AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

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1. SUBJECT CLASSIFICATION

A. PRIMARY: Public Health

B. SECONDARY: Nutrition

2. TITLE AND SUBTITLE

Nutritional value of chapatis with and without added lysine

3. AUTHOR(S)

Chaudhry, M. S.; MacMasters, M. M.; Hoover, W. J.

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1968	15 p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS

Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Kansas State University, Manhattan, Kansas 66502

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*

(In Studies on attas and chapatis, II, pp. 129-144)

9. ABSTRACT

This paper reports the methods and results of a study of the comparative nutritional value of various wheat flours (attas) and unleavened breads (chapatis) fed to rats during an eight-week trial period. Rats were fed attas of 80% and 95% extraction, and chapatis made from them, with and without vitamin and mineral supplementation, and with lysine at two levels of supplementation. Growth rates and protein efficiency ratios (PER) were evaluated weekly for eight weeks. The PER values were higher for chapatis than for the attas from which they were prepared. Vitamin and mineral supplements resulted in an increase in PER during the eight weeks. Diets with lysine added but no vitamins and minerals added resulted in improved PER during the first four weeks but a declining PER during the subsequent four weeks. Livers of rats fed lysine-supplemented diets had lower moisture content and higher protein content than those of rats on other diets. The results tend to show that fortification of cereal products with lysine, without concurrent fortification with vitamins and minerals, may not be of value when the products form essentially the only article of diet, as is often true in developing countries.

10. CONTROL NUMBER

PN-AAB-873

11. PRICE OF DOCUMENT

12. DESCRIPTORS

Additives
Chapatis?
Flours
Lysine
Nutritive value
Wheat

13. PROJECT NUMBER

14. CONTRACT NUMBER

CSD-1586 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

Studies on Attas and Chapatis II

Nutritional Value of Chapatis With and Without Added Lysine

M. Shafiq Chaudhry[1/], M. M. MacMasters, and W. J. Hoover

Summary

Rats were fed ground whole wheat, attas of 80% and 95% extraction and chapatis made from them with and without supplementation with vitamins and minerals and with lysine at two levels of supplementation but without supplementary vitamins and minerals. Growth rates and protein efficiency ratios (PER) were evaluated weekly over an eight week period. The PER values were higher for chapatis than for attas from which they were prepared. Supplementation with vitamins and minerals resulted in an increase in PER during the 8 week feeding trial. A similar improved PER resulted during the first four weeks on diets with added lysine but without added vitamins and minerals, but an adverse effect on PER during the second four week period was found using this diet. Livers of rats fed lysine-supplemented diets had lower moisture content and higher protein content than those of the rats on other diets. Fortification of cereal products with lysine in the absence of adequate concurrent fortification with vitamins and minerals may not be of value when the products form essentially the only article of diet as is often true in developing countries.

1/ Contribution No. _____. Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Kansas State University, Manhattan, Kansas 66502.

This paper is taken from a portion of the dissertation presented by M. Shafiq Chaudhry in partial fulfillment of the requirements for the Ph.D. degree at Kansas State University. Mr. Chaudhry's present address is Department of Food Science, West Pakistan Agricultural University, Lyallpur.

Improving the nutritional status of developing nations, most of which produce insufficient protein for the national need, is currently of much interest. Often a cereal food constitutes the major portion of the diet of most of the people and may even be essentially their sole source of nutrition. In West Pakistan and a large portion of India, unleavened bread, known as "chapatis", holds that position. Improvement of the nutritional status of the people in those areas will be most easily and acceptably accomplished by improvement of the nutritional value of chapatis.

Flour enrichment programs in the United States, Newfoundland, the Philippines and elsewhere have established the beneficial nutritional effects of vitamin and mineral supplementation of basic cereal foods. There have been very few studies made of the nutritional value of chapatis. The Protein Efficiency Ratio (PER) of chapatis was reported by Shayamala and Kennedy (1962) to be about 20% higher than that of unheated flour, and replacement of 10% flour with soy flour or dry milk solids was found to further increase the PER. Imtiaz (1962) reported that chapatis prepared from whole-wheat pastry flour with the addition of 15% medium fat soy flour and 10% dry skimmed milk supported excellent growth of rats.

In areas where chapatis form the staple food, it has been felt that the limiting amino acid in the diet is lysine, since the lysine content of wheat protein is known to be the limiting factor in that protein for humans. It is likely that vitamins and minerals are not at optimal levels in diets based largely on chapatis. The losses of thiamin that occur during milling of wheat to the coarse flour, called "atta", from which chapatis are made, was studied by Singh _et al_. according to Aziz and Bhatti (1962). Losses of 20% to over 50% of thiamine were reported, the loss being dependent upon the type of milling.

The present study was undertaken to explore the possibility of improving the nutritional value of chapatis by the fortification of atta with lysine and some vitamins and minerals.

Materials and Methods

Gaines variety White Club wheat was milled experimentally to produce two attas of 80% and 95% extraction respectively; the wheat and the milling procedure were described by Chaudhry *et al.* (1968).

Chapatis were prepared in batches, each from 1000 grams of atta (d.b.) to which 70-75% distilled water was added to produce a dough of the proper consistency. The dough was kneaded by hand, divided into balls weighing 50 grams each, and each ball shaped with a rolling pin into a chapati of 6- to 7-inch diameter. Each chapati was cooked on an ungreased hot plate (290° - 300°C.) for approximately two minutes.

Cooked chapatis were air-dried in the laboratory (65° - 75°F.) for 48-72 hours, then ground in a Wiley Hammer Mill No. 1 to pass through a 1-m sieve.

Diets containing the two attas and whole ground wheat, as well as the cooked, dried and ground chapatis, were prepared and fed to weanling male rats, as shown in Table I. Each prepared diet was analyzed for moisture content, and 2% sodium chloride and 5% refined cottonseed oil (d.b.) were added to each. The moisture content of each diet was then adjusted to 15% by the addition of distilled water. Vitamin and mineral supplementation levels were based upon multiple increments of the amounts available in the original grain, rather than upon known dietary requirements of the test animals.

Male weanling rats (Sprague-Dawley strain) were used in the studies They were fed a stock diet for one day before being housed individually.

Five randomly selected rats were maintained on each diet. Initial weight was taken after the animals had been on the diets for 5 days and weekly thereafter, for a total of 8 weeks. Food and water were provided ad libitum. At the end of 8 weeks, the rats were sacrificed and the liver of each was removed for analysis for moisture, crude fat and crude protein.

Whole wheat, attas and chapatis were analyzed for moisture, crude fat, nitrogen, ash, crude fiber, thiamine, niacin, riboflavin, calcium, and iron contents by methods 44-15, 30-20, 46-10, 08-10, 32-15, 86-80, 86-51, 86-70, 40-20, and 40-41 respectively, in Cereal Laboratory Methods (1962). The factor 5.7 was used to convert nitrogen to crude protein value. Lactobacillus plantarum NRRL B-531 was the organism used in determining niacin.

Each liver was wrapped in aluminum foil and frozen. The frozen liver was sliced rapidly, and appropriate amounts weighed for analysis. Moisture was determined by the vacuum oven method (100°C., 5 hrs.); dried samples were extracted for 8 hours with ethyl ether (high heat, Goldfish extractor) for crude fat determination. The Kjeldahl method was used to determine nitrogen.

Data on weight gain and on protein efficiency were analyzed by two way classification analysis of variance, Fryer (1966). Duncan's New Multiple Range Test, as outlined by Fryer (1966) was used to determine the significance of differences among means of percentage gain in weight and protein efficiency ratio.

Results and Discussion

Chemical analyses of the wheat, attas and chapatis are shown in Table II, where each value is an average of 4 to 6 replications. No significant difference in vitamin contents was found between the attas of 80% and 95% extraction. The three vitamins that were determined decrease

in amount during cooking of the chapatis, but the difference was not significant when atta of one extraction rate was compared with chapatis made from it. With the exception of minerals, atta of 80% extraction and chapatis prepared from it showed higher contents of nutrients than atta of 95% extraction and chapatis prepared from it.

Average cumulative weight gain curves are shown in Figs. 1, 2, and 3 Atta of 95% extraction (Diet III) promoted significantly better growth than that of 80% extraction (Diet II) and than whole wheat (Diet I) during the first week, otherwise the three diets yielded no significant differences, (Figs. 1 and 2).

Feeding studies to determine the nutritive value of vitamins and minerals added in making chapatis from atta of 80% extraction showed that supplementation at levels of 50% (Diet VIII) or 100% (Diet IX) above the level in the original wheat (Diet I) gave a significant improvement (Fig. 2). Similar results were obtained with chapatis made from atta of 95% extraction in which the vitamins and minerals were added to make them equal in the atta to the amounts present in the original whole wheat (Diet VII). Comparison of the data suggested that rats fed diets based on atta of 80% extraction (Diet IV) and chapatis made from it performed better than those fed diets based on atta of 95% extraction (Diet III) and chapatis made from it. The differences were, however, not statistically significant. Hepburn et al. (1960) found less than half as high a concentration of lysine in the best patent flour than in germ. Removal of 15% fines during production of atta of 80% extraction would therefore, mean removal of 15% lysine-poor material, with the result that the amino acid balance of the atta would be improved. The chapatis made from atta of 80% extraction also contained more vitamins than those made from atta of 95% extraction.

Supplementation with lysine led to unexpected results (Fig. 3). Addition of lysine at 0.2% (Diet X) and 0.4% (Diet XI) the weight of the atta significantly improved the performance of the test animals during the first four weeks, but caused a decline in the growth rate during the subsequent four weeks. Such an effect has not been reported in any of the numerous studies on lysine supplementations of foods. Rosenberg and Rohdenberg (1952) found significantly improved nutritional value to result from addition of lysine at 0.2% to 0.8% levels to a diet of which 90% was air dried bread; those workers considered 0.2% to 0.4% to be about the optimal level for lysine supplementation, and other workers have come to similar conclusions.

Lack of fortification with vitamins and minerals of the diets to which lysine was added might have caused the observed results. Fortification of wheat products with lysine to provide better nutrition for developing nations has generally been recommended. Apparently this is a promising procedure when the diet of the people contains other sources of vitamins, minerals and even small amounts of methionine. In economically poor areas of Pakistan and India, chapatis often form the sole article of food consumed over long periods of time. Little fruit, vegetables, fats or oils, meats or fish are eaten with the chapatis in such areas. In view of the results of the present study, it appears that atta supplementation with lysine is of questionable value unless adequate enrichment with vitamins, minerals and other amino acids in marginal supply is also practiced.

Data showing consumption of feed and of protein, gain in weight and PER are given in Table III, and data on analysis of variance are

shown in Table IV. Differences in PER among the diets in both four week periods were tested for significance by Duncan's NMRT. During the first four weeks, Diet XI (0.4% lysine supplemented) gave the highest PER, but that diet and Diet X (supplemented with lysine at 0.2% level) gave the lowest PER values during the second four week period. PER values were higher for chapatis than for the attas from which they were prepared. This may be due to nutrient availability rather than protein quality. Shayamala and Kennedy (1962) attributed a similar difference found in their studies to the destruction of Trypsin inhibitor during baking. Two other possible factors may be involved. First, Parihar and Chatterji (1956) determined by X-ray diffraction studies that starch is gelatinized during the baking of chapatis. The starch would therefore be more susceptible to the action of digestive enzymes. Second, although no information is available on the fate of phytin during the baking of chapatis, Kent (1966) states that phytin is hydrolyzed during the baking of bread. If hydrolysis occurs as chapatis are baked, phosphorus would be freed, and there would be less probable formation of complexes of calcium and iron with phytin.

During the first four weeks, supplementation with vitamins, minerals and lysine resulted in an increase of the PER and the increased value continued during the second four weeks, except in the cases of supplementation with lysine.

Data obtained on the livers at the end of the feeding experiment indicated that the supplementation of the diet with lysine increased protein content and decreased moisture content of the liver. No consistent effects were obtained as the result of rate of extraction of atta, baking or supplementation with vitamins and minerals.

(Acknowledgments)

The authors are grateful to the Northern Regional Research Laboratory, Peoria, Illinois, for the culture of *Lactobacillus plantarum*. The senior author appreciates the support of AID during the course of the study, and the provision by Kansas State University of all supplies and equipment used.

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TABLE 1

Composition of diets

(2% sodium chloride and 5% fat not shown in this table, were added to diets before feeding to rats)

Diet No.	Code	Description	The amount of various nutrients added (expressed as mg./100 g.)					
			Thiamine HCl	Niacin	Riboflavin	Calcium Carbonate	$Fe\ SO_4 \cdot 7H_2O$	Lysine HCl
I	WW	Whole ground wheat	-	-	-	-	-	-
II	AT-80	Atta, 80% extraction	-	-	-	-	-	-
III	AT-95	Atta, 95% extraction	-	-	-	-	-	-
IV	CH-80	Chapati from atta of 80% extraction	-	-	-	-	-	-
V	CH-95	Chapati from atta of 95% extraction	-	-	-	-	-	-
VI	CH-80 IX	Chapati from atta of 80% extraction	0.008	1.25	0.031	6.71	-	-
VII	CH-95 IX	Chapati from atta of 95% extraction	0.188	1.41	0.046	-	-	-
VIII	CH-80 1.5X	Chapati from atta of 80% extraction	0.368	2.35	0.056	93.64	11.67	-
IX	CH-80 2X	Chapati from atta of 80% extraction	0.735	4.70	0.112	187.28	23.34	-
X	CH-80 2L	Chapati from atta of 80% extraction	-	-	-	-	-	200
XI	CH-80 4L	Chapati from atta of 80% extraction	-	-	-	-	-	400

TABLE II

Analysis of wheat, attas and chapatis
(moisture free basis)

Sample	Moisture %	Crude protein %	Crude fat %	Crude fiber %	Ash %	Iron mg%	Calcium mg%	Niacin mg%	Thiamin mg%	Ribo-flavin
Whole wheat	9.97	12.22	1.70	2.62	1.713	4.89	58.4	4.70	0.733	0.112
Atta of 80% extraction	12.39	12.16	2.04	1.95	1.611	4.96	56.3	4.28	0.728	0.096
Atta of 95% extraction	12.57	12.07	1.81	1.72	1.482	5.07	53.9	3.78	0.680	0.074
Chapatis from atta of 80% extraction	7.28	12.15	1.21	2.02	1.643	5.77	56.5	3.58	0.728	0.081
Chapatis from atta of 95% extraction	6.54	12.09	1.00	1.79	1.491	7.52	65.1	3.42	0.548	0.066
LSD 0.01 level		n.s.	0.200	0.281	0.036	0.177	3.89	1.087	0.203	0.0199

TABLE III

Average amount of feed consumed, protein consumed, gain in weight and protein efficiency ratio (P.E.R. of different diets for two periods of four weeks each.

Diet No.	First four week period				Second four week period			
	Feed consumed (g.)	Protein consumed (g.)	Gain in weight (g.)	P.E.R.	Feed consumed (g.)	Protein consumed (g.)	Gain in weight (g.)	P.E.R.
I	225.4	21.66	22.20	1.031	201.2	19.34	13.20	0.680
II	205.0	20.40	22.60	1.107	200.2	19.92	18.80	0.797
III	235.2	22.44	22.20	1.011	198.6	18.95	16.40	0.873
IV	201.8	19.84	26.80	1.348	191.4	18.81	22.00	1.172
V	185.6	17.76	23.00	1.290	176.2	16.86	12.80	0.786
VI	217.2	21.52	27.20	1.266	197.8	19.60	22.60	1.156
VII	185.0	17.95	23.80	1.311	193.8	18.18	17.80	0.968
VIII	216.2	21.31	32.00	1.494	201.2	19.84	22.20	1.105
IX	261.2	25.62	19.20	1.535	272.8	26.76	35.00	1.317
X	200.2	20.00	16.40	1.724	190.0	18.98	11.00	0.544
XI	191.6	19.35	15.20	1.821	225.2	22.74	8.8	0.410

TABLE IV

Analysis of variance for protein efficiency ratio of various diets during two growth periods of four weeks each.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Periods	1	5.54268	5.5427	09.76**
Diets	10	2.6688	0.2669	5.285**
Periods X Diets	10	4.78756	0.4788	9.481**
Error	88	4.44127	0.0505	
Total	109	17.44031		

** significant at 0.01 level.

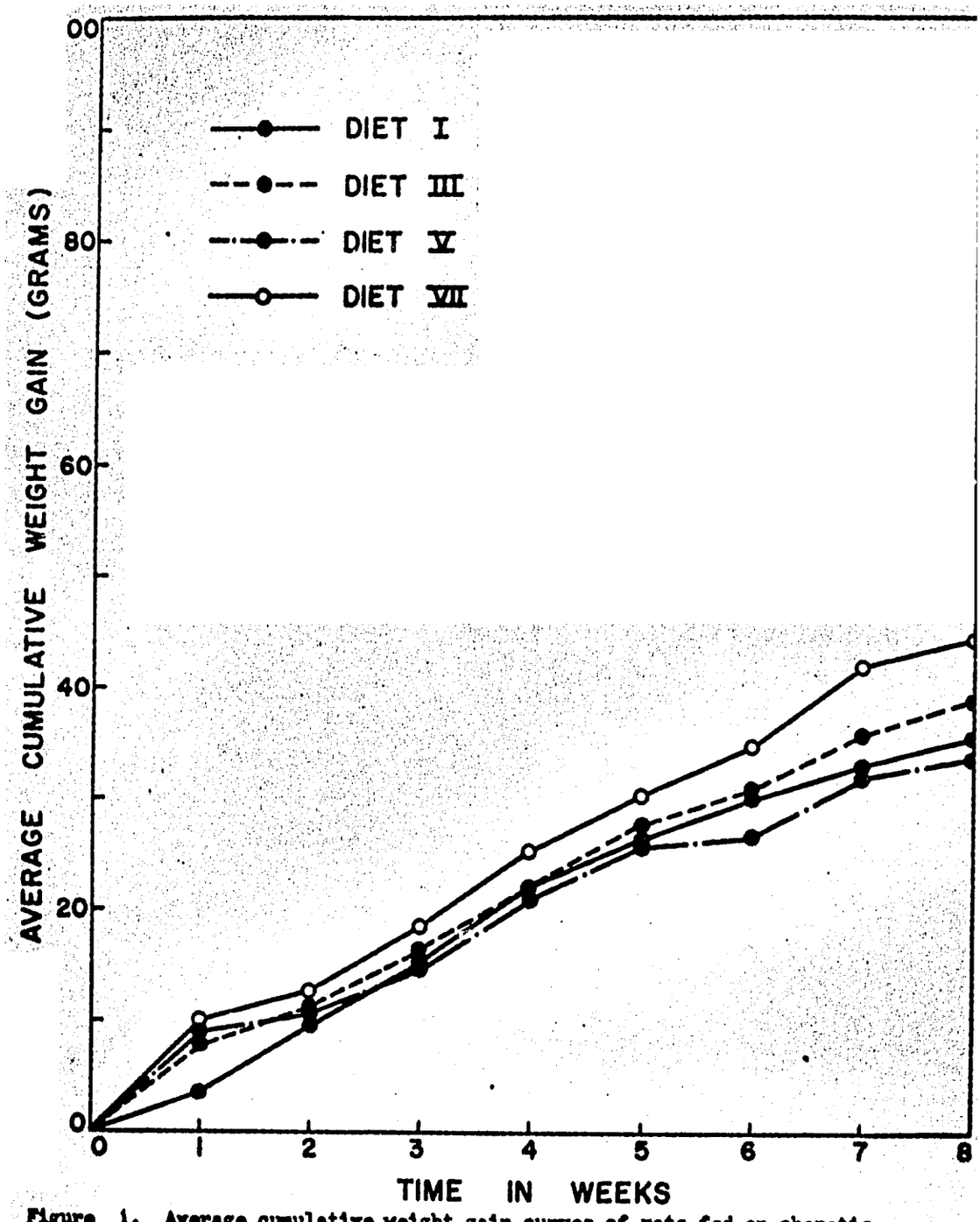

Figure 1. Average cumulative weight gain curves of rats fed on chapatis prepared from 95% extraction atta and supplemented with vitamins and minerals.

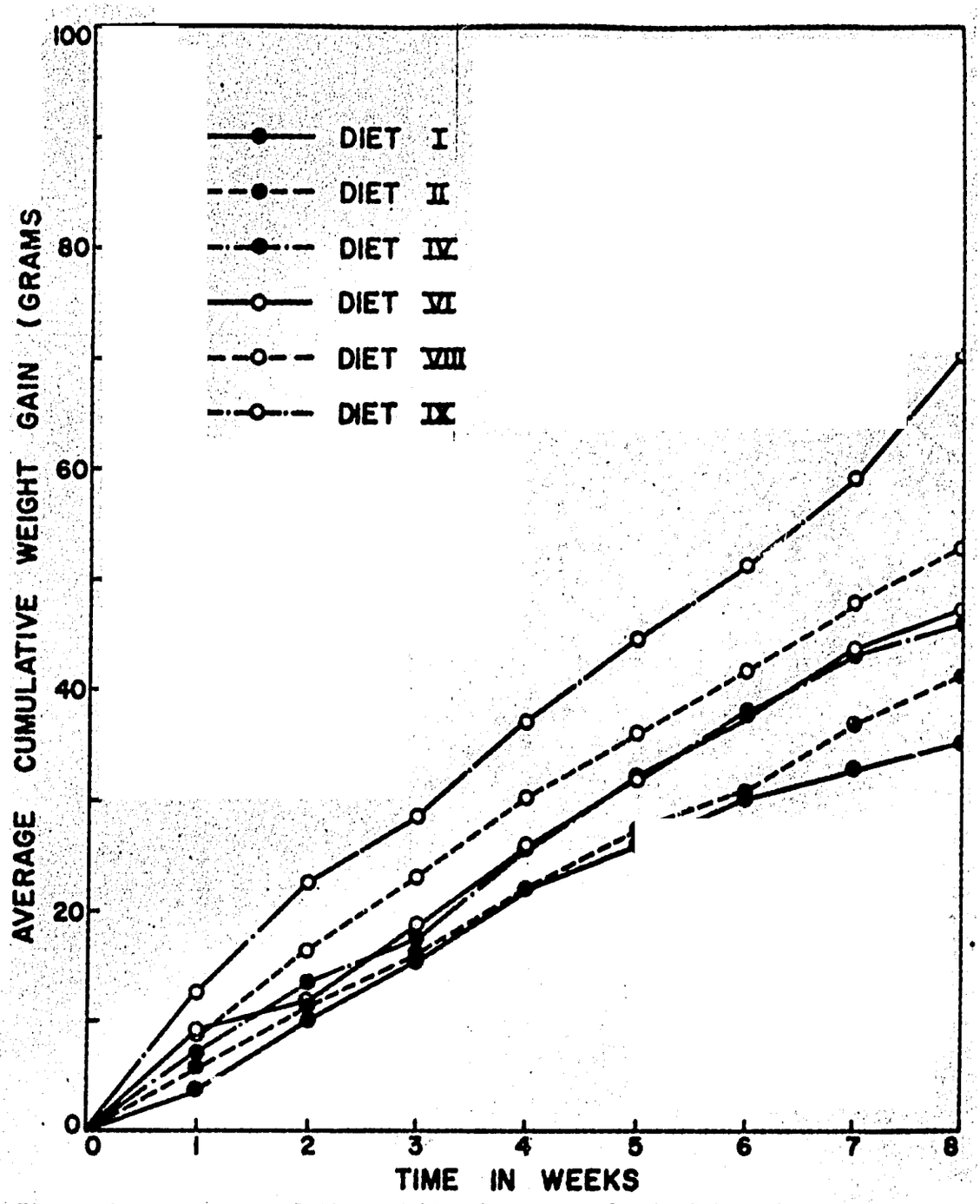

Figure 2. Average cumulative weight gain curves of rats fed on chapatis prepared from 80% extraction atta and supplemented with vitamins and minerals.

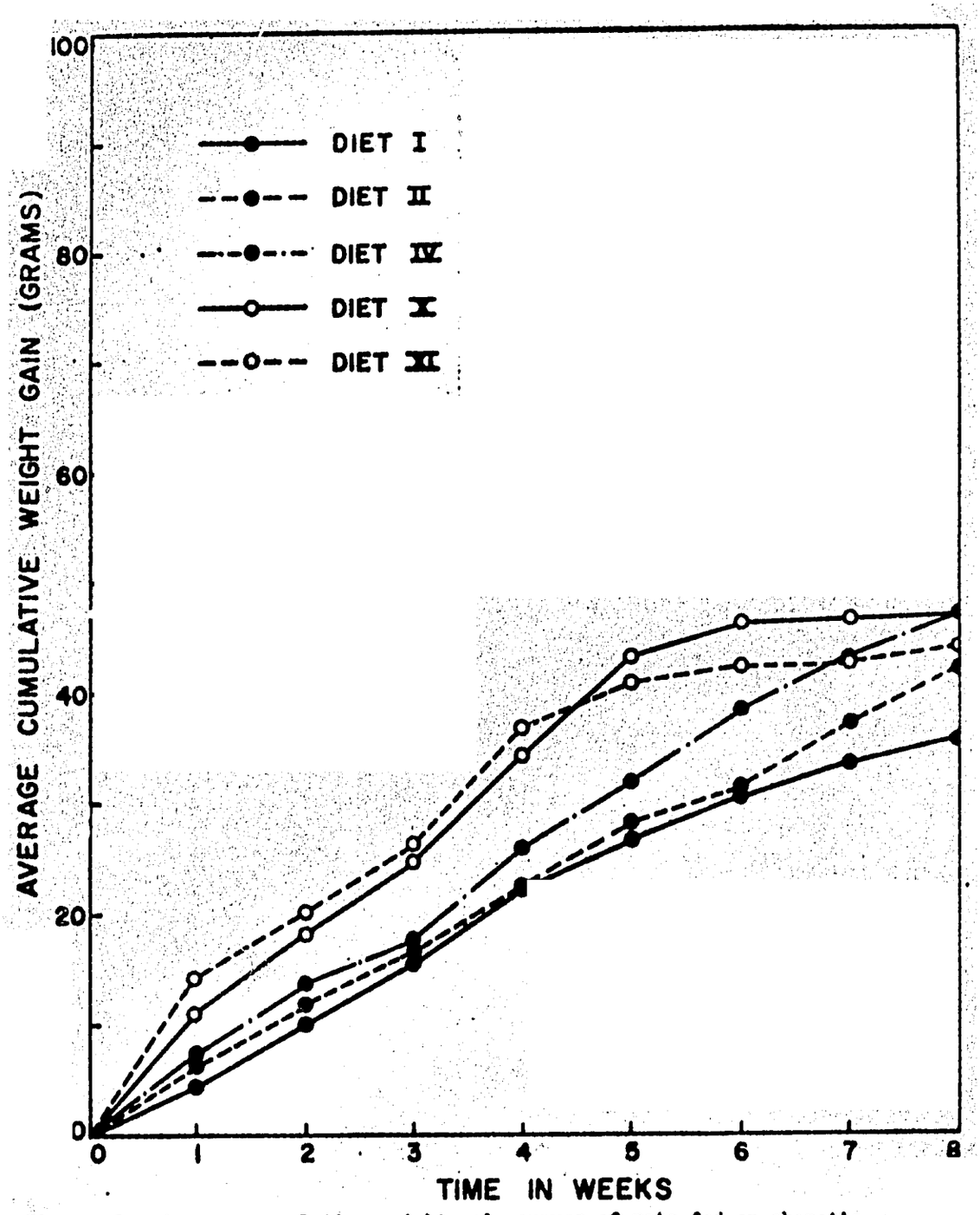

Figure 3. Average cumulative weight gain curves of rats fed on chapatis prepared from 80% extraction atta and supplemented with lysine.