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## NITROGEN BALANCE IN HUMAN SUBJECTS AS INFLUENCED BY CORN BREAD SUPPLEMENTED WITH PEANUT AND CHICKPEA FLOURS

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Results are presented on the nitrogen balance in six young human subjects fed corn bread alone and supplemented with either 10% peanut flour or 10% peanut-chickpea flour (1:1). The diets were iso-caloric (2800 k.cals/day) and iso proteic (10gN/day). Corn bread provided 70% of the protein intake and the remaining was derived from other vegetable sources. The average nitrogen balance of the subjects fed unsupplemented corn bread (-0.005gN/day) was significantly improved ( $P < 0.01$ ) when corn was supplemented with either 10% peanut flour (+0.025gN/day) or 10% of 1:1 peanut-chickpea flour (+0.045gN/day). These results showed that supplementation of corn with peanut and chickpea flours is desirable for raising the nutritional standard of the local rural population whose staple diet mainly consists of corn bread.

### INTRODUCTION

Cereals are the principal source of protein and calories in the diet of the people in the developing countries. Corn (*Zea mays* L.) is an important cereal crop consumed by a large segment of the population in Pakistan especially in the rural areas. Like other cereals, the protein content of corn is low and of poor nutritional quality and can not provide a balance diet for the proper nourishment and maintenance of the human body. This is especially true for the children and nursing mothers whose protein and caloric requirements are more than normal adults. Supplementation of corn with the rich protein sources can enhance the nutritive value of corn.

Food legumes are second in merit after animal proteins as a protein source for human use. Peanut (*Arachis hypogaea* L.), also called groundnut, is an important food legume famous for its high oil and protein contents. The residue left after the extraction of oil from peanuts contain about 50% protein and this can serve as a good protein supplement for corn bread. Chickpea (*Cicer arietinum* L.) is also produced in Pakistan in large quantities especially in the "barani" (rain-fed) area. It contains 17.5-28.0% protein [1] and thus can be used as a protein supplement in low protein diets such as corn.

Nutritional quality of protein depends upon the amount and kind of amino acids which become available to the body during digestion. If any one of the essential amino acids is deficient, it becomes the limiting factor in the utilization of protein inside the body. Corn is deficient in lysine and tryptophan while rich in sulphur amino acids [2,3]

On the other hand peanut and chickpea contain comparatively higher amounts of lysine and tryptophan but are deficient in methionine+cystine [4,5]. Combination of these protein sources can balance the essential amino acid contents in the diet. The nutritive value of such diets have been assessed by a number of workers [6,7,8], but their studies were restricted to laboratory animals. Since the digestive system and the amino acid requirements of man differ considerably from those of animals, it is necessary to test the protein quality of a diet intended for human use on human subjects.

The present work was undertaken to study the nitrogen balance in human subjects fed corn bread alone and supplemented with peanut and chickpea flours to assess the significance of these supplements in the improvement of low-cost diets based on corn.

### MATERIALS AND METHODS

*Collection of Samples.* 'Swabi white' cultivar of corn (*Zea mays* L.), which is the most popular local cultivar in the North West Frontier Province (NWFP), was selected for this study. Seeds of this corn cultivar were obtained from the local market, screened and finely ground in a Wiley Mill. Peanuts of local cultivar 'Kurram' were roasted for ½ hr at 110° in an oven, ground and the oil extracted with petroleum ether in a soxhlet apparatus. The resulting flour was sundried, ground and passed through a 100-mesh sieve. Chickpea or Kabli gram seeds were soaked in water overnight, sundried, finely ground and passed through 100-mesh

sieve. The flours were analysed for moisture, nitrogen and crude fat content by the standard methods of A.O.A.C.[9].

**Preparation of Breads.** Breads were prepared from whole corn flour serving as control (Diet-I), corn flour containing 10% peanut flour (Diet-II) and 10% of 1:1 peanut-chickpea flour (Diet-III). Breads were prepared as made locally in home. Water and table salt (1%) was added to the flour and the dough was prepared by mixing and kneading (5-7 min) with hands. The dough was divided into pieces of specific weight and converted into circular sheets of 1.5 cm thickness by hand. Each sheet was baked on a hot plate (tawwa) with slow uniform that (10-15 min). The bread were cooled to room temperature, wrapped in polyethylene bags and analysed for moisture, nitrogen and oil contents by the A.O.A.C.[9] methods.

**Nitrogen Balance.** Six human subjects, three male and three females, whose ages varied from 14-21 years, were selected for the N-balance experiments. All the subjects were healthy and continued their routine activities throughout the experimental period. Each subject received daily an iso-proteic (10 g N) and iso-caloric (2800 k.cals) diet in which 7g N was provided by the corn breads and 3g N was derived from other vegetable sources. In addition, a capsule of vitamins and mineral mixture was daily provided to each subject water was provided ad libitum. Each diet was fed for 7 days out of which the first 5 days were considered as the adjustment period and the remaining two days were recvoned as collection period in which the faeces and urine excreted during each 24 hr were collected from each subject. Nitrogen in 10g faeces and 10 ml urine was determined by the Kjeldhal method of A.O.A.C.[9]. Each determination was replicated thrice and form the average results, the total N excreted in faeces and urine during 24 h was calculated for each subject. The N-balance for each day was computed as follows:

$$\begin{aligned} \text{N absorbed} &= \text{N intake} - \text{fecal N} \\ \text{N retained} &= \text{N absorbed} - \text{Urinary N} \\ (\text{N-Balance}) \end{aligned}$$

The result were statistically analysed by the method of Snedecor and Cochran (10). The least significant difference (LSD) was computed at 5% and 1% levels of probability.

## RESULTS AND DISCUSSIONS

**Moisture, Protein and Oil contents of flour and Breads.** The moisture, protein and oil contents of corn, peanut and

chickpea flours and breads is shown in Table 1. The results for the moisture, protein and oil content of the various flours were similar to reports in literature (1,3,4,11). Peanut flour contained the maximum protein content of about five-fold while chickpea flour contained about two-fold more protein than the unsupplemented corn flour. The oil content did not show large variations in the flours. As expected, the protein content of corn bread increased by 64% with 10% peanut flour and by 51% with 10% of 1:1 peanut-chickpea flour. The increase in the protein content of bread is highly desirable because such breads can better meet the needs of the essential amino acids especially during growth, pregnancy and lactation.

**Nitrogen Balance.** Data on the individual nitrogen balances of the six human subjects is shown in Table 2. In the case of control diet (Diet-I) in which most of the dietary protein was derived from the unseupplemented corn bread, the nitrogen balance ranged from -0.06 to +0.03 g/day depending upon the age of the subject. The balance was negative in three individuals of lower age group (14-16 years) and positive in the remaining three subjtecs of higher age (17-21 years). This difference may be due to higher nitrogen requirement in younger subjects. The nitrogen balance of the subjects fed corn-bread supplemented with 10% peanut flour (Diet-II) varied from -0.04 to +0.07 g/day. Negative nitrogen balance was observed only in one subject of 14 years age while it was positive in the remaining five subjects. This indicated a slight improvement in the N-retention of the subjects with this diet. The corn bread containing 10% of a 1:1 Peanut-chickpea flour (Diet-III) resulted in negative nitrogen balce in one subject of lower age (14 years) but showed a positive nitrogen balance in the other subjects where the N-retention showed a gradual increase as the age of the subject increased. The nitrogen balance of this group was apparantly higher than the control Diet-I and the test Diet-II. A number of workers (12,13) have reported a negative N-balance in their subjects when fed dorn bread alone as a major source of dietary protein. On the other hand, Truswell and Brock (14,15) and Kies *et al.* (16) found a positive N-balance in adult human subjects when the N intake was 7.8 g/day of which 90% was derived from corn.

Data on the nitrogen-balance of each group (Table 3) revealed that average N-retention in the six human subjects was significantly ( $P < 0.01$ ) increased when corn (Diet-I) was supplemented with 10% peanut flour (Diet-II) and a further significant ( $P 0.01$ ) increase was observed when chickpea flour replaced half of peanut flour (Diet-III) in the bread. The group fed unsupplemented corn bread

Table 1. Moisture, protein and oil content<sup>1</sup> of corn, peanut and chickpea flours and breads

Flour/bread	Moisture	Protein <sup>2</sup>	Oil <sup>2</sup>
<b>A. Flour</b>			
(I) Corn	11.2±0.1	10.6±0.1	4.4±0.0
(II) Peanut	2.5±0.1	58.8±0.6	4.5±0.1
(III) Chickpea	7.8±0.3	23.2±0.8	5.1±0.5
<b>B. Breads</b>			
(I) Corn	28.1±0.5	8.8±0.6	4.4±0.3
(II) + 10 % Peanut	27.2±0.4	14.4±0.6	4.1±0.5
(III) + 10% Peanut – Chickpea (1:1)	27.2±0.5	13.3±0.3	4.4±0.2

1. Means ± Standard Deviations

2. Per cent of dry weight

Table 2. Nitrogen balance of human subjects fed corn breads

Subject	Sex	Age	Intake	Fecal	Urinary	Total	Absorbed	Nitrogen Balance
Diet – I. 100% Corn bread								
AG	Male	14	9.90	1.27	8.69	9.96	8.63	0.06
MI	---	16	9.90	1.13	8.79	9.92	8.77	-0.02
SH	---	19	9.90	0.99	8.90	9.89	8.91	+ 0.01
RB	Female	15	9.90	1.50	8.41	9.91	8.40	-0.01
BH	---	17	9.90	1.84	8.04	9.88	8.06	+ 0.02
JA	---	21	9.90	1.20	8.67	9.87	8.70	+ 0.03
Diet – II Corn bread containing 10% peanut flour								
AG	Male	14	9.90	1.58	8.36	9.94	8.32	-0.04
MI	---	16	9.90	1.79	8.10	9.89	8.11	+ 0.01
SH	---	19	9.90	1.81	8.05	9.86	8.09	+ 0.04
RB	Female	15	9.90	1.67	8.21	9.88	8.23	+ 0.02
BH	---	17	9.90	2.03	7.80	9.83	7.87	+ 0.07
JA	---	21	9.90	1.91	7.94	9.85	7.99	+ 0.05
Diet – III Corn bread containing 10% peanut-chickpea flour (1:1)								
AG	Male	14	9.90	1.75	8.16	9.91	8.15	0.01
MI	---	16	9.90	1.78	8.10	9.88	8.12	+ 0.02
SH	---	19	9.90	1.81	8.04	9.85	8.09	+ 0.05
RB	Female	15	9.90	1.58	8.28	9.86	8.32	+ 0.04
BH	---	17	9.90	1.92	7.90	9.82	7.98	+ 0.08
JA	---	21	9.90	1.18	8.63	9.81	8.72	+ 0.09

Table 3. Comparison of average nitrogen balance on the three types of corn diets

Diet	N-Intake (g/day)	Fecal-N (g/day)	Absorbed-N (g/day)	N-Retained (g/day)
I. Corn bread (unsupplemented)	9.90	1.32	8.58±0.13 a	0.005±0.01 A
II. Corn bread containing 10% peanut flour	9.90	1.80	8.10±0.02 b	+0.025±0.013 B
III. Corn bread containing 10% peanut-chickpea (1:1)	9.90	1.67	8.23±0.11 b	+0.045±0.015 C

Figures followed by similar letters are not significantly different. Capital letters indicate differences at 1% and small letters at 5% level of probability.

showed a negative N-balance whereas those fed with the supplemented breads showed a positive N-balance. These results showed that the supplemented breads provided a better balance of essential amino acids in the diets as compared to the unsupplemented diet. However, the N-absorption was significantly ( $P < 0.05$ ) higher with the control corn bread (Diet-I) than the supplemented breads (Diet-II and III). This is probably due to the higher fiber content in the peanut and chickpea flours resulting in lower digestibilities of these breads. Thus using peanut and chickpea flours with lower fibre content could lead to higher N-absorption and consequently higher N-retention. Even though peanut and chickpea supplementation resulted in a positive average N-balance, it is to be mentioned that the insensible N-loss is 5 mg/kg body wt/day (17) and 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. Nevertheless, to offset this loss of N (0.25-0.30g/day), a higher level of supplementation coupled with a low-fiber peanut and chickpea flours needs to be tested for the possible higher N-retention under these conditions.

In conclusion, the observations made in the present study on human subjects showed that the protein nutritional quality of the local corn cultivar 'Swabi white' can be improved by supplementation with peanut and/or chickpea flour as evident by the higher N-retentions with the supplemented diets.

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