*J. Nutr. Dietet.* (1965) 2, 28

# STUDIES ON PROCESSED PROTEIN FOODS BASED ON BLENDS OF GROUNDNUT, BENGAL GRAM, SOYABEAN AND SESAME FLOURS AND FORTIFIED WITH MINERALS AND VITAMINS

## III. SUPPLEMENTARY VALUE TO A POOR INDIAN KAFFIR CORN DIET

MYNA PANEMANGALORE, MANKARNIKA N. GUTTIKAR, M. NARAYANA RAO, D. RAJALAKSHMI AND M. SWAMINATHAN

*Central Food Technological Research Institute, Mysore*

*(Received 20 December 1964)*

Kaffir corn (jowar-*sorghum vulgare*) is one of the important millets consumed widely in India and Africa. Poor Indian diets based on kaffir corn are deficient in proteins, vitamin A, riboflavin and calcium.[1,3] The protein in poor kaffir corn diets are of moderate nutritive value.[1] The rate of growth of albino rats fed on such diets is low, being of the order of 4 to 8 g/week as compared with 15 to 20 g/week on well balanced stock diets. In an earlier paper from this laboratory, Joseph *et al.*[4] reported Indian multipurpose food (3 : 1 blend of groundnut flour and Bengal gram flour fortified with calcium salts and vitamins) when incorporated to provide about 5% extra protein supplemented to a marked extent a poor kaffir corn diet as judged by the growth of rats. In the previous papers of this series,[5,6] the results of studies on the preparation, shelf life and nutritive value of the proteins of two protein foods based on blends of groundnut, Bengal gram, soya bean and sesame flours have been reported. In the present investigation, the effect of supplementing a poor kaffir corn diet with the protein foods so as to provide 2.5% extra protein in the diet on the growth and composition of liver and carcass of albino rats has been studied.

### Experimental

*Materials*; The protein foods I and II used in the present study were the same as those described in a previous paper [5] Skim milk powder of good quality was used for for comparison. Kaffir corn obtained from the local market was cleaned of impurities and powdered in a flour mill to pass through 50 mesh sieve.

*Preparation of diets*: The composition of the poor kaffir corn diet used in this study was the same as that described by Joseph *et al*[4] The vegetables were dried, powdered and used in the diet. The required quantities of protein foods I and II (with and without fortification with l-lysine and dl-methionine) and skim milk powder were incorporated to provide 2.5% extra protein in the diet by replacing an equal amount of kaffir corn. Weighed amounts of these diets were mixed with three times the weight of water in feeding cups and cooked in steam at atmospheric pressure for 15 minutes and fed to the rats.

*Animal experiments*: Female albino rats (28 days old) weighing from 40 to 45 g. were alloted in a randomised block design to different groups. Each group contained 8 animals. The method adopted for the feeding of the animals was similar to that of Subrahmanyan *et al*[7] Records of the daily food intake and weekly increase in body weight were maintained. The experimental period was of 8 weeks duration, at the end of which haemoglobin and R. B. C. count in the blood were determined according to the methods of Joseph *et al.*[4] The animals were anaesthetised with ether and blood was drawn by heart puncture for serum protein determinations according to King and Wootton.[8] Liver was removed and a portion from the left lobe was fixed in 10% formalin for histological examination according to Paul Jayaraj *et al.*[9] The carcass and liver were analysed for moisture, fat and protein according to Joseph *et al.*[4] The initial protein contents of the carcasses of rats in the different groups were calculated from the values obtained for a group of

weanling rats of the same weight range. Using this data, the retention of protein in the carcass of rats receiving the different diets over a period of 8 weeks was calculated.

## Results

The results are presented in Tables I to III.

*Growth and composition of blood*: The average weekly increase in body weight of rats fed on the different diets is shown in Table I. The results show that incorporation of the protein foods I and II (with or without amino acid fortification) in the kaffir corn diet so as to provide 2.5% extra protein resulted in a highly significant ($P<0.001$) increase in the growth rate of rats, comparing well with that obtained with skim milk powder There were, however, no significant differences between the mean growth rates of rats receiving the protein foods fortified with l-lysine and dl-methionine and the unfortified protein foods. An increase in the serum proteins, haemoglobin and RBC count was also observed as a result of supplementation with protein foods.

*Composition of liver and carcass:* The results of analysis of liver and carcass of rats fed on the different diets is shown in Table II. There were no significant differences in the mean protein and fat contents of livers of rats receiving the different protein foods. The mean fat content of livers of rats fed on the poor kaffir corn diet was significantly ($P<0.05$) higher than those receiving the protein supplements. The mean protein content of the carcass of rats fed on the control poor kaffir corn diet was significantly less ($P<0.05$) than those of the carcasses of rats receiving the protein supplements.

*Histological structure of liver*: Livers of animals fed on the poor kaffir corn diet showed a mild degree of parenchymal damage of the protein deficiency type. The liver cells showed reduction in cytoplasmic proteins. Frozen sections stained with Sudan IV showed mild generalised and periportal fatty infiltration. Necrosis of the liver cells was absent. The liver sections of rats receiving the different protein foods were quite normal indicating thereby that the protein foods when providing 2.5% extra protein in the diets were effective in correcting the deficiencies of protein and other dietary essentials in the diet and in preventing liver damage.

*Intake and retention of protein*: The mean intake and retention of protein by the rats on the different diets are given in Table III. It will be observed that the amount of protein retained per 100 g. increase in body weight of rats receiving the different protein supplements is nearly the same. The amount of protein retained in the control poor kaffir corn diet was, however, significantly lower than those observed in ihe groups receiving the protein supplements.

## Discussion

A number of workers have reported that a poor kaffir corn diet promotes a low rate of growth in rats.[1] Sriramachari *et al.*,[10] observed that rats fed on a diet containing 10% protein from kaffir corn developed a mild to moderate generalised and periportal fatty liver which was prevented by the incorporation of 2% of legume proteins and 1% leaf protein in the diet. In the present study the livers of animals fed on the poor kaffir corn diet showed a mild degree of periportal and generalised fatty liver and the growth rate of rats was low (6.0g/week) Incorporation of the protein foods to provide 2.5 percent extra protein in the diet corrected the liver damage and almost doubled the growth rate (11-12g/week). The results indicate that protein foods based on oilseed meals and fortified with calcium salts and essential vitamins could be used as a low cost supplement for overcoming the deficiencies in poor kaffir corn diets.

## Summary

(1) Supplementation of a poor Indian kaffir corn (*surghum vulgare*) diet with protein foods based on 40 : 40 : 20 blend of groundnut, Bengal gram and sesame flours and 40 : 30 : 30 blend of groundnut, soya and sesame flours (fortified with calcium salts, vitamins A and D, thiamine and riboflavin) so as to provide 2.5% extra protein in the diet resulted almost in doubling the growth rate of rats. The

Table I: *Effect of supplementing*[1] *a poor Indian kaffir corn diet with Protein Foods and skim milk powder on the growth of rats*
(Mean values for 8 female rats per group; period of experiment—8 weeks)

| Group No. | Diet | Protein content of diet % (on dry basis) | Initial body weight (g) | Final body weight (g) | Weekly increase in body weight (g) | Average daily food intake (g) (on dry basis) | F. E. R. | Haemoglobin g/100 ml. blood | Red blood cells ($10^6$/cu. mm blood) | Serum Proteins[2] (g/100 ml) |
|---|---|---|---|---|---|---|---|---|---|---|
| I. | Poor Kaffir corn diet | 8.7 | 45.1 | 92.9 | 6.0 | 7.3 | 0.11 | 14.7 | 7.34 | 5.70 |
| II. | ,, + Protein Food I | 10.9 | 45.0 | 141.4 | 12.1 | 9.7 | 0.17 | 16.0 | 8.97 | 6.52 |
| III. | ,, + Protein Food I fortified with A. A.* | 11.0 | 44.9 | 139.9 | 11.9 | 9.3 | 0.17 | 15.7 | 8.88 | 6.10 |
| IV. | ,, + Protein Food II | 10.9 | 45.0 | 133.5 | 11.1 | 9.5 | 0.16 | 16.2 | 8.95 | 5.90 |
| V. | ,, + Protein Food II fortified with A.A.* | 11.0 | 45.0 | 133.9 | 11.1 | 8.8 | 0.17 | 16.6 | 9.17 | 6.30 |
| VI. | ,, + Skim milk powder | 10.9 | 44.9 | 145.8 | 12.6 | 9.3 | 0.18 | 15.8 | 9.10 | 6.12 |
| | Standard error (35 d.f.) | | | | ±0.53 | | ±0.007 | ±0.22 | ±0.22 | ±0.24 (25df) |
| | | | | | a b | | a b | a b | a b | a b |
| | Critical difference at 5% level | | | | 1.27 1.52 | | 0.017 0.020 | 0.52 0.63 | 0.52 0.63 | 0.58 0.70 |
| | ,, 1% ,, | | | | 1.83 2.04 | | 0.024 0.027 | 0 76 0.85 | 0 76 0.85 | 0.84 0.95 |
| | ,, 0.1% ,, | | | | 2.51 2.70 | | 0.033 0.036 | 1.04 1.12 | 1.04 1.12 | 1.17 1.27 |

1. The protein supplements provided about 2.5% extra protein in the diet.
2. Mean values for 6 rats per group.

*A.A. Amino acids — l – lysine and dl – methionine
a. one tailed test; b. two tailed test.

Table II. *The chemical composition of the carcass and liver of rats fed on a poor kaffir corn diet and the same supplemented with protein foods and skim milk powder*

(Mean values for 6 female rats per group ; duration of experiment, 8 weeks)

| Group No. | Diet | Body weight (g) | | Composition of liver | | | Composition of carcass | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | Moisture % | Protein (Nx6.25) % | Fat % | Moisture % | Protein (Nx6.25) % | Fat % |
| I. | Poor Kaffir corn diet | 45.1 | 92.9 | 70.0 | 18.88 | 4.56 | 64.1 | 16.46 | 10.58 |
| II. | ,, + Protein Food I | 45.0 | 141.4 | 70.9 | 19-34 | 3.28 | 56.7 | 17.34 | 19.17 |
| III. | ,, + Protein Food I fortified with A.A.* | 44.9 | 139.9 | 70.4 | 19.59 | 3.18 | 57.3 | 17.82 | 18.03 |
| IV. | ,, + Protein Food II | 45.0 | 133.5 | 70.8 | 20.32 | 3.16 | 57.9 | 18.00 | 17.08 |
| V. | ,, + Protein Food II fortified with A.A.* | 45.0 | 133.9 | 70.0 | 20.41 | 3.10 | 57.2 | 18.11 | 17.92 |
| VI. | ,, + Skim milk powder | 44.9 | 145.8 | 70.3 | 19.80 | 3.74 | 57.4 | 18.87 | 19.82 |
| | Standard error (25 df) | | | ±0.25 | ±0.43 | ±0.38 | ±0.66 | ±0.33 | ±0.87 |
| | | | | a b | a b | a b | a b | a b | a b |
| | Critical difference at 5% level | | | 0.61 0.73 | 1.03 1.25 | 0.92 1.10 | 1.58 1.91 | 0.80 0.97 | 2.14 2.58 |
| | 1% ,, | | | 0.88 0.99 | 1.50 1.69 | 1.33 1.49 | 2.30 3.58 | 1.16 1.81 | 3.12 3.49 |
| | 0.1% ,, | | | 1.22 1.33 | 2 09 2.25 | 1.85 2.00 | 3.19 3.45 | 1.12 1.75 | 4.33 4.67 |

a. One tailed test b. Two tailed lest

*A.A. Amino acids—l - lysine and dl - methionine

Table III : *Intake and retention of protein in rats fed kaffir corn diet supplemented with Protein Foods and skim milk powder*
(Experimental period : 8 weeks, Mean values for 6 female rats in each group)

| Group No. | Diet | Total protein intake (g) | Total increase in body weight (g) | Initial body protein (g) | Total body protein (g) | Protein retained (g) | Protein retained per 100g. increase in body weight (g) | | Protein retained % | |
|---|---|---|---|---|---|---|---|---|---|---|
| I. | Poor kaffir corn diet | 42.2 | 51.0 | 7.87 | 15.24 | 7.37 | 15.51 | | 17.29 | |
| II. | ,, + Protein Food I | 69.1 | 94.5 | 7.87 | 24.07 | 16.20 | 17.57 | | 23.41 | |
| III. | ,, + Protein Food I fortified with A.A.* | 67.6 | 93.2 | 7.87 | 24.45 | 16.58 | 18.06 | | 24.51 | |
| IV. | ,, + Protein Food II | 69.1 | 88.0 | 7.87 | 23.70 | 15.83 | 18.21 | | 22.91 | |
| V. | ,, + Protein Food II fortified with A.A.* | 64.7 | 92.3 | 7.87 | 24.80 | 16.93 | 19.15 | | 26.20 | |
| VI. | ,, + Skim milk powder | 66.9 | 102.2 | 7.87 | 27.70 | 19.83 | 19.68 | | 29.66 | |
| | | | | Standard error (25 df.) | | | ±0.69 | | ±1.34 | |
| | | | | | | | a | b | a | b |
| | | | | Critical difference at | 5% | | 1.67 | 2.02 | 3.23 | 3.89 |
| | | | | ,, | 1% | | 2.44 | 2.73 | 4.70 | 5.27 |
| | | | | ,, | 0.1% | | 3 38 | 3.65 | 6.52 | 7 04 |

a. one tailed test b. two tailed test

*A.A.—amino acids l-lysine and dl-methionine.

mean weekly growth rates on the different diets were as follows : (i) poor kafflr corn diet (PKD), 6.0g; (ii) PKD+protein food I, 12.1g; (iii) PKD+protein food II, 11.1g; and (iv) PKD+skim milk powder, 12.6g The supplementary value of the protein foods I and II fortified with l-lysine and dl-methionine to the kaffir corn diet was of the same order as the unfortified protein blends.

(2) The livers of rats on the control poor kaffir corn diet showed a mild degree of parenchymal damage of the protein deficiency type and mild generalised and periportal fatty infiltration. The livers of animals receiving the same diet supplemented with protein foods or skim milk powder were quite normal indicating thereby that the supplements when providing 2.5% extra protein corrected the deficiencies of protein and other dietary essentials in the diet.

**Acknowledgment**

This study was supported by grants-in-aid from the P. L. 480 funds, U. S. Public Health Service. Our thanks are due to Dr. H. A. B. Parpia, Director of the Institute for his kind interest and helpful suggestions.

REFERENCES

1. Kurien, P. P.. Narayana Rao, M,, Swaminathan, M. and Subrahmanyan, V. (1960), *Food Science*, **9**, 205,
2. Patwardhan. V. N. (1963), *Nutrition in India*, published by Indian Journal of Medical Sciences, Bombay.
3. Leela, R., Daniel. V. A.. Venkat Rao, S. Hariharan, K., Swaminathan, M. and Parpia, H. A. B. (1965), *J. Nutr. Dietet.*, **2**, (in press).
4. Joseph, K., Narayana Rao, M., Swaminathan, M. Sankaran, A. N. and Subrahmanyan, V. (1959), *Ann. Biochem. Exp Med.*, **19**, 87.
5. Guttikar, M. N., Panemangalore,M., Narayana Rao, M. and Swaminathan, M. (1965), *J. Nutr. Dietet.*, **2**, 21.
6. Guttikar, M. N., Panemangalore M., Narayana Rao, M., Rajalakshmi, D. and Swaminathan, M.(1965), *J. Nutr. Dietet.*, **2**, 24.
7. Subrahmanyan, V., Kuppuswamy, S., Rama Rao, G., Sankaran, A. N., Swaminathan, M. and Bhatia, D. S. (1954), *Bull. Cent. Fd. Tech. Res. Inst*, **3**, 187.
8. King, E. J. and Wootton, I. D. P. (1956), *Microanalysis in Medical Biochemistry*, 2nd Ed., J. and A. Churchill Ltd., London.
9. Paul Jayaraj, A., Joseph, K., Narayana Rao, M., Bhagavan, R. K., Swaminathan, M. and Subrahmanyan, V. (1960), *Ann. Biochem. Expt. Med.*, **20**, 341.
10. Sriramachari, S., Ramachandran, M., Phansalkar, S. V. and Patwardhan, V. N. (1959), *Indian J. Path. & Bact.*, **2**, 180.