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Biological evaluation of protein quality of home-processed supplementary foods for pre-school children

Saroj Dahiya & Amin C. Kapoor*

Department of Foods and Nutrition, Haryana Agricultural University, Hisar — 125 004, India

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Four supplements using wheat, pearl millet (bajra), bengal gram, green gram, groundnuts, jaggery and amaranth leaves were developed, employing roasting and malting techniques. The developed supplements and one commercial weaning food (Cerelac) were evaluated for protein quality, using rats as an experimental model. The values of protein efficiency ratio, food efficiency ratio, biological value, net protein utilization, net protein retention and protein retention efficiency of bajra-based supplements were significantly ($P < 0.05$) higher than those of wheat-based supplements, similar to Cerelac, but significantly ($P < 0.05$) lower than that of casein (standard protein). Rats fed on bajra-based supplements showed the best growth pattern throughout the feeding trial.

INTRODUCTION

Efforts to improve the health and nutritional status of growing children have focused primarily on the production of nutritious low-cost supplementary foods and their acceptability and shelf life. Such foods are often inadequate in energy and density, have high bulk properties and are of poor protein quality. Hence, there is a need for the development of supplementary foods with low viscosity and good quality protein (Chandrasekhar & Devadas, 1986). This paper reports the results of developing low-cost supplementary foods based on local foodstuffs of Haryana, and an attempt has been made to evaluate the biological quality of the protein of the developed home-processed supplement mixtures.

MATERIALS AND METHODS

Materials

The seeds of wheat (WH-283), pearl millet (CJ-104), green gram (K-851), and bengal gram (G-130) were obtained from the Directorate of Farms, Haryana Agricultural University, Hisar, in a single lot. Jaggery, groundnuts and amaranth leaves were procured in a single lot from a local market. The grains were cleaned from dust and other extraneous materials and stored at room temperature in plastic containers.

* To whom correspondence should be addressed.

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Processing procedures

Soaking
Bengal gram and green gram were soaked in a double amount of water at ambient temperature for 12 h.

Sprouting
The soaked seeds were wrapped in damp muslin cloth and allowed to sprout at room temperature (25–30°C) and relative humidity (70%) for 48 h (bengal gram) and 24 h (green gram). The sprouts were then fan-dried overnight.

Malting
Sprouted pulses were roasted in an oven at 70°C for 2 h each to develop malt aroma.

Roasting
Grains of wheat, pearl millet were roasted in an oven at 70°C for 2, 1 and 2 h, respectively.

Drying
Amaranth leaves were cleaned, washed, sun-dried and finally powdered.

Milling
The malted and roasted ingredients were ground in a cyclone mill separately (mesh size 0·5 mm).

Blending
The flour thus obtained and powdered jaggery were thoroughly blended.

Preparation of supplement mixtures

Cereal–legume–nut combinations, which could provide 400 kcal, 8–9 g of protein, 6·0 mg of iron and 0·4 g of calcium per day and an amino acid make-up similar to that of egg protein, were adopted. The following supplement mixtures were developed:

I. Roasted wheat (40 g), malted bengal gram (10 g), roasted groundnuts (10 g), dried amaranth (10 g) and jaggery (40 g).
II. Roasted wheat (40 g), malted green gram (10 g), roasted groundnuts (10 g), dried amaranth (10 g) and jaggery (40 g).
III. Roasted pearl millet (40 g), malted bengal gram (10 g), roasted groundnuts (10 g), dried amaranth (10 g) and jaggery (40 g).
IV. Roasted pearl millet (40 g), malted green gram (10 g), roasted groundnuts (10 g), dried amaranth (10 g) and jaggery (40 g).

Chemical analysis

The fresh supplement samples were analysed for moisture, nitrogen and fat content (AOAC, 1980). The amount of crude protein was calculated as total N × 6·25.

Animal experiments

The fresh supplements, Cerelac and casein were fed to rats for biological evaluation of protein quality.

Diet

The test diets contained supplement mixture and Cerelac so as to provide 10% protein, 4% salt mixture, 1% vitamin mixture, 10% oil, 10% sucrose and corn starch to make 100%. A control diet containing 10% casein protein was also prepared (Table 1).

Growth experiment

Protein efficiency ratio (PER) was determined by the method of Chapman *et al.* (1959). Young male albino Wistar rats aged 28 to 30 days and weighing 35 ± 5 g were obtained from the Disease and Germ Free Small Animal House, Haryana Agricultural University, Hisar. Forty-eight rats were divided into six groups each consisting of eight rats. Groups I, II, III and IV served as experimental groups.

Group V and the casein group received Cerelac diet and casein diet, respectively, and served as control groups. The rats were housed individually in polypropylene cages in an air-conditioned room (21 ± 2°C). Water and food were given *ad libitum*.

Weighed diet was given and unconsumed diet was collected, dried and weighed daily. The experiment was conducted for 28 days. The rats were weighed initially and then twice a week. Food efficiency ratio (FER) and PER were determined as follows:

$$\text{FER} = \frac{\text{Gain in body weight (g)}}{\text{Food intake (g)}}$$

$$\text{PER} = \frac{\text{Gain in body weight (g)}}{\text{Protein intake (g)}}$$

Nitrogen balance studies

Nitrogen balance studies were carried out by the method described by Chick *et al.* (1935). Forty-eight white albino Wistar male rats (weighing 70 ± 5 g) were distributed into six groups (each consisting of eight rats) and housed individually in polypropylene metabolic cages. One group of rats was fed a protein-free diet, and the remaining six groups (experimental groups I, II, III, IV, Cerelac and casein diets, respectively, as described earlier). Food and water were given *ad libitum*. The experiment was conducted for 14 days; the first 9 days were for acclimatization and the remaining 5 days as the collection period; urine and faeces were separately collected each day from each rat and pooled separately

Table 1. Composition of experimental diets (g/100 g diet)

Components	Casein	Supplement I	Supplement II	Supplement III	Supplement IV	Cerelac
Casein	11·76	—	—	—	—	—
Supplement I	—	80·25	—	—	—	—
Supplement II	—	—	78·30	—	—	—
Supplement III	—	—	—	85·47	—	—
Supplement IV	—	—	—	—	84·74	—
Cerelac	—	—	—	—	—	64·51
Sucrose	10·00	—	—	—	—	—
Groundnut oil	10·00	4·70	4·90	4·00	4·25	1·00
Mineral mixture I	4·00	4·00	4·00	4·00	4·00	4·00
Vitamin mixture II	1·00	1·00	1·00	1·00	1·00	1·00
Cellulose	5·00	—	—	—	—	—
DL-Methionine	0·30	—	—	—	—	—
Choline bitartrate	0·20	0·20	0·20	0·20	0·20	0·20
Corn starch	57·74	9·85	11·60	5·33	5·81	29·29

for 5 days. A few drops of H_2SO_4 (1 : 1) were added to urine to prevent any loss of ammonia. Samples of faeces were dried at 70°C, weighed and powdered. The concentrations of nitrogen in urine and faeces were estimated (AOAC, 1980). The change in body weight during the period was also recorded. The data obtained from this experiment were used to calculate various parameters.

True digestibility (TD) and biological value (BV) were calculated as follows:

$$TD = \frac{N_i - (NF_1 - NF_2)}{N_i} \times 100$$

$$BV = \frac{N_i - (NF_1 - NF_2) - (NU_1 - NU_2)}{N_i - (NF_1 - NF_2)} \times 100$$

where

N_i = nitrogen intake of animals fed test diet;
NF_1 = nitrogen excreted in faeces of animals fed test diet;
NF_2 = nitrogen excreted in faeces of animals fed protein-free diet;
NU_1 = nitrogen excreted in urine of animals fed test diet;
NU_2 = nitrogen excreted in urine of animals fed protein-free diet.

Net protein utilization (NPU) was determined according to the method of Platt *et al.* (1961):

$$NPU = \frac{BV \times TD}{100}$$

Net protein retention (NPR) and protein retention efficiency (PRE) were calculated according to the following formulae (Bender & Doel, 1957):

$$NPR = \frac{\text{Weight gain of test group + weight loss of protein free groups}}{\text{Weight of test protein consumed}}$$

$$PRE = NPR \times 16$$

Utilizable protein (UP) was calculated as follows (Gupta *et al.*, 1977):

$$UP = \frac{NPU \times N\ (\%\ \text{of dry matter}) \times 6.25}{100}$$

Statistical method

Data were statistically analysed for analysis of variance to know the significant differences among various groups (Snedecor & Cochran, 1967).

RESULTS AND DISCUSSION

FER and PER

Food intake of rats fed on different supplements, Cerelac and Casein varied from 165·2 ± 8·72 (supplement I) to 200·2 ± 12·42 g (Cerelac) (Table 2). It was significantly ($P < 0.05$) higher in the casein-fed group than in rats fed on supplements I and II and non-significantly higher than rats fed on supplements III and IV. Food intake of rats fed on supplements I and II was significantly ($P < 0.05$) lower than that of rats fed on supplements III and IV. Food intake of rats fed on Cerelac was significantly ($P < 0.05$) higher than those fed on supplements I, II and III, and non-significantly higher than those fed on supplement IV and the casein diet. A similar trend was observed in the protein intake of different groups, being highest (20·25 ± 1·22 g) in the Cerelac group and lowest (16·5 ± 0·81) in the supplement I fed group. Protein intake of rats fed on the casein diet was significantly ($P < 0.05$) higher than for those fed on supplements I and II and non-significantly higher than those fed on supplements III and IV and the Cerelac group.

The gain in body weight of growing rats was maximum in the casein (56·2 ± 8·98 g) fed group and minimum (38·2 ± 4·50 g) in rats fed on supplement I. The gain in body weight of rats fed on supplements I, II, and III was significantly ($P < 0.05$) lower than those fed on casein and Cerelac. The other groups (I, II and III) did not differ significantly among themselves with respect to their body weight (Table 3). Although the gains in body weight of rats fed on Cerelac and casein were almost the same, the food intake of the Cerelac group was non-significantly higher. This shows that the casein protein was slightly better assimilated than that of Cerelac.

Table 2. Food intake, protein intake and gain in body weight of rats, and food efficiency ratio (FER) of supplements (mean ± SD of eight rats)

Dietary group	Food intake (g)	Protein intake (g)	Gain in body weight (g)	FER	Corrected[a] PER
I	165·2 ± 08·72	16·50 ± 0·81	38·2 ± 4·50	0·22 ± 0·005	1·95
II	170·3 ± 16·50	17·20 ± 1·39	40·1 ± 3·10	0·23 ± 0·007	1·99
III	180·4 ± 13·50	18·10 ± 0·65	46·0 ± 5·25	0·25 ± 0·014	2·15
IV	182·5 ± 10·92	18·15 ± 0·92	48·7 ± 6·02	0·26 ± 0·08	2·27
Cerelac V	200·2 ± 12·42	20·25 ± 1·22	56·0 ± 7·15	0·28 ± 0·013	2·34
Casein	190·1 ± 06·49	19·10 ± 0·78	56·2 ± 8·98	0·29 ± 0·009	2·50
SE (m)	6·57	0·50	3·20	0·006	
CD ($P < 0.05$)	19·75	1·52	9·70	0·020	

[a] Based on values of 2·5 as standard for casein.
CD represents critical difference.

Table 3. True digestibility, biological value, net protein utilization, utilizable protein and dry matter digestibility of supplements, Cerelac and casein fed to albino rats (mean ± SD of eight rats)

Dietary group	Nitrogen consumed (mg/day)	Nitrogen absorbed (mg/day)	True digestibility (%)	Nitrogen retained (mg/day)	Biological value (%)	Net protein utilization (%)	Utilizable protein (%)	Dry matter digestibility (%)
I	94·1 ± 3·1	82·1 ± 6·1	88·2 ± 4·4	56·1 ± 2·1	74·8 ± 0·8	66·0 ± 2·3	6·58 ± 0·32	82·35 ± 0·95
II	96·2 ± 4·9	85·1 ± 8·0	89·4 ± 3·3	57·1 ± 2·0	76·9 ± 0·9	68·8 ± 3·3	6·83 ± 0·25	82·90 ± 1·20
III	97·1 ± 2·4	88·1 ± 10·2	83·2 ± 4·3	58·7 ± 1·6	79·1 ± 1·8	65·8 ± 3·4	6·52 ± 0·40	83·15 ± 1·05
IV	98·1 ± 6·3	89·0 ± 5·2	84·4 ± 2·1	59·0 ± 1·9	80·3 ± 1·7	67·8 ± 2·9	6·75 ± 0·20	83·78 ± 0·82
Cerelac V	108 ± 5·6	100 ± 7·2	90·3 ± 3·0	61·6 ± 1·8	82·9 ± 0·9	74·8 ± 1·9	7·42 ± 0·28	85·12 ± 0·79
Casein	115 ± 4·2	103 ± 4·3	93·3 ± 4·2	77·3 ± 1·7	89·5 ± 1·6	33·5 ± 2·7	8·30 ± 0·35	90·15 ± 0·68
SE (m)	2·64	4·64	1·50	0·73	0·76	1·20	0·16	0·43
CD ($P < 0{\cdot}05$)	7·95	12·42	4·50	2·20	2·29	3·60	0·50	1·30

CD represents critical difference.

Table 4. Net protein retention (NPR) and protein retention efficiency (PRE) of supplements, Cerelac and casein (mean ± SD of eight rats)

Dietary group	Gain in weight (g)	Weight loss (g)	Protein consumed (g)	NPR	PRE
I	10·60 ± 0·26	2·8 ± 0·08	5·75 ± 0·13	2·33 ± 0·06	37·3 ± 1·20
II	11·10 ± 0·59	2·8 ± 0·07	5·85 ± 0·10	2·37 ± 0·08	37·9 ± 1·45
III	12·40 ± 0·49	2·8 ± 0·09	5·99 ± 0·24	2·53 ± 0·10	40·5 ± 1·35
IV	12·90 ± 0·38	2·8 ± 0·05	6·10 ± 0·16	2·57 ± 0·09	41·1 ± 1·00
Cerelac V	14·05 ± 0·22	2·8 ± 0·02	6·25 ± 0·19	2·69 ± 0·07	43·0 ± 0·98
Casein	15·00 ± 0·27	2·8 ± 0·03	6·20 ± 0·27	2·87 ± 0·05	45·9 ± 0·86
SE (m)	0·21	—	0·11	0·04	0·72
CD ($P < 0·05$)	0·64	—	0·34	0·14	2·10

CD represents critical difference.

The corrected PERs of different diets varied from 1·95 (supplement I) to 2·50 (casein). The PERs of bajra-based supplements were significantly ($P < 0·05$) higher than those of wheat-based supplements. The PER of Cerelac was significantly ($P < 0·05$) higher than that of supplements I, II, III and IV. Similarly, the PER of casein was significantly ($P < 0·05$) higher than that of all the supplements and Cerelac.

The PER values of different supplements obtained in the present study are similar to those reported by Vaidehi & Godwa (1981), who have also obtained PERs of 2·17 to 2·64 in extruded weaning foods containing wheat flour, bengal gram, green gram, groundnut, sesame flours, whole milk powder and sugar. Malleshi *et al.* (1986) also found that a weaning food prepared from malted ragi and green gram (7:3) had a PER of 2·2, while Fashkin & Ogunsole (1962) reported lower PERs, 0·60, 0·70, 1·70, for Nutogi, Soy-ogi and Cerelac, respectively.

Bunce & King (1969) reported that the PER was maximum at 12% dietary protein when rats were fed on diets containing different levels of protein for 3 weeks. They further observed that PER of rats fed varying levels of dietary protein was maximum at 8% protein and decreased as the protein content of diet increased above this level.

TD, BV, NPU and UP

True digestibility of all the supplements varied from 83·2 ± 4·3 (supplement III) to 93·3 ± 4·2% (casein) (Table 4). The TD of wheat-based supplement (II) was significantly ($P < 0·05$) higher than those of bajra-based supplements (III and IV). However, non-significant differences were observed between wheat-based supplements (I and II) and between bajra-based supplements (III and IV). Casein and Cerelac had significantly ($P < 0·05$) higher true protein digestibility than bajra-based supplements (III and IV).

Supplement I had the lowest BV (74·8 ± 0·8%) and casein the highest (89·5 ± 1·6%). Bajra-based supplements (III and IV) had significantly ($P < 0·05$) higher BV than wheat-based supplements (I and II). Cerelac had a significantly ($P < 0·05$) higher BV than all the supplements except supplement IV. The BV of casein differed significantly ($P < 0·05$) from that of all the supplements and Cerelac.

Net protein utilization varied from 65·8 ± 3·4% (supplement III) to 83·5 ± 2·7% (casein) (Table 4). All the supplements did not differ significantly ($P < 0·05$) with respect to their NPU. However, Cerelac and casein had significantly ($P < 0·05$) higher NPUs than those of all the supplements. Casein had the highest NPU (83·5), which differed significantly ($P < 0·05$) from all the supplements and Cerelac. The same trend was followed in dry matter digestibility of all the supplements, Cerelac and casein diet.

Ahmed *et al.* (1981) also reported NPU of 66 to 75% in rice mixture with pulse, whereas Malleshi *et al.* (1986) reported lower NPUs (51·6%) in malted ragi and green gram weaning mixtures. The differences in NPU may be due to the different composition of the supplements, and absorption and digestibility effect in the biological system.

The lower values of PER, TD, BV and NPU in supplement I may be due to roasting as it adversely affects the availability of some amino acids. Similarly, Chopra & Hira (1986) reported that PER, TD, BV and NPU decreased on roasting, but the effect was greater with rice and maize as compared to wheat.

Among supplements, UP in supplement II was maximum (6·83%) followed by supplements IV, I and III (Table 4). Utilizable protein of supplements I, II, III and IV was significantly ($P < 0·05$) lower than that of casein and Cerelac. Values of UP for supplements II and IV were similar. Casein had the maximum UP (8·30%), which was significantly ($P < 0·05$) higher than those of all the supplements and Cerelac.

NPR and PRE

The average gain in weight on wheat-based supplements I and II was significantly ($P < 0·05$) lower than on bajra-based supplements (III and IV), Cerelac and casein. However, wheat-based supplements (I and II) and bajra-based supplements (III and IV) did not differ significantly ($P < 0·05$) between themselves in body weight gain. Casein-fed groups had the highest gain in body weight, which differed significantly ($P < 0.05$) from all the other groups.

A similar trend was observed in NPR of all the supplements. The NPR of wheat-based supplements (I and II) was found to be significantly ($P < 0.05$) lower than bajra-based supplements (III and IV), Cerelac and casein. However, bajra-based supplements and wheat-based supplements did not differ significantly between themselves with regard to NPR values. Casein had the highest NPR (2.87 ± 0.05), which differed significantly ($P < 0.05$) from all the other diets (Table 4).

Since PRE is obtained by multiplying NPR by 16, the trend of results obtained is similar to that of NPR (Table 4).

Malting and roasting of different cereals and pulses had significant effects on the NPR and PRE values of supplements. The NPR of Cerelac observed during the present study was higher than that estimated by other workers. NPR is a more accurate measure of protein quality than PRE as it allows the evaluation of maintenance requirement, and results are independent of food intake.

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