Nutritive Quality of Baladi Bread Supplemented with Fish Protein Concentrate, Green Algae, or Synthetic Amino Acids

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ABSTRACT

Amino acid analyses and feeding studies were conducted with rats to study the effects of fortification of baladi bread with fish protein concentrate (FPC), green algae, or pure amino acids. Baking at 400°C resulted in the loss of 12–21% of L-lysine in bread made with FPC or L-lysine. Diets based on baladi bread baked from a composite flour containing 6.6% FPC and 91.2% wheat flour (88% extraction) gave approximately the same growth rate for rats as did a diet based on bread from wheat flour (88% extraction) containing added 0.35% lysine, 0.13% DL-threonine, and 0.7% DL-methionine. In both breads, the protein efficiency ratio (PER) was 2.6, compared to a mean value of 1.0 in the unsupplemented bread. The PER for a reference diet containing 11% casein and 0.5% DL-methionine was 3.0. Diets based on bread containing algae (6% in the composite flour) did not improve the growth rate of rats.

In Egypt, wheat in bread form is the daily staple. The most popular type of bread is "baladi," a flat circular loaf composed of two crusty layers with almost no crumb. Baladi bread is usually prepared from highly-extracted (80–90%) flour. This bread is low in lysine content. When fed to rats, it gave a mean protein efficiency ratio (PER) value of 1.3 ± 0.16 (Hussein et al 1973, 1974) compared with 2.44 ± 0.14 for a casein reference diet. To correct for lysine deficiencies, baladi bread was fortified with broad beans or a mixture of synthetic amino acids, which resulted in higher PER values of 3.0 ± 0.10 and 3.3 ± 0.08, respectively.

Our objective was to assess the protein quality of bread prepared from formulas containing added fish flour, green algae, pure L-lysine, or a mixture of L-lysine, DL-threonine, and DL-methionine. The amino acid composition of the bread and percent lysine loss during baking were also determined.

MATERIALS AND METHODS

Preparation of Bread

Bread was made from the 87.5%-extracted flour, by a formula and procedure simulating commercial practice. The ingredients included 100 parts flour, 1.5 parts sodium chloride, four parts starter sponge, and variable water. Nine breads (A–L) were prepared, with or without fortification by fish flour, green algae (*Scenedesmus obliquus*), L-lysine, or a mixture of L-lysine, DL-threonine, and DL-methionine. The ingredients and compositions of the flours used in breads A–L are presented in Table I. The dough was mixed mechanically, fermented for 2 hr, flattened on a wooden board dusted with wheat bran, and baked at 400°C for 90 sec on the top surface of a stone oven heated by gasoline. The loaves were cooled, wrapped, and allowed to stand overnight at room temperature, then dried in a stream of unheated air, and ground. The breads were assayed for protein content (N × 5.7) by the semimicro Kjeldahl method of the AOAC (1965).

Amino Acid Composition

Acid hydrolysis was used in the preparation of samples for amino acid analysis. Samples containing about 32 mg of nitrogen were weighed in 1-L round-bottom flasks, and 800 ml of 6*M* aqueous hydrochloric acid was added. The contents of the flasks were heated for 24 hr under reflux in the presence of a gentle N_2 stream. After filtration, the filtrate was dried by evaporation under reduced pressure and redissolved in 100 ml of 0.1*M* hydrochloric acid. About 8 g of the hydrolysate was weighed exactly and diluted to 50 ml with 0.2*M* sodium hydroxide. A 5.0 ml aliquot was taken for amino acid analyses by the ion-exchange column chromatography technique of Spackman et al (1958), using an amino acid analyzer.

Results were expressed in milligrams per gram of nitrogen. Chemical scores were calculated, based on the lysine content of 343.75 mg/g of nitrogen in the 1973 reference amino acid pattern (World Health Organization).

TABLE I
Flour Composition and Lysine Lost During Baking of Baladi Bread

Formulas	Composition of Flour Used in Bread							Bread Protein (%)	Lysine Content (g/16 g of N)		
	Wheat Flour[a]	Bran[b]	FPC[c]	Algae[d]	L-Lys	DL-Thr	DL-Met		Calc.	Anal.	Loss (%)
A	97.80	2.2	···	···	···	···	···	13.1	2.94	2.94	0
B	95.90	2.2	1.9	···	···	···	···	14.4	3.75	3.01	19.8
C	94.20	2.2	3.6	···	···	···	···	15.9	4.32	3.40	21.3
D	91.20	2.2	6.6	···	···	···	···	17.0	5.08	4.45	12.5
E	97.00	2.2	···	···	0.15	0.05	0.60	13.3	4.10	3.24	20.9
F	96.6	2.2	···	···	0.35	0.13	0.70	14.1	5.40	ND	···
G	97.66	2.2	···	···	0.14	···	···	13.2	4.13	ND	···
K	97.52	2.2	···	···	0.28	···	···	13.2	5.30	ND	···
L	92.7	1.5	···	5.8	···	···	···	15.1	3.40	3.28	4.0

[a] Analysis of flour (87.5% extraction): protein, 10.9%; lysine, 2.9 g/16 g of N.
[b] The bran was picked up by the surface of the dough during flattening of the dough piece.
[c] Analysis of FPC: protein, 88%; lysine, 9.0 g/16 g of N.
[d] Analysis of algae: protein, 73%; lysine, 4.6 g/16 g of N.

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Feeding Trials

The protein values of the enriched bread samples were determined biologically. Male Sprague-Dawley rats aged 21 ± 2 days were housed individually in wire flour cages. The experiments corresponded completely with the experimental procedure described by the German Group on Protein Evaluation (Müller 1964). For the first three days, the rats were fed a mixture of one part of a commercial rat diet and one part composed of equal portions of all 10 experimental diets. After this the food was removed, and 6 hr later the rats were weighed and distributed into 10 blocks of six rats of equal weight. In each block, diets and cages were randomized. All experimental diets were isonitrogenous (10% total protein) and included a test bread at levels of 59–75% of the diet. A casein diet containing 11 parts casein (86% crude protein) and 0.5 part DL-methionine per 100 parts diet served as a reference. Other nonvariables in the diets were a 6% salt mixture (Müller 1964), a 2% vitamin mixture (Müller 1964), 5% cottonseed oil, and 10% sucrose. In the next three weeks, body weight and food intakes of the rats were recorded at weekly intervals to permit calculation of weight gain and PER. On the last day of the experiment the food

TABLE II
Amino Acid Composition in Six Bread Formulas (mg/g Nitrogen) with Reference to the FAO Pattern

Amino Acid	FAO Reference Pattern	Casein	Bread Formula[a] A	B	C	D	E	L
Essential Amino Acids								
Lys	344	524	139	188	212	278	202	205
Met	···	162	101	143	127	132	362	109
Cys	···	21	161	141	136	136	147	122
Total Sulphur	219	183	262	284	263	268	509	231
Phe	···	344	291	350	304	279	281	277
Tyr	···	362	182	189	199	201	189	203
Total aromatic	375	706	473	539	503	480	470	480
Leu	437	634	436	479	456	432	555	438
Ile	250	555	223	242	233	238	201	231
Val	312	478	259	258	271	276	252	276
Thr	250	273	192	197	213	213	201	211
Trp	62	83	67	70	72	75	68	65
Total	2,250	4,105	2,051	2,256	2,224	2,280	2,301	2,138
Nonessential Amino Acids								
Asp	···	···	315	315	363	381	299	356
Glu	···	···	1,933	1,731	1,687	1,575	1,722	1,557
Ala	···	···	256	286	278	273	227	283
Pro	···	···	641	596	556	532	602	554
Ser	···	···	307	299	302	281	277	295
Gly	···	···	247	266	272	238	240	259
Arg	···	···	311	209	311	368	274	346
His	···	···	146	134	136	149	126	142
Total	···	···	4,126	3,852	3,906	3,800	3,788	3,792
E/N[b] %	···	···	49.8	58.6	56.9	60.0	61.1	56.4

[a]Flour replaced with: A, no replacement (control); B, 1.9% fish protein concentrate (FPC); C, 3.6% FPC; D, 6.6% FPC; E, L-lysine/DL-threonine/DL-methionine (0.15, 0.05, 0.6%); L, 5.8% algae.
[b]E/N = essential/nonessential amino acids.

TABLE III
Protein Efficiency Ratios (PER) and Productive Protein Values (PPV) in the Reference Casein Diet and Nine Bread Diets

Diet[a]	Food Intake (g) $\bar{X}$[b]	Food Intake (g) $S_{\bar{x}}$[b]	Gain (g) Body Weight $\bar{X}$	Body Weight $S_{\bar{x}}$	Gain (g) Carcass Nitrogen $\bar{X}$	Carcass Nitrogen $S_{\bar{x}}$	PER[c] $\bar{X}$	PER[c] $S_{\bar{x}}$	PPV[c] $\bar{X}$	PPV[c] $S_{\bar{x}}$	Utilizable Protein[d] (%)
A	99	6.5	9.8	2.1	0.28	0.03	1.0	0.18a	17	0.32	3.84
B	121	7.1	13.8	1.8	0.46	0.05	1.3	0.11a	25	2.69	5.64
C	132	10.0	18.0	2.5	0.54	0.07	1.6	0.10a	28	2.15	7.50
D	170	7.8	42.5	4.2	1.14	0.07	2.6	0.16b	40	1.36	13.06
E	112	10.0	14.6	1.8	0.45	0.16	1.6	0.12a	27	2.02	6.15
F	156	9.4	33.8	4.3	0.82	0.02	2.6	0.24b	36	1.76	10.79
G	129	8.4	18.3	2.4	0.49	0.03	1.6	0.20a	25	0.93	6.11
K	118	0.8	26.2	0.8	0.68	0.03	2.3	0.06b	36	1.71	9.12
L	98	2.9	10.0	0.5	0.34	0.01	1.2	0.05a	24	1.24	5.33
M	193	22.0	71.2	14.3	2.12	0.43	3.4	0.49	67	2.30	···

[a]Flour replaced with: A, control; B, 1.9% fish protein concentrate (FPC); C, 3.6% FPC; D, 6.6% FPC; E, L-lysine/DL-threonine/DL-methionine (0.15, 0.05, 0.6%); F, L-lysine/DL-methionine (0.35, 0.13, 0.7%); G, 0.14% L-lysine; K, 0.28% L-lysine; L, 5.8% algae; M, casein/DL-methionine reference diet.
[b]$\bar{X}$, mean; $S_{\bar{x}}$, standard deviation of the mean.
[c]Six male rats, 24–45 days old, per dietary group, for three-week experiment. In this column, values that do not share a common letter are significantly different ($P<0.05$). PPV = Productive protein values = $\frac{\text{body N (end)} - \text{body N (start)}}{\text{nitrogen intake}} \times 100$

was removed; 6 hr later the rats were killed with chloroform, the abdomens opened, and the carcasses dried at 95° C and ground. Carcass nitrogen was analyzed in individual animals by the semimicro Kjeldahl method (AOAC) 1965.

In addition, a group of animals was analyzed for carcass nitrogen at the start of the experiment, and those values were used to calculate the original body nitrogen of each animal and the gain in body nitrogen of each animal during the experiment.

Daily protein and lysine intakes were calculated from dietary protein content and food intakes. Gain in carcass nitrogen and nitrogen intake data for a given protein source were calculated to permit subsequent computation (Müller 1964) of the "produktiver proteinwert" or "productive protein value" (PPV):

$$\frac{\text{Body N (end of experiment)} - \text{Body N (start)}}{\text{Nitrogen intake}} \times 100$$

PERs were analyzed by Duncan's multiple range test (1955). The 0.05 level of probability was accepted as the criterion for statistical significance.

RESULTS

The protein content and amino acid composition of bread prepared from six different formulas are presented in Tables I and II. The protein content of the control bread (A) was 13%, of fish protein concentrate (FPC) 88%, and of green algae 73%. Addition of 6.56% FPC to formula D increased the lysine content of the bread to 278 mg/g of nitrogen, which is 81% of the reference FAO pattern (World Health Organization 1973). The lysine contributed 12.6% of the total essential amino acids, a value comparable to its proportion in whole egg (12.8%).

In formula L, algae was incorporated at a 5.8% level, and its protein contributed 28% of the bread protein. This bread had a lysine content of 203 mg/g of nitrogen, 53% of the reference amino acid pattern.

The lysine contents of bread formulas C, E, and L, enriched with FPC, L-lysine, and algae, respectively, were the same when expressed as mg/g of nitrogen (202–212). That level of lysine corresponds to 53% of the reference amino acid pattern (chemical score 53–55%).

The lysine content in the six bread formulas A, B, C, D, E, and L was calculated from the lysine content of wheat flour (Hussein et al 1979), wheat bran (Farrel et al 1967), FPC,[1] green algae,[2] and L-lysine-hydrochloride.[3] The calculated values were compared with the lysine content found by analysis of the baked bread (Table I). The baking loss of lysine was negligible (4%) in formula L (wheat flour-green algae combination) but was higher in formulas B, C, D, and E, fortified with either FPC (12–21% losses) or pure L-lysine-hydrochloride (20% loss).

Table III and Fig. 1 summarize the data obtained in the feeding experiments. Replacement of wheat flour with 1.9, 3.6, and 6.56% FPC in bread formulas B, C, and D, respectively, produced gradual increase in weight gain, PER, and PPV values. When 6.56% FPC was used, the rate of weight gain was significantly higher ($P < 0.001$) than that from control diet A. The results with 6.56% FPC were similar to those with 0.347% lysine combined with threonine and methionine (diet F), and slightly higher (but not to a statistically significant level) than those obtained with 0.28% lysine (diet K).

Analysis of variance (Table III) showed significant differences in the PER values (F-test, $P < 0.01$). The PER values of diets D, F, and K were not different from one another but differed significantly from the six other diets ($P < 0.01$). The PPV and PER values were highly correlated, and the results of statistical analyses of PPV and PER data were similar.

[1]According to the manufacturer, the FPC had the following amino acid composition (g/16 g of N): Lys, 9.0 (available, 8.6); threonine, 5.1; total sulphur amino acids, 4.9.
[2]According to the manufacturer, the green algae had the following composition (g/16 g of N): Lys, 4.6 (available, 4.4); threonine, 5.2; total sulphur amino acids, 3.59.
[3]L-lysine-hydrochloride was a commercial product containing 78% lysine.

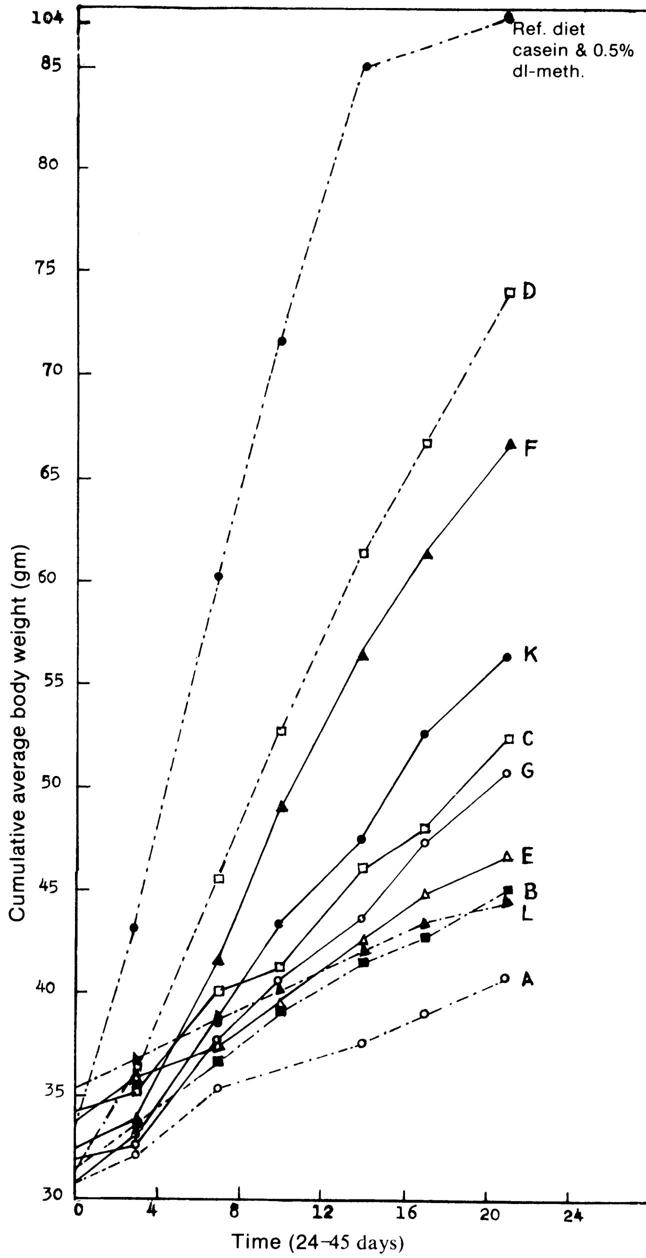

Fig. 1. Growth curves of rats fed diets based on bread prepared from formulas without fortification (A) or fortified with fish protein concentrate (B, C, D), pure algae (L), lysine (G, K), or a mixture of lysine, threonine, and methionine (E, F).

Figure 2 shows total amount of protein in the rat carcasses. With 1.9% FPC in bread (diet B), the total protein content of the carcass was similar to that obtained with either 0.14% lysine (diet C) or a mixture of 0.154% lysine with threonine and methionine (diet E). As is evident from Fig. 2, comparable amounts of carcass protein resulted from diets containing FPC or lysine.

The lysine intake in milligrams was computed from the lysine content of the diet and the food consumption of the rats, assuming 100% availability of lysine from the bread, as shown by Calhoun et al (1960). An equation for best fit line of dose-response relationship (gain in weight) was formulated by the least squares method. The data included in the equation were derived from 36 animals consuming diets with a lysine content ranging between 0.29% (diet A) and 0.92% (diet M). Figure 3 illustrates the linear relationship between gain in body weight and lysine intake. When the line was extrapolated arbitrarily to zero lysine intake, it intercepted the Y-axis at exactly zero weight gain. In other words, the body weight is maintained at zero lysine intake.

DISCUSSION

The nutritive value of the baladi bread (diet A) is slightly lower than values we reported earlier (Hussein et al 1973, 1974). The present data clearly show that bread formulas supplemented with 5.8% algae (diet L), 1.96 or 3.6% FPC (diets B and C, respectively), 0.138% lysine (diet G), or 0.154% lysine, 0.058% threonine, and 0.67% DL-methionine (diet E) did not improve the protein quality of the bread significantly. Supplementation with FPC or lysine at higher levels, however, led to PER and PPV values significantly higher than the respective values obtained with diet A ($P < 0.01$). This means that baladi bread should be supplemented with lysine at the level of 0.28% of the diet if significant improvements are to be detected.

Analysis of the bread indicates that part of the lysine added to bread in pure form (L-lysine-HCl) or in bound form (FPC) was destroyed during baking. The percent loss during baking ranged between 12 and 21%. This loss is higher than values reported by Stillings et al (1971). They reported only 3% lysine loss when FPC was the source of lysine in a bread formula and 9–16% lysine loss when pure lysine was the source.

Matthews et al (1969) tried to fortify chapatties, an Indian unleavened bread prepared from highly extracted wheat flour. They found a 25% average lysine loss. Similar results were obtained by Rosenberg and Rohdenburg (1951) and by Ericson et al (1961) for white bread. White bread is usually baked at an oven temperature of 250–300°C, in contrast to the high temperature necessary for baking baladi bread (400°C).

Diets E and F had mean PER values of 1.56±0.12 and 2.58±0.24; both were much lower than those obtained earlier (Hussein et al 1973). When the baladi bread was supplemented with the same amino acids at the same levels after baking, the respective PER values were 2.20 and 3.3.

The supplementary value of algae protein in wheat flour converted into baladi bread was very poor. It could not replace the high-quality proteins from fish or the pure amino acids. Poor palatability, as shown from the very low food intake, is the most undesirable characteristic of algae proteins. Differences in the protein quality among batches of algae seem to be the reason for conflicting results (Lee et al 1967, Müller-Wecker and Kofranyi 1973).

The utilizable protein of the nine different bread formulas is shown in Table III. This index is found by multiplying the percent relative nutritive value of a bread by the protein content in that bread (Hegsted 1968). The relative nutritive value is calculated as 100 times the ratio of the PER of the bread to the PER of the reference diet (casein and DL-methionine). This index ranks the bread formula with 6.56% FPC as the highest, with a utilizable protein of 13.1%. That value is about 21% higher than the utilizable protein (10.8%) found for the other significantly improved bread, that contains 0.347% lysine, 0.128% threonine, and 0.7% DL-methionine.

From an economic viewpoint, FPC is a good supplement because it adds protein as well as amino acids to bread. Another point to consider is that children have limited stomach capacity and therefore need high nutrient density in the diet.

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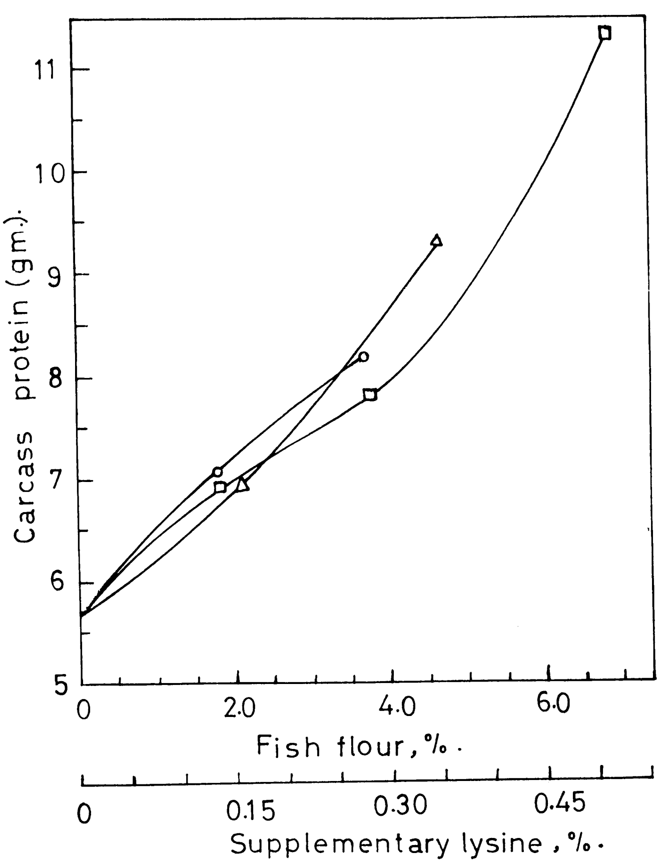

Fig. 2. Typical responses of body protein to fortification of bread with either fish flour (diet B, □—□); lysine (diet C, o—o); or a mixture of lysine, threonine, and methionine, (diet E, Δ—Δ).

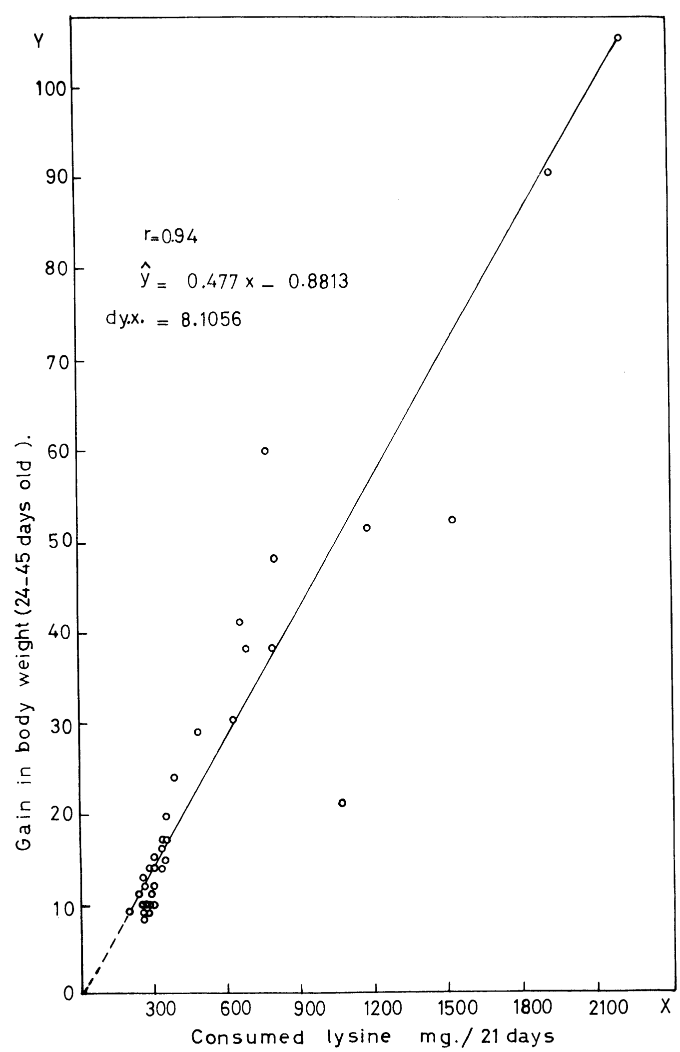

Fig. 3. Gain in body weight in relation to consumed lysine.

of Petroleum. The bread formulas were prepared and baked in the bakeries of the General Organization of Mills and Bakeries-Miet Okbah, Giza, by permission of El-Shehry.

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