PER, weight gain and feed consumption of rats fed lysine-and threonine-supplemented flours and breads fortified with different levels of methionine.

fed flours fortified with 0.15 and 0.35 percent methionine were 72.6 g and 81.8 g, respectively, and 58.6 g and 55.8 g, respectively, in the case of the corresponding breads. There was no substantial difference between the weight gain of rats fed flour supplemented with 0.28 percent methionine (64.5 g) and that of rats fed the bread made from this flour (61.2 g).

As shown in Table 2, the recovery of added methionine was 91-95 percent of the calculated value, which confirms the values obtained in Experiment II.

The results of this experiment indicate that: (i) addition of 0.15 percent methionine to flour and bread resulted in growth similar to that of rats fed flours and breads supplemented with higher levels and (ii) baking in most cases caused a substantial decrease in the protein quality of breads supplemented with lysine, threonine and methionine.

Computor evaluation of mixed diets: This theoretical study was undertaken to investigate the supplementary effect of commonly consumed food items on the protein quality of bread. Table 2 (p.23) shows the formulated diets that were studied; the protein quality of such mixtures was estimated by calculating net dietary protein calories as percent of total calories (NDpCal%). Of particular interest was to find the limiting amino acid in each one of the mixtures studied by the use of the FAO amino acid scoring method (23, p.30).

In Table 9 are shown values for NDpCal% of the mixtures and the calculated limiting amino acid in each. The bread alone had a NDpCal% of 4.1, which is low, considering that the minimum value for adults should be 5.0 and for growing children 8.0 (24, p.45). The limiting amino acid in bread, as expected, was lysine. Addition of 25 percent of a dairy product, such as concentrated yoghurt (lebneh), increased the NDpCal% to 5.9; a further improvement was observed when yoghurt was replaced with cheese (NDpCal% = 7.0). When a small part of bread in the bread-lebneh mixture was substituted by olive oil, there was no change in NDpCal%, while the addition of 15 percent tea with sugar to the bread-cheese mixture decreased the value from 7.0 to 6.0. In all these mixtures the sulfur amino acids were limiting.

A mixture of bread and grapes (40-60 percent) had a considerably lower NDpCal% (2.2) than bread alone; the limiting amino acid in this mixture was lysine. The value of NDpCal% increased to 4.8 when one-third of the grapes were replaced by cheese and the limiting amino acid in the mixture was total sulfur amino acids. A NDpCal% of 5.2 was found for a 75-25 percent mixture of bread and walnuts and lysine was the limiting amino acid; addition of cheese to this mixture increased the NDpCal% to 7.4 and changed the limiting amino acid to total sulfur amino acids.

It is interesting that in a bread-egg mixture of

Table 9. The NDpCal% of mixtures of bread and common dietary items and the limiting amino acid in these mixtures.

Mixture No.	NDpCal%[1]	Limiting amino acid
1	4.1	Lysine
2	5.9	S-AA[1]
3	5.9	S-AA
4	7.0	S-AA
5	6.0	S-AA
6	2.2	Lysine
7	4.8	S-AA
8	5.2	Lysine
9	7.4	S-AA
10	7.3	Neither
11	6.7	Lysine
12	6.8	S-AA
13	7.6	S-AA

1. Total sulfur amino acids.

65-35 percent neither lysine nor sulfur amino acids were limiting; the NDpCal% was 7.3. Substituting a portion of egg with vegetable oil reduced the NDpCal% to 6.7 and made lysine limiting. "Falafel" and "Shawwarmah" sandwiches, two common dietary items in Lebanon, had NDpCal% values of 6.8 and 7.6, respectively. Again sulfur amino acids were limiting.

The results of this study show that, in bread alone as well as in mixtures of bread and some foods of plant origin, and in rare cases in mixtures of bread and animal food stuffs, lysine is the limiting amino acid; the NDpCal% of most of these mixtures is relatively low. However, in mixtures of bread and most foodstuffs of animal origin, total sulfur amino acids are the limiting amino acid, the NDpCal% of most of these mixtures being relatively high. The fact that lysine was calculated to be limiting in bread confirms the experimental findings of Experiment I.

V. SUMMARY AND CONCLUSIONS

Breads were prepared from flours supplemented with three levels of lysine, lysine and threonine, lysine and three levels of methionine or lysine, threonine and methionine. Total content of each of these amino acids in all flours and breads and available lysine in unsupplemented and lysine-supplemented flours and breads were measured. Of the two methods tried for the determination of available lysine, the one reported by Bruno and Carpenter (8) gave more successful results in this work. Also, the protein quality of all the flours and breads mentioned above was determined biologically. In addition, the protein quality of mixtures of bread and common dietary items was estimated by calculation of NDpCal% and amino acid scores with the use of a computor.

Supplementation of flour and bread with 0.30 percent l-lysine resulted in considerable improvement in the protein quality; higher levels had no effect. When dl-threonine was added to lysine-supplemented flour at a level of 0.62 percent, a sustantial increase was observed in the protein quality of the flour as well as the corresponding bread. Addition of methionine to lysine-supplemented flour did not cause further increase in protein quality. These observations indicate that, among

the amino acids studied, threonine is the second limiting amino acid in flour and bread. Baking had no effect on the protein quality of lysine-supplemented and lysine-and threonine-supplemented breads, although the threonine content decreased due to baking in the latter.

While there was no further improvement in the protein quality of lysine-and threonine-supplemented flour and bread due to addition of methionine, the protein quality of the corresponding bread was decreased appreciably by baking.

Computor calculations showed that bread, and in some cases, mixtures of bread and dietary food items of plant origin usually were limited by lysine, and that they had a low NDpCal%. However, when bread was supplemented with foodstuffs of animal origin, total sulfur amino acids in most cases became limiting, and the NDpCal% increased considerably. In general, substantial improvement was observed in the protein quality of bread supplemented with other dietary food items.

It was concluded that wheat flour used for baking Arabic bread should be supplemented with 0.30 percent l-lysine and 0.62 percent dl-threonine based on flour weight. Although methionine did not seem to be limiting in bread, its importance becomes apparent when it is observed that most mixed diets having animal protein sources are limiting in sulfur amino acids. Based on this fact, methionine

supplementation of flour seems to be logical and necessary. More work is needed to suggest the optimum level of methionine supplementation of bread for use in mixed diets.

VI. SELECTED BIBLIOGRAPHY

1. Allison, J.B. Biological evaluation of proteins. Physiol. Rev. 35, 664, 1955.

2. Allison, J.B. The efficiency of utilization of dietary proteins. Protein and Amino Acid Nutrition. Ed. Albanese, A.A. Academic Press Inc., New York, 1959.

3. Baliga, B.P., M.E. Bayliss and C.M. Lyman. Determination of free lysine E-amino groups in cottonseed meals and preliminary studies on relation to protein quality. Arch. Biochem. Biophys. 84, 1, 1959.

4. Barnes, R.H., and D.K. Bosshardt. The evaluation of protein quality in the normal animal. Ann. N.Y. Acad. Sci. 47, 273, 1946.

5. Bender, A.E. Nutritive value of bread protein fortified with amino acids. Science, 127, 874, 1958.

6. Bender, A.E., and B.H. Doell. Biological evaluation of proteins: a new aspect. Brit. J. Nutr. 11, 140, 1957.

7. Block, R.J., and K.W. Weiss. Amino Acid Handbook. Charles Thomas publisher, Springfield, Illinois, 1956.

8. Bruno, D., and K.J. Carpenter. A modified procedure for the estimation of available lysine in food proteins. Biochem. Jour. 67, 13 , 1957.

9. Campbell, J.A. Methodology of Protein Evaluation. Division of Food Technology and Nutrition, Faculty of Agricultural Sciences, American University of Beirut, Publication No. 21, 1963.

10. Carpenter, K.J. The estimation of the available lysine in animal-protein foods. Biochem. J. 77, 604, 1960.

11. Carpenter, K.J., and G.M. Ellinger. Estimation of "available" lysine in protein concentrates. Biochem. J., 61, XI, 1955.

12. Chapman, D.G., R. Castillo, and J.A. Campbell. Evaluation of proteins in foods. 1. A method for the determination of protein efficiency ratio. Can. J. Biochem. Physiol. 37, 679, 1959.

13. Clarke, J.K., and B.M. Kennedy. Availability of lysine in whole-wheat bread and in selected breakfast cereals. J. Food Sci. 27, 609, 1962.

14. Clegg, K.M., and N. Davis. "Available lysine" in wheat, flour and bread. Proc. Nutr. Soc. 17, X, 1958.

15. Conkerton, E.J., and V.L. Frampton. Reaction of gossypol with free E-amino groups of lysine in proteins. Arch. Biochem. Biophys. 81, 130, 1959.

16. Derse, P.H. Evaluation of protein quality. II- Methods. J. Assoc. Off. Agric. Chemists. 41, 192, 1958.

17. Deshpande, P.D., A.E. Harper, and C.A. Elvehjam. Nutritional improvement of white flour with protein and amino acid supplements. J. Nutrition. 62, 503, 1957.

18. Ericson, L.E. Studies on possibilities of improving the nutritive value of Swedish wheat bread. II- The effect of supplementation with lysine, threonine, methionine, valine and tryptophan. Acta Physiol. Scand. 48, 295, 1960.

19. Ericson, L.E., S. Larsson, and G. Lid. The loss of added lysine and threonine during the baking of wheat bread. Acta Physiol. Scand. 53, 85, 1961.

20. Ericson, L.E., S. Larsson, and G. Lid. A comparison of the efficiencies of free lysine and of roller-dried skim milk, fish protein and soya bean protein for the supplementation of wheat bread. Acta Physiol. Scand. 53, 366, 1961.

21. Ericson, L.E., and C. Ovenfors. Studies on the possibilities of improving the nutritive value of Swedish wheat bread. I. The effect of lysine supplementation. Acta Physiol. Scand. 47, 44, 1959.

22. Evaluation of Protein Quality. National Academy of Sciences - National Research Council. Washington, D.C. Pub. No. 1100. 1963.

23. Food and Agriculture Organization. Protein Requirements. Nutrition Studies No. 16. FAO. Rome. 1957.

24. Food and Agriculture Organization / World Health Organization. Protein Requirements. FAO. Rome. 1965.

25. Feldberg, C., and C.P. Hetzel. The role of lysine in cereal food products. Food Technology. 12, 496, 1958.

26. Flodin, N.W. Amino acids and proteins, their place in human nutrition problems. Agr. Food Chem. 1, 222, 1953.

27. Food Composition Tables for Use in the Middle East. Faculty of Agricultural Sciences, Pub. No. 20. Beirut.

28. Ford, J.E. A microbiological method for assessing the nutritional value of proteins. 2. The measurement of "available" methionine, leucine, isoleucine, arginone, histidine, tryptophan, and valine. Brit. J. Nutr. 16, 409, 1962.

29. Friedman, L., and O.L. Kline. The relation of the ámino acid-sugar reaction to the nutritive quality of protein hydrolyzates. J. Nutrition. 40, 295, 1950.

30. Frost, D.V. Methodology of measuring the nutritive value of proteins, protein hydrolyzates and amino acid mixtures. The repletion method. In Protein and Amino Acid Nutrition. Ed. Albanese, A.A. Academic Press Inc., New York. 1959.

31. Gates, J.C., and B.M. Kennedy. Protein quality of bread and bread ingredients. J. Amer. Diet. Assoc. 44, 374, 1964.

32. Guthneck, B.T., B.A. Bennett, and B.S. Schweigert. Utilization of amino acids from foods by the rat. J. Nutr. 49, 289, 1953.

33. Hepburn, F.N., C.W. Lewis, and C.A. Elvehjem. The amino acid content of wheat, flour and bread. Cereal Chem. 34, 312, 1957.

34. Hepburn, F.N., W.K. Calhoun, and W.B. Bradley. The biological availability of essential amino acids in wheat, flour bread and gluten. Cereal Chem. 43, 271, 1966.

35. Hoagland, R., and G.G. Sinder. Nutritive value of the protein in voluntary muscle, heart, liver and kidney from cattle, sheep and hogs. J. Agr. Res. 32, 1025, 1926.

36. Howe, E.E., G.R. Jansen, and E.W. Gilfillan. Amino acid supplementation of cereal grains as related to the world food supply. Amer. J. Clin. Nutr. 16, 315, 1965.

37. Hundley, J.M. Algae as sources of lysine and threonine in supplementing wheat and bread diets. Science. 124, 536, 1965.

38. Hutchinson, J.B., T. Moran, and J. Pace. Effect on the growth-rate of weanling rats of supplementing the protein of white bread with l-lysine. Nature, 178, 46, 1956.

39. Hutchinson, J.B., T. Moran, and J. Pace. Bread and the growth of weanling rats; the lysine-threonine balance. Nature, 181, 1733, 1958.

40. International Committee on Nutrition for National Defense. Republic of Lebanon. Nutrition Survey, February-April 1961. Washington. Government Printing Office. 1962.

41. King, K.W., W.H. Sebrell, Jr., E.L. Severinghaus, and W.O. Stovick. Lysine fortification of wheat bread fed to Haitian school children. Amer. J. Clin. Nutr. 12, 36, 1963.

42. McDermott, E.E., and J. Pace. The contents of amino acids in white flour and bread. Brit. J. Nutr. 11, 446, 1957.

43. Miller, D.S., and G. Donoso. Relationship between the sulfur/nitrogen ratio and the protein value of diets. J. Sci. Fd. Agric. 14, 345, 1963.

44. Mitchell, H.H. The dependence of the biological value of food proteins upon their content of essential amino acids. In Nutr. Abs. Rev. 25, 996, 1955.

45. Morrison, A.B., and J.A. Campbell. Evaluation of protein in foods. V. Factors influencing the protein efficiency ratio of foods. J. Nutrition. 70, 112, 1960.

46. Orr, M.L., and B.K. Watt. Amino Acid Content of Foods. U.S.D.A. Washington, D.C. 1957.

47. Osborne, T.B., and L.B. Mendel. Amino acids in nutrition and growth. J. Biol. Chem. 17, 325, 1914.

48. Osborne, T.B., L.B. Mendel, and E.L. Ferry. A method of expressing numerically the growth promoting value of a protein. J. Biol. Chem. 37, 223, 1919.

49. Ousterhout, C.R.G., and B.D. Lundholm. Biological availability of amino acids in fish meals and other protein sources. J. Nutr. 69, 65, 1959.

50. Pelshenke, P.F. Report to the Government of Lebanon on the Bread Situation. Food and Agriculture Organization of the United Nations. Rome. 1964.

51. Platt, B.S. Tables of Representative Values of Foods Commonly Used in Tropical Countries. Medical Research Council Special Report Series No. 302. London. Her Majesty's Stationary Office. 1962. (Reprint 1965).

52. Rao, S.R., F.L. Carter, and V. Frampton. Determination of available lysine in oilseed meal proteins. Anal. Chem. 35, 1927, 1963.

53. Rao, M.N., H. Sreenivas, M. Swaminathan, K.J. Carpenter, and C.B. Morgan. The nutritionally available lysine and methionine of heated casein-glucose mixtures. J. Sci. Fd. Agric. 14, 544, 1963.

54. Rerat, A., and R. Jacquot. Note préliminaire sur la double supplémentation de la farine de blé par la dl-lysine et l'aurémycine. (Preliminary note on the double supplementation of wheat flour with dl-lysine and aureomycine). Ann. Nutrit. Alimentation 11, Mém. 25, 1957. In Nutr. Abs. Rev. 27, 1184, 1957.

55. Rosenberg, H.R., and E.L. Rohdenberg. The fortification of bread with lysine. I. The loss of lysine during baking. J. Nutrition. 45, 593, 1951.

56. Rosenberg, H.R., E.L. Rohdenberg, and J.T. Baldini. The fortification of bread with lysine. III. Supplementation with essential amino acids. Arch. Biochem. Biophys. 49, 263, 1954.

57. Sabiston, A.R., and B.M. Kennedy. Effect of baking on the nutritive value of proteins in wheat bread with and without supplements of nonfat dry milk and of lysine. Cereal Chem. 34, 94, 1957.

58. Sakr, A. Amino Acids in Wild Plants. M.S. Thesis. American University of Beirut, 1961.

59. Sure, B. Influence of lysine, valine and threonine additions on the efficiency of the proteins of whole wheat. Arch. Biochem. Biophys. 39, 463, 1952.

60. Sure, B. Improving the nutritive value of cereal grains. J. Nutrition. 50, 235, 1953.

61. Sure, B. Relative nutritive values of proteins in foods and supplementary value of amino acids in pearled and peanut flour. J. Agr. Food Chem. 3, 789, 1955.

62. Wretlind, K.A.J., and W.C. Rose. Methionine requirement for growth and utilization of its optical isomers. J. Biol. Chem. 187, 697, 1950.

63. Yang, S.S., H.E. Clark, and G.E. Vail. Effects of varied levels and a single daily supplements of lysine on the nutritional improvement of wheat flour proteins. J. Nutrition. 75, 241, 1961.

APPENDIX

The vitamin and mineral mixtures had the following compositions per g:

1. Vitamin mixture:

Vitamin A	900 I.U.
Vitamin D	100 I.U.
Alpha-tocopherol	5 mg
Vitamin C	45 mg
Inositol	5 mg
Choline chloride	75 mg
Vitamin B_2	1 mg
Menadione	2 mg
Niacin	4 mg
Pyridoxine-HCl	1 mg
Thiamin-HCl	1 mg
Ca-pantothenate	3 mg
Biotin	20 ug
Folic acid	90 ug
Vitamin B_{12}	1 ug

2. Mïneral mixture:

NH_4-alum	90 ug
$Ca_3(PO_4)_2$	100 mg
$CaCO_3$	70 mg
Ca-citrate	300 mg
$CuSO_4$	80 mg
$FeNH_4(SO_4)_2$	15 mg
$MgCO_3$	35 mg
$MgSO_4$	240 ug
KCl	125 mg
KI	40 ug
KH_2PO_4	200 mg
NaCl	80 mg
NaF	500 mg