Pakistan J. Sci. Ind. Res., Vol. 30, No. 12, December 1987

NITROGEN RETENTION BY ADULT HUMANS ON MAIZE BREAD SUPPLEMENTED WITH PEANUT, CHICKPEA AND PEANUT-CHICKPEA FLOURS

Jehangir Khan Khalil* and M.I.D. Chughtai

Division of Biochemistry, Institute of Chemistry, Punjab University, Lahore

(Received November 2, 1985; revised December 3, 1987)

The nitrogen balance of six adult human subjects fed on maize bread alone and supplemented with 20 % each of peanut flour, chickpea flour and peanut-chickpea flour (1 : 1) was studied. The diets were isonitrogenous (10 g N/day) and isocaloric (2900 kcal/day). Maize bread in each diet provided 70 % of the protein intake and the rest was derived from other fruits and vegetable sources (20 %) and milk (10 %). The results showed that the average nitrogen balance of subjects fed unsupplemented maize bread (+ 0.13 g/day) was significantly ($P < 0.05$) improved with all the supplemented breads (plus 0.34 – 0.39 g/day). It was concluded that fortification of maize bread with the above mentioned protein sources can enhance the nutritive quality of maize bread for adult human subjects.

Key words: Nitrogen balance, *Zea mays* L., Protein content of maize bread.

INTRODUCTION

Maize or corn (*Zea mays* L.) is a cereal of regional economic importance in the North West Frontier Province of Pakistan (NWFP) where approximately half of the total maize production of the country is harvested [1]. It is a dietary staple for most people in the rural areas of the NWFP. The kernels of maize are low in protein quantity and the quality of protein is limited by its low content of lysine and tryptophan [2,3].

In a previous communication, we showed that the protein content of maize flour (MF) blended with 10–30 % peanut flour (PNF) obtained by direct solvent extraction increased by 30–91 % with a substantial increase in the lysine and tryptophan content [4]. The amount of these limiting aminoacids increased further when a mixture (1 : 1) of PNF and chickpea flour (CPF) was used as a supplement at 10–30 % replacement level [5]. The supplemented flour showed higher nutritional quality with rat bioassays. The choice for PNF and CPF was based on their local production and availability. Since the amino-acid requirement of man differs from those of laboratory animals, the results obtained with laboratory animals cannot be strictly applied to human beings. Here food products intended for human consumption should be evaluated with human subjects. In a previous paper [6], we showed that a significant increase in the nitrogen balance of six human subjects when maize bread was supplemented with 10 % PNF or 10 % of a mixture of PNF + CPF (1 : 1) had occurred. Even though statistically significant, the increase in nitrogen balance was quantitatively not large. In the present investigation, the nitrogen retention of six adult male human subjects was studied when fed maize bread alone or supplemented with a 20 % supplementation level of PNF, CPF or a mixture of PNF + CPF (1 + 1) termed PCF.

MATERIALS AND METHODS

Preparation of samples. Samples of maize, peanut and chickpea were purchased from the local market. Procedures for the preparation of whole maize flour (MF), PNF and CPF have been reported earlier [6]. PCF was prepared by mixing equal weights of PNF and CPF. Maize breads were prepared from MF serving as control (diet I), MF supplemented with 20 % PNF (diet II), 20% CPF (diet III) and 20 % PCF (diet IV). Breads were baked for 15-20 min. on a hot plate called the "tawa". Proximate analyses of the breads were carried out according to AOAC [7].

Nitrogen balance experiments. The experimental procedure was similar to that reported earlier [6]. Nitrogen (N) balance experiments were performed on a group of six apparently healthy young college students (aged, 16–19). The study consisted of four experimental periods of 14 days each. The first 9 days were used as an adjustment period and the last 5 days for collecting the urine and faeces. A two-week interval separated the test periods.

*Present Address: Department of Human Nutrition NWFP Agricultural University, Peshawar.

The source of test protein was maize bread in test diet I, maize bread supplemented with 20 % each of PNF, CPF and PCF (1 : 1) flours, respectively, in diets II, III and IV. In addition, each subject received a daily dose of a vitamin and mineral mixture and a weekly dose of 50,000 I.U. of Vitamin A. Each diet provided approximately 10 g N and 2900 kcal per day per subject. The average daily intake of food, protein and calories, as calculated from food composition tables [8], is shown in Table I. All the test diets were so designed as to provide 70 % of the total protein intake from the breads. The remaining protein was derived from vegetable sources (20 %) plus some amount [10] from milk. Diet I was regarded as the control diet. All diets were isonitrogenous and isocaloric.

Table 1. Composition of experimental diets[a] (Diet. 1–IV).

Food served	Amount (g)	Protein (g)	Energy[b] (kcal)
Maize bread	311-567	43.7	908-1622
Vegetables[c]			
Okra or	350	7.7	172
peak or	110	7.7	172
potato	428	7.7	347
Ghee (fat)	50	–	450
Sugar	50	–	200
Tomato	100	0.8	25
Onion	100	1.5	41
Radish (white)	50	0.6	15
Banana	one	1.6	102
Orange juice	100	0.6	46
Soft drink (7-Up)	one	–	36
Milk	150	6.0	150
Total	–	62.5	2900

[a]The composition of the four diets was similar except for the amount of maize bread which was 567 g for diet I (unsupplemented control), 311 g in diet II (20 % peanut-maize bread), 455 g in diet III (20 % chickpea-maize bread) and 358 g in diet IV (20 % peanut-chickpea (1:1) maize bread). The amount of each type of maize bread was calculated to provide 7 g N (43.7 protein) in a total intake of 10 g N/day (62.5 g protein/day). Each experimental diet was divided into breakfast, lunch and evening.

[b]The caloric intake was adjusted to 2900 kcal/day with protein-free foods.

[c]Each vegetable was given in rotation.

24-hour urine specimens were collected from each subject in glass jars containing a small amount of toulene (I ml/quart jar) for the last five days. Faeces were collected in pre-weighed plastic bags during the last five days of each experimental period.

Analysis for nitrogen. Nitrogen was determined by Kjeldahl's method [7] in urine on a 10–80 ml aliquot of each 24 hr. sample, and on a 5 g sample of faeces. Nitrogen in food was also determined to check the protein con-

Table 2. Effect of various maize diets on nitrogen balance in human subjects.

Subject	N-intake (g/day)	Urinary N (g/day)	Feacal N (g/day)	Total N excreted (g/day)	Nitrogen balance (g/day)
Diet I. Unsupplemented maize bread (control)					
BA	10.19	8.05	2.06	10.11	+ 0.08
SA	10.25	8.52	1.79	10.31	– 0.06
AM	10.20	8.30	2.03	10.33	– 0.13
MO	10.20	8.17	1.67	9.84	+ 0.36
MU	10.26	8.10	1.88	9.98	+ 0.28
FZ	10.21	8.07	1.87	9.94	+ 0.27
Average	10.21	8.20	1.88	10.09	+ 0.13 ±0.18a
Diet II. Maize bread containing 20 % peanut (PNF)					
BA	10.12	7.89	1.89	9.78	+ 0.34
SA	10.20	8.22	1.69	9.91	+ 0.29
AM	10.18	8.39	1.53	9.92	+ 0.26
MO	10.16	8.02	1.66	9.68	+ 0.48
MU	10.20	8.31	1.71	10.02	+ 0.18
FZ	10.22	7.98	1.74	9.72	+ 0.50
Average	10.18	8.13	1.70	9.83	+ 0.34 ±0.11 b
Diet III. Maize bread containing 20 % chickpea (CPF)					
BA	10.25	8.06	2.00	10.06	+ 0.19
SA	10.20	7.98	1.70	9.68	+ 0.52
AM	10.26	7.88	1.95	9.83	+ 0.43
MO	10.22	8.12	1.89	10.01	+ 0.21
MU	10.20	7.76	1.99	9.75	+ 0.45
FZ	10.22	8.03	1.82	9.85	+ 0.37
Average	10.22	7.97	1.89	9.86	+ 0.36 ±0.12 b
Diet IV. Maize bread containing 20 % P-C flour (PCF)					
BA	10.00	7.91	1.66	9.57	+ 0.43
SA	10.00	7.80	1.88	9.68	+ 0.32
AM	10.00	7.98	1.56	9.54	+ 0.46
MO	10.00	7.66	1.79	9.45	+ 0.55
MU	10.00	7.86	1.89	9.75	+ 0.25
FZ	10.00	7.81	1.82	9.63	+ 0.37
Average	10.00	7.83	1.76	9.60	+ 0.39 ±0.09 b

tent. The N balance was computed as follows :

$$\text{N balance} = \text{N intake} - (\text{faecal N} + \text{urinary N})$$
$$= \text{N intake} - (\text{total N excreted})$$

The data was statistically analysed [9] and least significant difference (LSD) was computed at 5 % and 1 % levels of probability.

RESULTS AND DISCUSSION

The protein content of maize bread (unsupplemented) increased from 7.7 (29.9 % moisture) to 13.6 % (25.1 % moisture) by supplementation with 20 % PNF, to 9.7 % (26.3 % moisture) with 20 % CPF and to 11.6 % (27.2 % moisture) with 20 % PCF.

Nitrogen equilibrium is defined as nitrogen excretion within 95-105 % of nitrogen intake [10]. According to this definition, most of the subjects in all the four types of diets had values within the above range and were in nitrogen equilibrium (Table 2).

In test diet I, in which most of the dietary protein was supplied by the unsupplemented maize bread, all the subjects but two were in positive nitrogen balance. The negative nitrogen balance shown by the two subjects was well within the experimental variation seen in this type of experiments. The average nitrogen balance for the group (Table 2) was + 0.13 g/day. This showed that at 10 g N/day, of which 7 g N was supplied by maize bread, the bread could maintain nitrogen balance in young human subjects. This is in conformity with previous reports in literature [11-14].

Results of nitrogen balance with the supplemented maize breads (diets II, III and IV) showed that all the three supplements were about equally effective in increasing significantly ($P < 0.05$) the nitrogen balance of the subjects.

Both PNF and CPF have been reported to increase the nitorgen retention in human subjects fed on diets supplemented with these flours [14-16].

The increase in the nitrogen balance of subjects receiving the supplemented breads may be due to improvement in the amino acid balance of these breads. It might be just as well to mention that the insensible N loss, which is reported to be 5 mg/kg body weight/day [17] does not show up in the actual N balance calculations. However, even with such a loss, the relative improvement in the N retention with the supplemented breads will not be affected due to the presence of internal control.

A comparison of the N balance data was made with that obtained with rat-bioassays reported earlier [4,5,18]. Both types of assays indicated significant increase in the protein quality of maize bread fortified with protein supplements. However, a significant ranking effect with rat-bioassays was observed for the three supplements with CF flour showing significantly higher improvement in the protein quality of maize than PCF which in turn was significantly better than PF. The present results of N balance studies did not show such ranking. It is known that the requirement of lysine for a human adult is different from that of rat and may account for this difference in ranking.

In conclusion, it can be stated that supplementation of maize bread with 20 % level of PNF, CPF or PCF (1 : 1) can improve significantly the nutritive value of maize bread for adult human subjects. However, further studies are need to investigate whether these supplemented breads could also maintain a positive N balance in children, whose requirements for different essential amino acids especially lysine are more than adult subjects. Fortification of maize bread with 20 % PCF, in particular, is recommended for improving the protein nutriture of maize consuming individuals. Alternatively, CPF and PNF can also be used at the same replacement level.

REFERENCES

1. Anonymous, Agr. Statistics of Pakistan, Fd. and Agr. Div. (Planning Unit), Min. Fd. Agr. and Cooperatives, Islamabad (1978).
2. R. Bressani and E.G. Marenco, J. Agr. Fd. Chem., **11**, 517 (1963).
3. E.E. Howe, G.R. Jansen and M.L. Anson, Am. J. Clin. Nutr., **20**, 1134 (1967).
4. J.K. Khalil and M.I.D. Chughtai, Pakistan J. Biochem., **15**, 83 (1982).
5. J.K. Khalil and M.I.D. Chughtai, Qual. Plant Plant Foods for Hum. Nutr., **34**, 285 (1984).
6. J.K. Khalil, B. Ahmad, I. Ahmad and T. Hussain, Pakistan J. Sci. Ind. Res., **26**, 83 (1983).
7. *AOAC, Official Methods of Analysis* (Assoc. Off. Anal. Chem. Washington, D.C., 1980), 13th ed.
8. P.L. Pellet and S. Shadarevian, *Food Composition Table for Use in the Middle East* (Am. Univ. Beirut, Labanon).
9. G.W. Snedecor and W.G. Cochran, *Statistical Methods* (Iowa State Uni. Press, Ames, Iowa, 1980).
10. R.M. Leverton, M.R. Gram, E. Brodovsky, M. Chaloupka, A. Mitchell and N. Johnson, J. Nutr., **58**, 83 (1956).

11. C. Kies, E. Williams and H.M. Fox, J. Nutr., **86**, 350 (1965).
12. A.S. Truswell and J.F. Brock, Am. J. Clin. Nutr., **9**, 725 (1961).
13. A.S. Truswell and J.F. Brock, Am J. Clin. Nutr., **10**, 142 (1962).
14. T. Hussain, Pakistan J. Biochem, Prof. M.I.D. Chughtai Commemoration Vol., pp. 22-31 (1979).
15. E.M. De Maeyer and H. Vanderborght, J. Nutr., **65**, 335 (1958).
16. R.F. Hashmi, A.H. Gilani and M.B. Sial, Res. Studies (Agr. Univ. Faisalabad), I, 298 (1972).
17. FAO/WHO, Energy and Protein Requirements, FAO Nutritional Meeting Series No. 52, FAO/Rome (1973).
18. J.K. Khalil and M.I.D. Chughtai, Sarhad J. Agr. (1986), (in press).