

# Protein evaluation of weaning foods processed from wheat and rice supplemented with green gram and lentil by using weanling rats (*Rattus norvegicus* strain Albino)

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**Summary.** Although breast milk is adequate to meet the energy and nutrient requirements of an infant up to four to six months of age, thereafter it is insufficient to sustain normal growth and needs to be supplemented with other foods, so called weaning or supplementary foods. Plant protein sources such as legumes can be used in child feeding as an inexpensive and available source. Germination is often used as an applicable and easy natural method to improve nutritional value through availability of minerals and digestibility of protein and starch and reduce or remove anti-nutritional factors in legumes' grain. The current study was conducted to evaluate the protein quality of two different weaning foods using bioassay procedures. The weaning foods were formulated from roasted wheat, rice, germinated and de-hulled green gram and lentil, skim milk powder (SMP) and carrot powder and used for animal assays with different proportions. Briefly, there were ten groups of laboratory rats in total, three groups of rats received reference diets containing three different levels of SMP (3, 6 and 9%) and six groups received weaning foods supplemented with three levels of SMP. Basal diet (0% protein) served as control treatment. Relative protein value (RPV) and relative nitrogen growth index (RNGI) were registered 0.9, 0.85 and 0.78, 0.95 for wheat and green gram based (WG) and rice and lentil based (RL) weaning foods, respectively. Both weaning foods showed high net protein ratio (NPR) and relative net protein ratio (RNPR) values. The results confirm that, although RL weaning food suggests higher protein quality, both formulations can be used safely as weaning or supplementary foods.

**Key words:** cereal, legumes, protein quality, weaning food.

## Introduction

Weaning is the process of gradually introducing a mammal infant to what will be its adult diet and withdrawing the supply of its mother's milk. Weaning foods are processed food given to babies from age of four month and above to supplement breast milk (1). Since breast milk is insufficient to sustain normal growth and needs after four to six months of age (2), it is necessary to supply sufficient calories and protein for the growing child by weaning or supplementary foods. During weaning period also known as food-ac-

customed period (3), children need nutritionally balanced, calorie-dense supplementary foods in addition to mother's milk because of the increasing nutritional demands of the growing body (4,5,1). Thus, weaning food plays a vital role in the all-round growth development and mental health of children (6). On the other hand, the capacity of a supplementary diet to meet the protein and energy requirements of infants depends on its nutritional quality as well as its dietary bulk (2).

Under the present socio-economic situation in developing countries, where weaning foods are relatively expensive and often out of reach of a majority of

the people (7), the best means of helping enhance the nutritional status of the people is by encouraging increased use of inexpensive and available plant protein sources such as legumes in child feeding (8). Combination of commonly used cereals with inexpensive plant protein sources like legumes can be used. Cereals are deficient in lysine but have sufficient sulphur containing amino acids which are limited in legumes (9,10) whereas legumes are rich in lysine (11). A mutual complementation of amino acids and consequent improvement in protein quality is therefore achieved when legumes are blended with cereals in the right proportions (12). The effects of the supplementation are highly beneficial, since nutritive value of the product is also improved.

However, the positive role of combination appears to be limited because of several factors including low protein and starch digestibility (13,14), poor mineral bioavailability (15,16) and high anti-nutritional factors such as protease inhibitors, hemagglutinins, and poly-phenolic compounds (17,18; 19). There are many low cost methods to combat this problem, for instance, soaking, cooking, de-cortication and germination. The soaking and wet extraction operations cause nutrient losses (20). When cooked, the starch granules break open; absorb water and swell, making the gruel usually unpalatable and too thick for babies to swallow. The use of germination is considered the best, since dietary bulk reduction results from the formation of amylases that break down starch. During germination,  $\alpha$ -amylase is synthesized within the aleurone layer and utilized in the starch endosperm, where hydrolysis of the starchy granules occurs. In addition to bulk reduction, germination increases the thiamin, riboflavin, niacin, folic acid, ascorbic acid and iron contents, diastase ratio and biological value of seeds (21). Furthermore, it reduces anti-nutritional factors (decreases phytin) and flatulence-producing factors. Therefore, germinated and de-hulled legumes are the best choices for supplementary foods due to better digestibility and availability of nutrients. Such formulations along with a vegetable were prepared and analyzed for their nutritional composition earlier by Ahmadzadeh and Prakash (22). In addition, it has been reported that protein and thiamin (23), mineral bioavailability (15,24) and protein and starch digestibility (13) increased, whereas phytic acid

(13,23,25) and tannin (17,25) decreased during germination of legumes.

There are many standard bioassay procedures for the evaluation of the nutritive value of proteins utilizing rats. The slope-ratio technique using weight gain as the response and nitrogen intake as the measure of dose appears to be most satisfactory (26). The purpose of this study was to evaluate the protein nutritional quality of the formulated cereal/legume as potential weaning supplements.

## Materials and methods

### *Experimental procedure*

All the raw materials were purchased from local markets. Salt mixture (Bernhart, Tommarelli) used for feed formulation was obtained from Sisco Research Laboratories PVT. LTD, Bombay, India. Other chemicals used for the study were AR grade (A standard grade of analytical reagents) and were purchased from SD Fine, Qualigen Limited, India.

Two different weaning foods were formulated by mixing roasted wheat (*Triticum aestivum* L.) flour (60%), germinated and de-hulled green gram (*Phaseolus aureus* L.) flour (25%), skim milk powder (10%) and carrot (*Daucus sativus* L.) powder (5%) (WG) and rice (*Oryza sativa* L.) flour (60%), germinated and de-hulled lentil (*Lens culinaris* L.) (25%), skim milk powder (10%) and carrot powder (5%) (RL). The formulated weaning foods were made to 30% slurry and then roller dried (double drum drier, Model NS-06, Esher Wyss, Germany). They were tested previously for nutritional qualities (22). Nutrient profiles of the formulations were optimized to satisfy the energy and nutrient requirements of a weaning rat.

### *Animal assay*

Sixty 21-23 days old weanling rats (*Rattus norvegicus* strain Albino) were divided into ten groups. The sex and weight distribution of each group was equalized so that average body weight was not greater than 1 g. All rats were fed by 8% protein reference standard diet for two days. Afterwards, one group of rats received the basal diet (0% protein), three groups of rats received reference diets containing three levels

of SMP (3, 6 and 9%) and finally six groups received weaning foods supplemented with three levels of SMP.

#### *Protein assay and data collecting*

The protein content of formulated diets was analyzed by Kjeldahl method. The rats were given *ad libitum* access to food and water for two weeks. Body weight changes and food intake were monitored twice a week and daily, respectively.

#### *Composition of feed*

The basal diet composition is presented in Table 1. Diets were then made to contain three levels of protein (3, 6 and 9%) by use of formulated supplementary foods. To adjust to 100 percent, corn starch was used.

#### *Protein quality*

##### *Nitrogen growth index (NGI)*

Using regression analysis,  $Y = mX + C$ , where  $Y$  was weight gain and  $X$  was protein intake, the quality of the protein was estimated to be equal to  $m$ , the coefficient of regression which was Nitrogen growth index (NGI).

##### *Relative nitrogen growth index (RNGI)*

It was calculated by dividing NGI of test protein to reference standard protein.

##### *Relative protein value (RPV)*

It was calculated by dividing slope of the test material to slope of the test protein (skim milk powder).

##### *Net protein ratio (NPR)*

Net protein ratio was weight gain of test group of animals plus mean weight loss of non-protein control group per gram protein consumed.

##### *Relative net protein ratio (RNPR)*

Relative Net Protein Ratio was the NPR of the test material expressed relative to the NPR of the reference standard.

#### *Statistical analysis*

The correlation coefficients were computed and regression equations were made.

## **Results and discussion**

Individual protein intake ( $\text{g day}^{-1}$ ) and individual weight gain ( $\text{g day}^{-1}$ ) due to protein levels are shown in Tables 2 and 3. The calculated RPV, NGI and RNGI values are also presented. At 3% protein level, the diets of both weaning foods were just able to maintain the body weight of the rats, because the average weight gain falls to 0-1.0  $\text{g day}^{-1}$ ; whereas the rats gained body weight with superior response at 6 and 9% protein levels. The calories in a diet are provided by protein, fat and carbohydrates (27). (2). In some cases, reduction in weight gain might be attributed to the anti-nutritional factors and toxic constituents present in the legumes (28). It has been reported by Steinke (29) that 5% protein is required as maintenance level for rats. Some researchers have reported that a large population of nursing mothers utilized soybean (30) and pasta as source of protein to feed their children. This trend is caused by the high price of animal proteins and commercial weaning foods that a large proportion of low-income families cannot afford. These mothers typically believe that fortifying local weaning diets with soybean ensures that infants and children consuming these supplemented diets receive their required protein and other nutrients (12,30).

Regression equations were developed for estimating relationship between weight gain and consumed protein. If non-protein data was not included, RPV could be calculated by dividing the slope of test protein to slope of SMP protein. By comparing RPV values of two test proteins, it could be concluded that RL weaning food contains higher RPV value than WG supplementary food. These values are higher than those reported by Malleshiet *al.*(31) for malted ragi and green gram weaning food which had RPV of 0.84. If regression equations with inclusion of non-protein data were derived, the slope of each equation would be accounted as NGI. But the significance of NGI values can be

**Table 1.** Basal diet composition

Ingredient	Amount (%)
Sucrose	10.0
Ground nut oil	9.9
Salt mixture	3.0
Vitamin mixture	1.0
Vitaminized oil	0.1

**Table 2.** Calculation of RPV and NGI from rat growth and protein intake data: 14-day experiment for WG weaning food

Level of protein fed	Individual protein intake g day-1 (X)						Individual weight gain g day-1 (Y)					
	1	2	3	4	5	6	1	2	3	4	5	6
SMP protein												
3%	0.14	0.13	0.12	0.11	0.12	0.13	0.75	0.85	0.64	0.82	0.89	1.0
6%	0.38	0.4	0.36	0.4	0.39	0.43	2.1	2.5	2	2.14	2.17	2.32
9%	0.64	0.66	0.66	0.53	0.64	0.76	3.17	2.53	2.78	2.17	3.17	2.92
Test protein												
3%	0.088	0.11	0.1	0.09	0.14	0.09	0.32	0.21	0.07	0.39	0.21	0.21
6%	0.32	0.36	0.27	0.29	0.22	0.23	1.21	1.6	1.2	0.78	0.64	0.67
9%	0.75	0.76	0.6	0.66	0.6	0.55	2.46	2.32	2	2.21	1.85	1.89
Non-protein	0	0	0	0	0	0	-0.25	-0.53	-0.43	-0.39	-0.43	-0.36

*Regression equations relating weight gain (Y) to protein consumed (X):*  
*A: Non-protein data not included (RPV); I: SMP protein  $Y = 3.74 X + 0.48$ ;  $R = 0.95$   $N = 18$ ; II: Test protein  $Y = 3.36 X - 0.04$ ;  $R = 0.98$   $N = 18$ ;  $RPV = 3.36/3.74 = 0.90$ ; B: Non-protein data included (NGI); I: SMP protein  $Y = 4.75 X - 0.03$ ;  $R = 0.96$   $N = 24$   $NGI$  (SMP protein) = 4.75; II: Test protein  $Y = 3.73 X - 0.22$ ;  $R = 0.98$   $N = 24$   $NGI$  (Test protein) = 3.73;  $RNGI = 3.73/3.74 = 0.78$ ;  $N =$  Number of pairs of observations;  $R =$  Correlation Coefficient*

**Table 3.** Calculation of RPV and NGI from rat growth and protein intake data: 14-day experiment for RL weaning food

Level of protein fed	Individual protein intake g day-1 (X)						Individual weight gain g day-1 (Y)					
	1	2	3	4	5	6	1	2	3	4	5	6
SMP protein												
3%	0.14	0.13	0.12	0.11	0.12	0.13	0.75	0.85	0.64	0.82	0.89	1.0
6%	0.38	0.4	0.36	0.4	0.39	0.43	2.1	2.5	2	2.14	2.17	2.32
9%	0.64	0.66	0.66	0.53	0.64	0.76	3.17	2.53	2.78	2.17	3.17	2.92
Test protein												
3%	0.1	0.11	0.1	0.09	0.09	0.09	0.35	0.53	0.35	0.07	0.5	0.07
6%	0.26	0.35	0.27	0.33	0.27	0.25	1.21	1.32	1.21	1.21	0.71	1.17
9%	0.63	0.68	0.6	0.65	0.61	0.6	2.07	2.53	2.03	2.21	2.17	2.42
Non-protein	0	0	0	0	0	0	-0.25	-0.53	-0.43	-0.39	-0.43	-0.36

*Regression equations relating weight gain (Y) to protein consumed (X):*  
*A: Non-protein data not included (RPV); I: SMP protein  $Y = 3.74 X + 0.48$ ;  $R = 0.95$   $N = 18$ ; II: Test protein  $Y = 3.57 X - 0.04$ ;  $R = 0.98$   $N = 18$ ;  $RPV = 3.57/3.74 = 0.95$ ; B: Non-protein data included (NGI); I: SMP protein  $Y = 4.75 X - 0.03$ ;  $R = 0.96$   $N = 24$   $NGI$  (SMP protein) = 4.75; II: Test protein  $Y = 4.03 X - 0.2$ ;  $R = 0.97$   $N = 24$   $NGI$  (Test protein) = 4.03;  $RNGI = 4.03/4.75 = 0.85$ ;  $N =$  Number of pairs of observations;  $R =$  Correlation Coefficient*

appreciated only when they are expressed in relative terms. Thus, considering milk as 1.0, WG and RL supplementary foods would have relative growth indices of 0.78 and 0.85, respectively.

Means of initial weights and gain in weights, food intake and mean protein intake for SMP, test proteins and non-protein group are presented in Table 4. The calculated NRP and RNPR are also shown. Net protein retention

**Table 4.** Calculation of net protein ratio (NPR) and relative net protein ratio (RNPR) from rat growth for two supplementary foods

Diet	Average initial weigh (g)	Average gain in weigh (g)	Average Food intake (g)	Average protein intake (g)	NPR	RNPR
SMP protein (9%)	26.0±0.8	39.2±1.0	100.9±1.6	9.1±0.3	4.92	1.0
WG weaning food protein (9%)	27.0±0.7	29.7±0.9	100.3±1.6	9.1±0.3	3.88	0.78
RL weaning food protein (9%)	27.0±0.5	31.4±0.8	98.7±1.5	8.8±0.2	4.2	0.85
Non-protein	26.08±0.6	-5.6±0.4	35.7±1.0	-	-	-

is defined as the ratio of sum of weight gain of test protein group and weight loss of non-protein group to that of protein intake of test protein group. The RNPR is the NPR of the test material expressed relative to the NPR of the reference standard, SMP protein that has been defined as 1.0. RNPR is a better measure for comparing different test proteins. From data shown, it is obvious that RL supplementary food had higher RNPR value compared with WG supplementary food and also both weaning foods showed greater RNPR compared with that of malted barley and chickpea weaning food (RNPR=0.72) which has been reported by Wondimu and Malleshi (32). It has also been reported that NPR of different kinds of cowpea (3.2-3.3) and soybean (3-3.1) (30) and the NPR of some different kinds of barley (2.3-2.6) (33) are lower than our tested diets. However, they are near to both kinds of pasta and soybean diets. The higher NPR for commercial weaning food could be due to its quality of protein not its amount.

The difference between RPV, RNGI and RNPR of two studied supplementary foods might be due to this fact that, rice protein quality is higher than wheat. The amino acids compositions of rice and wheat show that essential amino acid content of rice per gram of protein is greater than that of wheat (3, 34). However, a number of studies have shown that the protein content of plant-based food materials is inadequate to meet the protein requirements of individuals compared with food material produced from animal sources (35,36).

## Conclusions

It can be concluded from this study that formulated weaning foods exhibited good protein nutritional quality when tested with slope ratio technique utiliz-

ing weanling rats. The WG and RL weaning foods indicated RPV of 0.9 and 0.95, respectively which are very close to the ideal reference rate of 1.0. The results of this study conclusively show that both formulations can be used safely as weaning or supplementary food for young children.

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