**Protein Digestibility of Weaning Foods Prepared
from Rice-Minced Meat and Rice-Mungbean
Combination in Infants Using a Short
Term Nitrogen Balance Method¹**

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Summary Three diets based on rice-dehulled mungbean, rice-minced meat and rice-mungbean with hull were tested with infants 11 to 20 months of age using a short term nitrogen balance technique. The results indicate that with isocaloric and isonitrogenous intake, all the subjects given either of the three diets were in positive nitrogen balance. The protein quality, in terms of nitrogen absorption and true digestibility, of rice-meat diet was superior to that of rice-bean diets. Among the rice-bean diets, it was noted that rice-mungbean with hull had a lower digestibility as compared to rice-dehulled mungbean diet. The poor digestibility of rice-mungbean with hull diet is the first limiting factor in its utilization by infants. Dehulling of mungbean before cooking is recommended for preparing weaning food for infant feeding.

Key Words protein digestibility, weaning foods, rice and mungbean

It is well recognized that malnutrition affects both physical and mental development. The most evident manifestation during the first year of life is physical growth retardation (1, 2). Recently it has been shown that it affects central nervous system and gives rise to low intellectual capacity and behavioral abnormalities. Children with severe malnutrition also show decrease in adaptation capacity and defense to environment, (3-5) leading to infection and increased mortality.

In Pakistan, malnutrition among infants and young children is one of the nations most severe health problems. The nutrition survey conducted in 1976 (6) and the dietary survey of the North West Frontier province in 1979 (7) have all pointed toward the prevalence of protein energy malnutrition (PEM) among

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children of low socio-economic group in both the rural and urban population.

It has been reported that 56% of the children in Pakistan suffer from various degrees of malnutrition, with over 7% and about 10% suffering from severe and moderately severe malnutrition respectively. Of every 10 children born, one dies before his first birthday and 25 in every 100 die before reaching their fifth year of life. Some 80% of the children under 5 years suffer from diarrhea and respiratory infection. Acute dehydration caused by diarrhea kills 30% of malnourished as against 2% of well nourished babies (8). Malnutrition is prevalent due to inadequate food intake, illiteracy, poverty, lack of clean drinking water, infectious diseases, improper weaning and feeding practices.

The best time to introduce solid food is 4-6 months, but in Pakistan 80% of the mothers breast feed their children up to 2 years, and introduce solid food at the age of 1½-2 years. This late introduction of weaning food to the infants has been one of the major factors contributing to PEM and high incidence of infant mortality (7).

Since the high cost of weaning food available in the market is also one reason for withholding solid food till the age of 2 years, it was considered appropriate to investigate the formulation of home prepared weaning food for infants from locally available plant and animal sources taking into consideration the normal diet pattern, habitual weaning food, price structure and acceptability of the products.

Cereal like wheat is the staple diet in Pakistan. Rice is commonly available. However, these cereals are deficient in lysine. As more than 80% of the children are dependent on cereal as a major source of protein and energy at the weaning age, it would give rise to nutritional deficiencies and adverse health (9). It has been recognized that legumes are comparatively rich in lysine and therefore a combination of cereal and legume can provide an ideal source of dietary protein, and their increased consumption is widely recommended for infants and children.

Formulation and nutritional quality of home prepared weaning food have been described previously (10). Many workers have also reported the nutritional quality of infant foods based on cereal and legume combination (11-16).

The digestibility of weaning food plays an important role in the overall acceptability and quality of the product.

People mostly in the rural communities withhold legumes from the diet of the weaned infants, presumably due to the poor digestibility of the bean, which may be another factor contributing to protein deficiency in this age group.

The poor digestibility of common beans and other food legumes in comparison with animal protein has also been recognized by various investigators (9, 17), but not many works have been done with human subjects.

In the present paper, protein digestibility of 3 infant food mixtures prepared from rice-mungbean dehulled, rice-meat and rice-mungbean with hull, using a short term nitrogen balance method in human infants, has been reported and discussed.

MATERIALS AND METHODS

Subjects. Six male children 1–2 years of age were selected with the help of medical staff from Rajavithree orphanage. The subjects were initially treated for any bacterial and parasitic infection with proper medication, and were rehabilitated and fed milk-egg and other foods for at least 6–8 weeks. When the subjects were normal weight for height, healthy and without any symptom of infection the study was undertaken. The objective and design of the study were approved by the committee on clinical investigation of the Faculty of Medicine, Ramathibodi Hospital Bangkok, before the beginning of the study. The initial characteristics of the subjects are given in Table 1.

Diets. Rice (*Oryza sativa*), mungbean (*Vigna radiata*), meat and vegetable oil were purchased from the local market. Three diets were prepared. The formulation and methods of preparation of the diets are given in appendix 1. The approximate

Table 1. Initial characteristics of subjects studied for metabolic evaluation of weaning foods.

Subjects	Age (month)	Weight (kg)	Height (cm)
ND	20	9.96	78.4
PP	18	10.76	76.2
SK	11	9.90	75.5
DP	17	9.66	73.4
AC	14	9.89	76.5
TP	18	8.53	69.5
Mean $\pm$ SE	16.3 $\pm$ 1.3	9.8 $\pm$ 0.3	74.9 $\pm$ 1.3

Table 2. Proximate compositions of the weaning foods.

Weaning food		Protein (g/100 g)	Fat	Energy (kcal/100 g)
Diet I	Rice: dehulled mungbean: oil (60: 30: 10)	12.2	13.7	412
Diet II	Rice: minced meat: oil (60: 30: 10)	13.6	14.2	430
Diet III	Rice: mungbean with hull: oil (60: 30: 10)	12.5	13.3	408

Numbers in parentheses indicate the proportion of ingredients.

Table 3. Nutrient compositions per 100 kcal of weaning foods.

Weaning food	Protein (g)	Fat (g)
Diet I Rice: dehulled mungbean: oil (60: 30: 10)	2.96	3.32
Diet II Rice: minced meat: oil (60: 30: 10)	3.23	3.30
Diet III Rice: mungbean with hull: oil (60: 30: 10)	3.06	3.25

Numbers in parentheses indicate the proportion of ingredients.

Table 4. Protein, fat and energy contents of the cooked weaning foods.

Weaning food	Protein (g/100 g)	Fat (g/100 g)	Energy (kcal/100 g)
Diet I Rice: dehulled mungbean: oil (60: 30: 10)	1.89	3.04	89
Diet II Rice: minced meat: oil (60: 30: 10)	2.57	2.97	97
Diet III Rice: mungbean with hull: oil (60: 30: 10)	1.92	2.66	82

Numbers in parentheses indicate the proportion of ingredients.

compositions of the weaning foods are presented in Table 2, while the nutrient compositions per 100 kcal are shown in Table 3.

The experimental diets were based on rice-dehulled mungbean-oil for diet I, rice-minced meat-oil for diet II and rice-mungbean with hull and oil for diet III. The diet I and diet III were given to infants to compare the effect of dehulling on digestibility of rice-bean combination. The ingredients in these diets were chosen considering availability cost and acceptability.

The protein, fat and energy contents per 100 g of the cooked food mixtures are shown in Table 4.

Level of protein and energy intake. The protein intake was 1.7 g/kg/day, while the energy intake was 110 kcal/kg/day (5). The levels of protein and energy intake were kept constant throughout the entire study.

Vitamin intake. The vitamin supplement was given daily during the metabolic period. The composition of multivitamin syrup is shown in Table 5. Each subject was orally given 0.6 ml of this preparation daily.

Table 5. Compositions of the multivitamin preparation (Deca-vi-sol drops).

Vitamin	Per 0.6 ml
Vitamin A, U.S.P. units	3,000
Vitamin D, U.S.P. units	400
Vitamin C, mg	60
Thiamine (B ₁), mg	1
Riboflavin (B ₂), mg	1.2
Niacinamide, mg	8
Pyridoxine (B ₆), mg	1
Cyanocobalamin (B ₁₂), µg	1
Panthenol, mg	3
Biotin, µg	30

Fig. 1. Experimental design for metabolic study. A, adaptation period; B, balance period.

Methods. i) Chemical analysis. The protein and fat contents of the weaning food mixtures were determined by the methods described in A.O.A.C. (18), while the energy density was measured by a bomb calorimeter.

ii) Metabolic evaluation. Experimental design: The subjects were studied for 3 consecutive metabolic periods with 3 day resting period in between. Each metabolic period lasted for 7 days, with 4 day adaptation and 3 day balance periods. The experimental design for the metabolic evaluation is given in Fig. 1, during the balance period the subjects were placed in the metabolic bed as designed by Fomon (19) for complete urine and fecal collection.

Nitrogen balance studies. In the balance period daily urine samples were collected in bottles to which 5 ml of toluene was previously added as preservative and analyzed for nitrogen. Feces were collected and pooled for 3 days. Carmine dye, 50 mg was used as a stool marker and was given at the beginning and end of each balance period. Pooled feces were mixed and diluted with water to a fixed volume. An aliquot was analyzed for total nitrogen. Anthropometric measurements were done at the beginning and at the end of one week experimental period except for body weight which was measured daily in the morning before breakfast. The nitrogen balance, apparent digestibility or nitrogen absorption as percent of intake and true digestibility were calculated from the nitrogen balance data as follows (20).

(a) Nitrogen balance = N intake – fecal N – urinary N – skin N

(b) Apparent digestibility
or

$$\text{Nitrogen absorption} = \frac{\text{N intake} - \text{fecal N}}{\text{N intake}} \times 100$$

$$(c) \text{ True digestibility} = \frac{\text{N intake} - (\text{fecal N} - \text{fecal obligatory N})}{\text{N intake}} \times 100$$

The values of obligatory N excretion were obtained from the data recommended by FAO/WHO/UNU expert committee (21). They recommended obligatory N losses in urine, feces and skin as 41, 21 and 10 mg N/kg/day respectively.

RESULTS AND DISCUSSION

Nitrogen balance

The results on the mean nitrogen balance data of the infants given the three weaning foods are presented in Table 6. It was noted that at a similar level of protein and energy intake of 1.7 g/kg/day and 110 kcal/kg/day, all the subjects given either of 3 diets were in positive nitrogen balance. Subjects given diet II prepared from rice-minced meat showed a significantly higher mean nitrogen balance of 102.3 mg/kg/day than diets I and III prepared from rice dehulled mungbean and rice mungbean with hull, which were found to have a mean nitrogen balance of 68.6 and 74.6 mg/kg/day respectively. No significant differences in the mean of nitrogen balance between diets I and II and diets I and III were observed, while the difference was statistically significant between diets II and III. The mean nitrogen retention as percentages of intake was lower, 25.9 and 29.0, for diets I and III made from rice-mungbean combination as compared to 38.7 observed for diet II, but there were no statistical differences in the mean nitrogen retention of the three diets. Graham *et al.* (17) have also reported lower nitrogen retention of beans as compared to methionine enriched beans fed to infants.

A significantly higher positive nitrogen balance in infants given diet II and a positive nitrogen balance with diets I and III indicate that while diet II is superior in overall protein adequacy, diets I and II are also adequate for supporting body nitrogen balance.

Protein digestibility

The protein digestibility of the three experimental diets is shown in Table 7. The results indicate that the mean of apparent digestibility or nitrogen absorption as percentages of intake was 78.2 for diet II and 66.6 and 64.4 for diets I and III respectively. There were no significant differences in the mean nitrogen absorption of diets I and II and diets I and III, while significant difference was observed in diets II and III. Calloway and Kretsch (21) reported protein digestibility of 69% for subjects fed a diet based on cereal and bean combination. Their results are

Table 6. Nitrogen balance of the infants fed the three weaning foods.

Weaning food	N intake (mg/kg/day)	Urinary N (mg/kg/day)	Fecal N (mg/kg/day)	Skin N (mg/kg/day)	N retention as % of intake	N balance (mg/kg/day)
Diet I Rice: dehulled mungbean: oil (60:30:10)	265.6±2.0	98.2±11.7	88.7±15.7	10	25.9±2.7	68.6±6.9
Diet II Rice: minced meat: oil (60:30:10)	264.8±2.2	95.0±14.5	57.5±15.7	10	38.7±5.1	102.3±13.4
Diet III Rice: mungbean with hull: oil (60:30:10)	255.0±6.5	80.3±11.3	90.1±15.2	10	29.0±2.8	74.6±8.4
Statistical analysis						
I vs II					NS	NS
II vs III					NS	$p<0.05$
I vs III					NS	NS

Table 7. Protein digestibility of the three weaning foods fed to infants.

Weaning food	Number of subjects	N intake (mg/kg/day)	Fecal N (mg/kg/day)	Apparent digestibility or N absorption as % of intake	True digestibility (%)
Diet I Rice: dehulled mungbean : oil (60 : 30 : 10)	6	265.6 ± 2.0	88.7 ± 15.7	66.6 ± 5.9	74.6 ± 5.9
Diet II Rice: minced meat : oil (60 : 30 : 10)	6	264.8 ± 2.2	57.5 ± 15.7	78.2 ± 6.0	86.1 ± 5.9
Diet III Rice: mungbean with hull : oil (60 : 30 : 10)	6	255.0 ± 6.5	90.1 ± 15.2	64.4 ± 6.2	72.7 ± 6.1
Statistical analysis					
I vs II				NS	NS
II vs III				$p < 0.01$	$p < 0.01$
I vs III				NS	NS

somewhat similar to the apparent digestibility values of 66.6% and 64.4% noted in the present work, though there were differences in the protein intake and subject age.

True digestibility was calculated using an endogenous fecal nitrogen value of 21 mg N/kg/day (22). As expected the values for all the preparation increased. Diet II, a rice-meat combination, showed a higher mean true digestibility of 86.1% as compared to the mean true digestibility of 74.6% and 72.7% observed for diets I and III, a rice-bean combination. A statistically significant difference in the true digestibility was found in diets II and III, but there were no significant differences in diets I and II and diets I and III. Mean fecal nitrogen was correspondingly low 57.5 mg/kg/day in subjects fed diet II, while it was higher 88.7 mg/kg/day and 90.1 mg/kg/day in subjects given diets I and III respectively. These results agreed well with those of Naverrete and Bressani (14) who found higher fecal output of nitrogen in the bean fed subjects as compared to subjects fed ground meat. The same authors reported that the apparent and true digestibilities of beans were lower than those of meat, which are similar to the observation recorded in this study. The large fecal output of nitrogen in subjects given rice-bean diet and those reported elsewhere has been considered to be due to the poor digestibility of beans.

The present results showed that diet II prepared from rice-meat had a higher protein digestibility than diets I and III prepared from rice-bean combination. In the case of the two rice-bean diets, it was found that rice-dehulled mungbean, diet I has a higher nitrogen absorption and true digestibility than rice-mungbean with hull of diet III. It has been reported that the level of fat as a source of energy intake has no significant effect on the protein utilization provided that the body received adequate intakes of protein and energy (15). The dietary intakes in the present studies were isocaloric and isonitrogenous, therefore it seems that the improved digestibility with diet II may be due to a better amino acid pattern and the availability of amino acids for digestion and absorption with rice-meat protein, while in rice-bean diets I and III, the presence of hull which is mostly fibre may be a reason for the low digestibility of diet III. These observations are similar to those of Graham *et al.* (17) and Navarrete and Bressani (14).

Body weight changes

The results of the body weight changes in individual subjects given the 3 experimental diets are shown in Table 8. It was noted that all the subjects gained weight during the experimental period in which diets I and II were given. The body weight increased significantly with the first two diets as compared to diet III. The highest mean weight gain of 66 g/day was noted for diet II followed by an increase of 50 g/day with diet I, though there were no statistically significant differences in the mean weight gain of the subjects with diet I and diet II. For diet III, four subjects lost and two gained weight. The energy intake was isocaloric being 110 kcal/kg/day and isoproteinic 1.7 g/kg/day, which is the recommended level of energy and protein requirements of this age group (22). In our study it was noted

Table 8. Daily weight change of infants during metabolic studies (g/day).

Subjects	Experimental period		
	Diet I	Diet II	Diet III
NP	+48	+100	-23
PP	+44	+76	-34
SK	+44	+82	-12
DP	+75	+16	+8
AC	+68	+30	+34
TP	+22	+90	-17
Mean $\pm$ SE	+50.2 $\pm$ 7.8	+65.7 $\pm$ 14.0	-7.3 $\pm$ 10.0
Statistical analysis			
I vs II	NS		
II vs III	$p < 0.0025$		
I vs III	$p < 0.0025$		

that even with the recommended level of energy and protein intakes, subjects lost weight during the experimental period in which diet III was given. The reason for the net loss in body weight may be due to the presence of hull, which is mostly crude fibre in diet III. A high fibre diet may decrease the availability of energy from the diet, moreover a short term balance period of 7 days may not be fully indicative of the dietary effect on body weight (23). A long term balance study is required to be made with infants comparing the three diets and possibly increased the number of subjects studied, because there were considerable variations in the subjects in their response to the diets. Nevertheless, it is clear from the results of this metabolic evaluation that protein quality and digestibility of rice-meat diet are superior to those of rice-bean diets. The rice-mungbean diets also show a fairly good protein quality but it is necessary to process the legume more extensively and it is recommended to remove the hull before cooking for preparing weaning food for infant feeding.

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APPENDIX

*Formulation and preparation of the three weaning foods for metabolic evaluation.**Diet I.*

Ingredients	Amount (g)
1. Rice	60
2. Mungbean dehulled	30
3. Oil	10

Method. Soak mungbean in water for overnight. Remove the hull from mungbean. Clean and wash rice. Put rice and dehulled mungbean in a pot with 2-3 cups of water, boil, add oil and cook till soft and tender, blend and serve.

Diet II.

Ingredients	Amount (g)
1. Rice	60
2. Minced meat	30
3. Oil	10

Method. Clean and wash rice, add minced meat and rice in a pot with 2-3 cups of water and boil, add oil and cook till soft and tender, blend and serve.

Diet III.

Ingredients	Amount (g)
1. Rice	60
2. Mungbean with hull	30
3. Oil	10

Method. Soak mungbean in water for overnight, the hull was not removed from mungbean. Clean and wash rice. Put rice and mungbean with hull in a pot with 2-3 cups of water, boil, add oil and cook till soft, blend and serve rice-mungbean with hull diet.