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Lysine-Supplemented Wheat and Growth of Preschool Children[1,2]

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CEREALS form the major source of protein and calories in the food eaten by the mass of people in many of the developing countries of the world (1). In most of these countries, the chances of improving the quality of protein intake by the conventional means of increased dairy and poultry production are remote. The availability of proteins from oilseeds and other vegetable sources is currently being explored and the results encourage a cautious optimism (2).

However, the rapidly increasing populations of these countries make it urgent that all means of improving protein quality are utilized. In diets based mainly on grain, the low content of amino acids like lysine, threonine, and methionine is responsible for the poor quality of the protein. Supplementation of grains that lack amino acids in the natural state has improved the quality of cereal proteins as judged by the growth of weanling rats (3). This method has its limitation and applies mainly to those sections of population resident in cities or those consuming cereal products that are centrally processed. However, the numbers of people resident in towns or within reach of such a program run into millions and make its implementation well worthwhile.

The preschool child in emerging countries is offered a high cereal weanling diet, and, as a result, runs the risk of developing protein malnutrition. Improvement of the quality of cereal protein would, theoretically, result in the improved growth and nutritional status of this vulnerable section of the population.

The following studies were undertaken to assess the effect of lysine supplementation of wheat on the growth of preschool children.

MATERIALS AND METHODS

Subjects

Fifty-two children, aged 2–5 years and resident in an orphanage, took part in the study. All subjects were in apparent good health and were maintained on a diet that provided 2 g vegetable protein and 100 kcal/kg body wt a day for a preliminary period of 3 months. At the start of the feeding trial, the children were nutritionally assessed and divided into two groups (the experimental and control groups) on the basis of height, weight, age, sex, and rate of growth during the preceding 3 months.

Blood was drawn for estimation of hemoglobin and serum proteins at the start and again at the end of the trial which lasted for 6 months.

Records of minor illnesses among the children were maintained.

Biochemical Methods

Three-day nitrogen balances were carried out on 24 children (12 statistical pairs) from the control and experimental groups during the latter half of the trial. Each day's collection was analyzed separately. Urinary and fecal nitrogen were determined by the micro-Kjeldahl method. Creatinine was estimated by Jaffe's reaction.

Every week, on a day randomly selected, the cooked food consumed by a child from each

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group was taken for the estimation of lysine. The homogenized foods were stored in the frozen state until analyzed.

At the end of the trial, 48 samples, each representing a day's intake of cooked food from the experimental and control groups, had been collected (24 samples from each group).

Twelve pairs of the 24 paired food samples from both groups were analyzed for lysine content.

Ten-gram aliquots of each sample were weighed into ampules and 60 ml of 3 N HCl was added. The ampules were sealed and hydrolyzed for 16 hr at 121 C. The contents were cooled and filtered through a medium-porosity sintered glass funnel with vacuum and the filtrate transferred to a 100-ml volumetric flask. Hydrolysates representing the food samples of the experimental group of 3 randomly selected days were pooled and analyzed for lysine content. The corresponding samples of hydrolysate for the control group were similarly mixed and analyzed. Two-milliliter aliquots were pipetted into 25-ml beakers and evaporated on a water bath. The residue was taken up in water and evaporated again to remove excess acid. This process was repeated twice more. The amino acids were taken up in 2 ml of citrate buffer, pH 2.2, and passed through a small column containing activated charcoal. The eluate was collected into 2-ml volumetric flasks. Lysine estimation was carried out on a 0.5-ml aliquot by running the samples through a Beckman amino acid analyzer (model 120 B) using 0.38 N citrate buffer, pH 5.28. The resin used was PA-28.

Hemoglobin was estimated by the oxyhemoglobin method using a spectrophotometer calibrated and checked at regular intervals with a hemoglobin standard.

Total serum proteins were estimated by the microbiuret method (4) and the protein fractions by paper electrophoresis at pH 8.6 followed by staining with bromphenol blue and elution of the dye with 0.01 N sodium hydroxide.

Diet

The orphanage diet provided 2 g vegetable protein and 100 kcal/kg body wt per day. This diet was maintained throughout the 6 months feeding trial.

Each child's portion of the cooked food was weighed out at each meal. Plate wastage, records of which were maintained, was negligible throughout.

The children in the experimental and control groups ate the same diet; the only difference between the two groups was that the children in the experimental group were given lysine-supplemented wheat. The wheat flour used was supplemented at 0.25% level with lysine,[7] and broken wheat used in some dishes was mixed with lysine-impregnated wheat grains[8] to provide supplementation at 0.1% level (5).

The wheat used in the diets of the experimental and control groups came from the same lot and provided 54% of daily calories and 85% of the daily protein. Animal foods were excluded entirely.

The wheat flour and the broken wheat grains were either boiled, roasted, or fried, as baked foods are alien to South Indian cooking.

For the first 3 months of the trial, the lysine-fortified wheat was colored with turmeric to distinguish the cooked foods from those made from the unsupplemented wheat. For the latter 3 months, turmeric was used to color the unsupplemented wheat given to the control group.

RESULTS

The composition of the diet and the average daily consumption of nutrients and lysine of the children in the experimental and control groups are given in Tables I and II.

The cereal eaten by the children in the control group provided a daily intake of 0.68 g lysine, as calculated from standard food tables (6). The supplemented wheat provided the children in the experimental group with an additional 0.37 g/day (total, 1.05 g).

From analyses of samples of cooked foods, the children in the experimental group had an intake of 0.73 g lysine/day and those in the control group, 0.54 g/day. The losses of lysine in cooking were 30% in the experimental group and 21% in the control group.

[7] *l*-Lysine monohydrochloride.

[8] Kindly supplied by the Director, Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

TABLE I

Composition of diet and intake of nutrients in grams per child per day

Wheat flour	127.5
Broken wheat	52.5
Pulses (*Phaseolus mungo*)	8.0
Peanut oil	41.0
Root vegetables	25.0
Vegetables	59.0
Fruit	25.0
Brown sugar	22.5
Spices	10.0
Protein	25.5
Fat	42.7
Calories	1,140

TABLE II

Lysine content of the experimental and control diets

	Lysine, per child per day, g	
	Experi-mental	Control
As calculated from raw foodstuffs		
Wheat	0.85	0.48
Pulses	0.15	0.15
Other sources	0.05	0.05
Total intake	1.05	0.68
As estimated in cooked foods		
Average of 12 days' samples	0.73	0.54
Percentage losses in cooking	30	21

Two children from the experimental group and four from the control group were excluded from the study because of illness that necessitated hospitalization for more than 15 days or because they left the orphanage. The results, therefore, pertain to the 24 children (10 boys and 14 girls) in the experimental group and the 22 children (9 boys and 13 girls) in the control group who completed the study.

The average increase in weight of the children in both groups is shown in Fig. 1 and the average increase in height in Fig. 2 and Table III.

At the end of 6 months of feeding, the children on the lysine-supplemented wheat had grown significantly in height compared to the children in the control group ($P < 0.05$). Statistically significant differences in increases in weight were not observed between the two groups of children. The children in both groups lost weight during the last month of the trial; April and May are the hottest months of the year.

At the start of the trial, three children in the control group and two in the experimental group had angular stomatitis. At the end of the study, all had healed and new cases were not observed.

During the trial there were 18 episodes of diarrhea among the children in the experimental group compared with 12 episodes among the children in the control group. In other respects, there was no significant difference in the pattern of minor illnesses between the two groups.

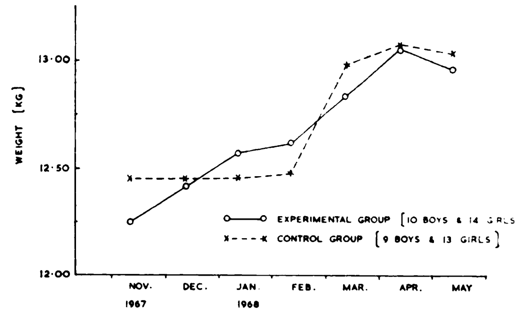

FIG. 1. Average weights of children in the experimental and control groups.

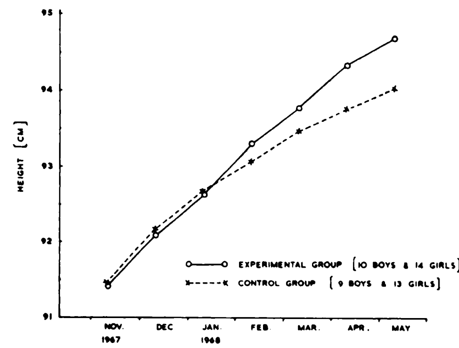

FIG. 2. Average heights of children in the experimental and control groups.

The hemoglobin, packed cell volume, total serum proteins, and serum albumin were estimated in 21 children in the control group and in 20 children in the experimental group at the beginning and end of the study. The data are presented in Table IV. Statistically significant differences were not observed between the two groups.

Data on the 3-day nitrogen balances carried out on 12 statistical pairs of children from both groups are presented in Table V. Retention of absorbed nitrogen in the children in the control and experimental groups was similar. The daily creatinine excretion of both groups of children was the same. The children in the experimental

TABLE III

Averages of the heights and weights of the children in the experimental and control groups (mean ± 1 SD)

Subjects	Height, cm		Weight, kg	
	Initial	Increase	Initial	Increase
Experimental group (10 boys and 14 girls)	91.4 ± 8.2	3.26 ± 1.19	12.25 ± 2.23	0.75 ± 0.68
Control group (9 boys and 13 girls)	91.4 ± 8.0	2.61 ± 0.99	12.45 ± 2.06	0.58 ± 0.81

Differences in the increases in height between the two groups were statistically significant ($P < 0.05$)

TABLE IV

Initial levels and changes during the experiment of total serum proteins, albumin, hemoglobin and packed cell volume (mean ± 1 SD)

	Protein, g/100 ml	Albumin, g/100 ml	Hemoglobin, g/100 ml	PCV, %
Experimental group (9 boys and 11 girls)				
Initial level	6.95 ± 0.64	3.55 ± 0.46	10.2 ± 1.18	33.6 ± 2.5
Change	−0.56 ± 0.55	0.22 ± 0.53	0.03 ± 0.86	0.32 ± 2.79
Control group (9 boys and 12 girls)				
Initial level	7.29 ± 0.78	3.98 ± 0.63	10.4 ± 1.1	34.0 ± 2.8
Change	−1.03 ± 0.90	−0.23 ± 0.69	0.20 ± 1.44	1.02 ± 2.99

Differences between the two groups were not statistically significant.

TABLE V

Results of nitrogen-balance studies (mean ± 1 SD)

	Intake of N, g	Creatinine		Nitrogen, g/day		% Retention of		Urinary N/ creatinine	Absorption, %
		mg/24 hr	mg/kg body wt	Urinary	Fecal	Intake	Absorbed N[a]		
Experimental group (12)	4.07	135±32	10.5±2.5	1.78±0.34	1.51±0.21	18.4±9.8	28.4±13.8	13.4±2.1	62±6
Control group (12)	4.06	132±20	9.7±1.5	1.65±0.25	1.44±0.29	22.5±8.0	33.4±8.6	12.5±2.3	64±7

Differences between the two groups were not of statistical significance (t test).
Numbers in parentheses are numbers in the group. [a] Difference between intake and fecal excretion.

group excreted slightly more creatinine per kilogram body weight compared with the children in the control group; the differences were of no statistical significance. The balances were carried out during the latter half of the feeding trial, which happened to be the hottest time of year.

DISCUSSION

The feeding trial was designed to study the effect of lysine-supplemented wheat on the growth of preschool children and to compare it with the effect of unsupplemented wheat. For that reason, the diets were so composed that the cereal formed the main source of protein and, therefore, of lysine; the lysine contributed by the other foodstuffs was negligible. Over a period of 6 months observation, the children on the lysine-supplemented wheat grew significantly in height compared to the controls.

One of the contentions against the supplementation of cereals in Indian diets, is that, pulses are invariably eaten and that the lysine from this source makes up for its deficiency in the cereal.

In two recent diet surveys, the actual average consumption of pulses by preschool children was 15 g a day in an urban area (unpublished data) and 6 g daily in the rural population (7). The children in the orphanage were each given 8 g of pulses a day. The quantity eaten by the children in the orphanage was not very different from the amounts eaten by the children surveyed. Lysine-supplemented cereal in the orphanage diet induced better growth than in the control group and apparently would have the same effect if added to the diets of children in this geographic area.

There is a difference between the orphanage diet and the diets eaten by the majority of preschool children in South India. The cereal used in the orphanage was wheat while that eaten by the local population is rice.

The differences in cereal are not relevant when their lysine contents are studied. Wheat contains 0.14 g lysine/g of nitrogen whereas rice contains 0.23 g lysine/g nitrogen (6). However, wheat has a protein content very nearly double that of rice. The lysine content of both is comparable per 100 g of cereal.

An increase in nitrogen retention in children has been reported on the addition of lysine to basal diets (8, 9). In the reported studies, the children served as their own controls and had increased retention of nitrogen on the lysine-supplemented diet compared with their nitrogen retention on the basal diets. In the present study, the children did not serve as their own controls. The nitrogen retention of children on the lysine-supplemented diet was compared to that of children on the control diet. Statistically significant differences in retention were not observed. The nitrogen balances were carried out during the hottest months of the year, at a time when 11 of the 24 children studied were losing weight. A loss in weight during the hot season has been noted in previous trials (unpublished data) and may have contributed to the equivocal results obtained.

Previous workers (10) were unable to prove conclusively that a supplement of lysine-enriched bread to the diets of Haitian schoolchildren resulted in improved growth compared with the controls. The lysine-enriched bread provided about one-third of the estimated daily intake of protein and about a quarter of the daily calories. The proportion of calories and protein derived from the bread was small. In addition, the study was conducted in schoolchildren whose growth is not as rapid as that of preschoolers.

In the study reported here, the supplemented cereal formed the major source of calories and proteins and the effect was observed in young children; the growth in height of the children on the lysine-supplemented wheat was unequivocally greater than that of the children who served as controls.

SUMMARY

The effect of supplementing wheat with lysine was studied in preschool children resident in an orphanage.

There was a statistically significant increase in height ($P < 0.05$) in the children fed with lysine-supplemented wheat compared with the children who served as controls on unsupplemented wheat.

Significant differences in hemoglobin, packed cell volume, total serum protein, serum albumin, and nitrogen retention were not observed between the two groups.

The value of lysine supplementation of diets composed largely of cereal is discussed.

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