

## Originalarbeiten

### **The application of protein concentrates from locally available legumes in the development of weaning foods**

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*Summary:* The effect of mixing different sources of vegetable proteins from legumes in the preparation of infant weaning foods was investigated. Melon, cowpea and soya as sole protein sources or as a mixture were fed to 60 albino rats. A milk powder-based commercial product (Cerelac) was used as control diet. The mixture of the vegetable protein diet compared favourably with the control diet in terms of growth rate, protein efficiency ratio (PER) and net protein ratio (NPR) and also ensured optimum nitrogen content in liver, kidney and muscle tissues. In contrast the use of individual protein sources failed to support satisfactory growth and were inferior to those in animals fed with the control or mixed diets. It was thus concluded that in the developing countries an application of such a mixture of vegetable proteins may be suitable in the preparation of weaning foods.

*Zusammenfassung:* Das Hauptziel dieser Arbeit ist es, den Einfluß von pflanzlichem Eiweiß aus verschiedenen Hülsenfrüchten in der Ernährung von Kleinkindern aufzuzeigen.

In parallelen Versuchen wurden Proteine aus der Melone, aus Raps und Sojabohnen sowie aus einer Mischung derselben insgesamt 60 Albinoratten verfüttert, um das Wachstum und den Stickstoffmetabolismus im Gewebe zu bestimmen. Cerelac® diente als Kontrollsubstanz. Mit der Proteinmischung wurden hinsichtlich des Proteinwirkungsverhältnisses (Protein efficiency ratio, PER) und des Nettoproteinverhältnisses (net protein ratio, NPR) Ergebnisse erzielt, die gut mit der Kontrollgruppe korrelieren. Wird jedoch das jeweilige Protein allein verabreicht, sinkt die Stickstoffretention in der Zelle, und das Wachstum der Tiere ist geringer.

Deshalb sollten vor allem in Entwicklungsländern pflanzliche Eiweiße in Form von Mischungen zur Herstellung von Babynahrung eingesetzt werden.

*Key words:* vegetable proteins, weaning foods

### **Introduction**

The main source of dietary vegetable proteins are cereals and grain legumes. The cereals have a protein content varying from 7 to 12 per cent (1), whereas the legumes usually reach considerably higher (20–40%)

content (2). In addition, these latter proteins provide the two essential amino acids lysine and threonine, considered as first limiting amino acids in cereals (1). Thus legume proteins may supplement the cereal proteins by compensating for the deficit of lysine in these proteins and reducing that of sulphur amino acids.

Teply et al. (3) applied a soy isolate formula and showed it to be beneficial (4, 5) in the treatment of malnourished infants, well comparable to results achieved with commonly used cow milk formula as far as recovery and weight gain are concerned. In other investigations (6) spray dried blends containing two parts of soy bean milk and one part of peanut milk fortified with methionine showed protein efficiency ratio (PER) comparable to that of milk. Also in clinical trials (7) no differences were found using this mixture and milk formula with regard to growth and nitrogen retention.

Akinrele (8) found that the biological quality of the protein of fermented corn (locally called "ogi") was so poor that it did not support growth in rats. Similarly, in previous animal experiments from our laboratory (9) we reported that the single source of vegetable protein acquired from ogi and groundnut was inadequate in supporting growth and maintaining nitrogen equilibrium. We thus proceeded to examine other sources of vegetable proteins using a mixture of different proteins instead of relying on individual protein performance.

## Materials and Methods

### *Cowpea, melon, soy and maize grains*

The cowpea, melon and maize grains that were used in these experiments were obtained from the agricultural research farm of the University of Ife (Nigeria), while the soy seeds were received from the Institute for Agricultural Research and Training (IAR and T), Ibadan (Nigeria). In all cases the products were cleaned and dehulled, and then they were minced and defatted, using the soxhlet extractor. The resultant products were milled, followed by sieving and subsequently dried as flour products. They were used as supplements either as sole source or mixed sources of protein.

### *The mixture*

Corn flour was mixed with flours from cowpea, melon and soya at a ratio of 9:1:1:1 and water was added (1.25 ml/g) to obtain a slurry which was spray-dried and labelled as "cowpea-melon-soy-ogi"<sup>1</sup>).

### *The basal, experimental and control diets*

The nutrient composition of the basal diet is shown in Table 1 while that of the dietary regimen is shown in Table 2. The preparation of the basal diet was based upon the methods of Bernhart et al. (10). The basal diet was mixed with individual protein source as recommended to achieve an isonitrogenous diet at 10 % protein level. A similar diet was prepared using the mixture of melon-cowpea soy proteins. A commercial diet based on defatted milk powder (Cerelac®) was used as a comparison to the vegetable diets. The protein content of the various vegetable products, as estimated from nitrogen determinations by modified micro Kjeldahl methods (12),

<sup>1</sup>) "ogi" has been described (8, 9, 11) as fermented corn flour

Table 1. The nutrient composition of the basal diet.

	g/kg
Protein	—
Corn flour	800
Sugar	60
Vegetable oil	100
Vitamin mix	10
Salt mix	30
Cod liver oil	5
Energy kcal %	438.5

was as follows (Table 2): melon  $18.40 \pm 1.20\%$ , soya  $20.50 \pm 0.23\%$ , the mixture of cowpea-melon-soya  $23.63 \pm 0.25\%$ , and the commercial product (Cerelac)  $11.50 \pm 0.33\%$ . To adjust the variable protein levels to the desired 10% level each diet was mixed with the basal diet lacking protein. This standardization facilitates the subsequent studies with regard to growth, nitrogen and tissue changes in (protein) content.

#### *The experimental animals*

For this investigation, sixty albino rats weighing between 55 and 65 g were obtained from the zoological breeding centre of the University of Ife, Nigeria. They were weighed, randomly distributed in metabolic cages and were adapted to the basal diet containing 4% casein over a period of seven days. After this period the animals were re-weighed and re-grouped. The average weight per group was approximately the same as shown by the initial weights in Table 3. One group of 5 animals served as control for the experimental groups were sacrificed and tissue samples from liver, kidney and the plantaris muscle of the hind leg were weighed and frozen (at  $-70^\circ\text{C}$ ) until nitrogen was determined. The remaining animals were placed on the experimental diets as described in Table 2, over a period of 28 days. Water was supplied *ad libitum*. During this period dietary intake and growth were recorded. After the accomplishment of the experiment the animals were anaesthetized and sacrificed. Tissue specimens were obtained and kept (at  $-70^\circ\text{C}$ ) until nitrogen determination by the modified micro Kjeldahl method (12).

## **Results**

### *Nutritional adequacy of diets*

An examination of the composition of the various vegetable proteins (Table 2) show that melon protein is adequate with regard to certain essential nutrients such as thiamine and ascorbic acid. In addition, its protein is also claimed to be highly digestible (13). The high nutritive quality of soy protein (4, 5, 11, 14, 15) is repeatedly documented, although the low thiamine content of this protein is to be noted. Therefore, in the present study the soya bean diet was fortified with a multi-vitamin mixture. As previously reported (9), the commercial product, Cerelac, is adequate in all nutrients and supports normal growth and optimum nitrogen content in tissues. The rates of growth in the respective groups during the experiment are given in Table 3. Compared with the commercial product (Cerelac), feeding with the mixture of vegetable proteins, a favourable

Table 2. Nutrient composition in per cent of 100 g of diet (Mean  $\pm$  SEM).

	Melon ogi	Soy ogi	Cowpea-melon- soy ogi	Cerelac
Protein % (N $\times$ 6.25)	18.40 $\pm$ 1.20	20.50 $\pm$ 0.23	23.63 $\pm$ 0.25	11.50 $\pm$ 0.33
Moisture %	8.12 $\pm$ 0.42	7.00 $\pm$ 0.17	5.40 $\pm$ 0.15	1.50 $\pm$ 0.41
Fat %	12.00 $\pm$ 0.61	9.50 $\pm$ 0.14	10.00 $\pm$ 0.01	7.80 $\pm$ 0.51
Ash %	3.50 $\pm$ 0.12	3.00 $\pm$ 0.52	3.35 $\pm$ 0.17	2.00 $\pm$ 0.61
Carbohydrates %	65.20 $\pm$ 1.11	58.50 $\pm$ 0.51	57.62 $\pm$ 0.23	77.20 $\pm$ 0.23
Thiamine (mg/100 g)	1.80 $\pm$ 0.20	1.12 $\pm$ 0.10	0.42 $\pm$ 0.12	0.28 $\pm$ 0.12
Ascorbic acid (mg/100 g)	8.90 $\pm$ 0.64	10.80 $\pm$ 0.50	17.60 $\pm$ 0.11	17.00 $\pm$ 0.13

Table 3. Mean weight gains in rats (g) during the experimental period (Mean  $\pm$  SEM).

Dietary groups of animals	Time in days									
	1	4	7	10	13	16	19	22	25	28
Melon ogi	58.05 $\pm$ 0.30	60.30 $\pm$ 1.22	63.88 $\pm$ 1.10	67.20 $\pm$ 1.10	70.32 $\pm$ 1.50	72.50 $\pm$ 1.50	74.80 $\pm$ 1.25	76.62 $\pm$ 1.67	78.01 $\pm$ 1.48	79.80 $\pm$ 1.82
Soy ogi	57.95 $\pm$ 1.23	61.39 $\pm$ 0.31	64.78 $\pm$ 1.23	68.18 $\pm$ 0.18	71.96 $\pm$ 0.32	74.99 $\pm$ 0.71	77.64 $\pm$ 0.62	79.60 $\pm$ 0.18	80.60 $\pm$ 0.51	85.76 $\pm$ 0.16
Cowpea-melon- soy ogi	58.06 $\pm$ 1.02	62.53 $\pm$ 1.21	67.07 $\pm$ 1.12	71.27 $\pm$ 1.71	74.17 $\pm$ 1.22	77.30 $\pm$ 1.62	82.71 $\pm$ 1.16	83.92 $\pm$ 1.26	85.55 $\pm$ 1.71	93.05 $\pm$ 0.81
Cerelac	58.50 $\pm$ 1.37	60.56 $\pm$ 1.11	65.93 $\pm$ 1.17	73.70 $\pm$ 1.26	78.58 $\pm$ 1.27	81.74 $\pm$ 1.51	84.69 $\pm$ 1.17	86.30 $\pm$ 1.22	87.98 $\pm$ 1.62	95.20 $\pm$ 1.28
Basal	57.98 $\pm$ 1.21	56.23 $\pm$ 2.42	54.61 $\pm$ 1.21	52.25 $\pm$ 3.62	50.15 $\pm$ 2.51	48.20 $\pm$ 2.61	46.15 $\pm$ 2.27	43.75 $\pm$ 2.52	42.45 $\pm$ 1.73	41.15 $\pm$ 1.68

\* Significant difference with Cerelac

+ Significant difference with Cowpea-melon-soy ogi

\* = P &lt; 0.05 = +

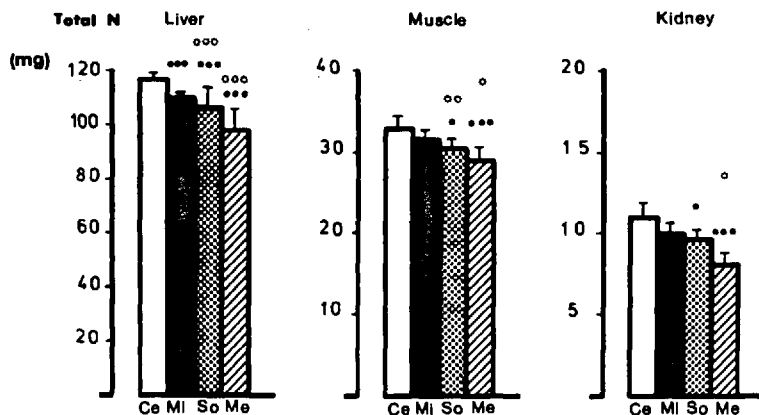
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\*\*\* = P &lt; 0.001 = +++

Table 4. Total weight of selected tissues of rats (g) during the experimental period (Mean  $\pm$  SEM).

Dietary samples	Liver	Kidney	Muscle (plantaris)
Stabilizing diet	2.40 $\pm$ 0.31	0.20 $\pm$ 0.11	0.62 $\pm$ 0.22
Melon ogi	2.50 $\pm$ 0.25	0.23 $\pm$ 0.07	0.65 $\pm$ 0.12
Soy ogi	2.64 $\pm$ 0.51	0.25 $\pm$ 0.01	0.67 $\pm$ 0.12
Cowpea-melon-soy ogi	2.65 $\pm$ 0.26	0.26 $\pm$ 0.16	0.68 $\pm$ 0.22
Cerelac	2.70 $\pm$ 0.01	0.28 $\pm$ 0.07	0.68 $\pm$ 0.22
Basal	1.33 $\pm$ 0.21	0.15 $\pm$ 0.03	0.27 $\pm$ 0.13

growth rate was apparent, while the use of the individual proteins was not associated with satisfactory weight gain. Actually, the differences in growth rate were statistically significant with Cerelac compared with the individual proteins (soya and melon) from the 10th day of the experiment. Significant differences between growth rates of animals fed the mixed diet and the individual proteins commenced only after the 16th day. No significant difference however was observed between the animals fed, the control and the mixed diets throughout the experiment.

Fig. 1. Total nitrogen (mg N) in tissues of rats during the experimental period (mean  $\pm$  SEM)

Ce = Cerelac, Mi = mixture of vegetable proteins (cowpea-melon-soy), So = soy, and Me = melon

• statistically significant difference between Cerelac and other individual proteins tested

° statistically significant difference between the mixture and other individual protein tested

• or ° (p < 0.05)

•• or °° (p < 0.01)

••• or °°° (p < 0.001)

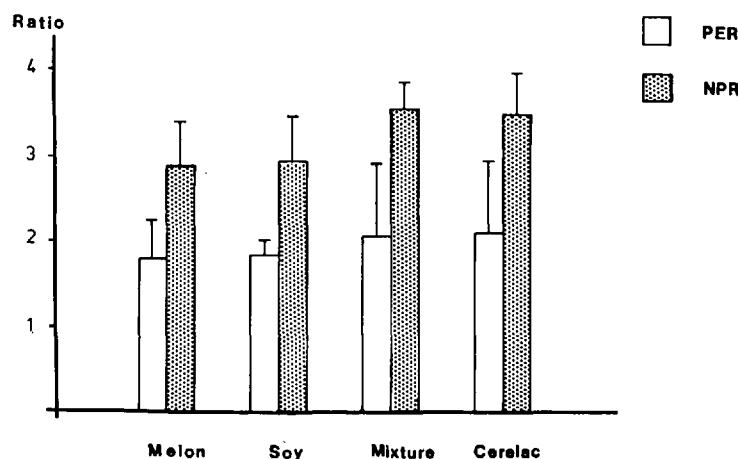


Fig. 2. The protein efficiency ratio (PER) and net protein ratio (NPR) during the experimental period (mean  $\pm$  SEM).

### *Tissue response to dietary changes*

The efficacy of the mixture of vegetable proteins over the use of individual proteins was further investigated by the total weight gain of the various tissues as given in Table 4. In this respect, however, no significant difference could be observed. On the other hand, as shown in Figure 1, the increase in tissue nitrogen content in the group of animals receiving the mixed diet and those kept on the control was similar. In contrast significant differences were observed with groups treated with the individual proteins compared with either of the aforementioned diets.

In the present work PER and NRP were calculated in the various dietary groups (Fig. 2). The most favourable values were apparent in groups treated with Cerelac (control) and the mixed diets, whereas PER and NPR were inferior in groups receiving individual protein sources.

### **Discussion**

It might be conceivable to explain these observations on the basis of the amino acid contents in the respective diets as well as ratios of the essential to non-essential amino acids. Melon protein contains high contents of methionine, tryptophan, and arginine in proportions that are much higher than the other vegetable proteins, that were tested (16). On the other hand, cowpea protein is known to be rich in lysine while soya bean protein contains appreciable amounts of cystine. Feeding with a mixture of these proteins may therefore approach a profile equal to that of an ideal protein. The results obtained in the present work support the above hypothesis since dietary treatment with the mixed diet resulted in adequate growth and normal tissue nitrogen contents.

In the developed world, milk and dairy products are cheap and readily accessible to needy mothers. On the other hand the developing world

lacks an adequate supply of these products. In tropical, dense forest regions, where parasites make the breeding of cows almost impossible, the access to dairy products is practically absent. During weaning the mothers may produce low quantities of breast milk because of inadequate food intake (17). Since growing infants need adequate protein during the period of weaning, they constitute a highly vulnerable target (17, 18). In the search for alternative sources of proteins to milk proteins it is proposed in this communication that a mixture of digestible, vegetable proteins may be applied in the preparation of weaning foods.

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### *References*

1. Partwardhan VN (1962) Pulses and beans in human nutrition. *Am J Clin Nutr* 11:12-13
2. Parpia HAB, Swaminathan N (1972) Supplements of cereals and cereal diets with grain legumes and limiting amino acids. *Proc 9th Int Congr Nutr Mexico* 4:139-148
3. Teply LT, György P (1962) Vegetable protein in infants feeding. *P Paed* 61:-925-933
4. Chi-yuan Chow (1983) Studies on the use of soybean food in infant feeding in China and the development of formula 5410. *UNU Food and Nutr Bull* 5:43-52
5. Graham GG (1971) Dietary protein quality in infants and children. Corn-soy-milk blend. *Am J Clin Nutr* 24:416-422
6. Soma K, Chandrasekhara MR, Sankra M (1962) Evaluation of infants foods. *J Food Sci* 11 (1):12-14
7. Doraiswamy TR, Chandrasekhara MR, Subbaraya J (1963) Infant feeding trials. *Ind J Paed* 30:365-367
8. Akinrele IA (1967) The nutritive value of "ogi", a Nigerian infant food. *J Trop Med Hyg* 70:279-280
9. Fashakin JB, Ogunsola F (1982) The utilization of local foods in the formulation of weaning foods. *J Trop Paed* 28:93-96
10. Bernhart FW, Tomarelli RM (1966) A salt mixture supplying national research council estimates of the mineral requirements of the rat. *J Nutr* 89: 495-500
11. Akinrele IA, Edwards CA (1971) An assessment of maizesoya mixture, "soy-ogi" as a weaning food in Nigeria. *Brit J Nutr* 26:177-185
12. AOAC Official methods of analysis (1970) 11th ed. Association of official analytical chemists, Washington DC
13. Oyenuga VA (1968) Nigerian foods and feeding stuffs. *Ib Univ Press*, p 59
14. Desikachar HSR, Subrahmayan V (1949) Utilization of soy milk in feeding infants. *Ind J Med Res* 13:77-79
15. Shurpalekar SR, Lahiry NI, Chandrasekhara MR (1960) Biological value of spray dried infant foods. *Ann Biochem Exp Med* 20 (6):145-148
16. FAO (Rome) (1970) The amino acid content of foods and biological data on proteins, p 59

17. Collis WRF, Deni IS, Omololu A (1962) The ecology of child nutrition in Nigerian villages. *Trop Geogr Med* 14:140-201
18. Ransome-Kuti O, Gbajumo WO, Olaniyan MO (1972) Some socio-economic conditions predisposing to malnutrition in Lagos. *Nig Med J* 2:111-113

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