

Original Article

Protein quality evaluation of two rice- and milk-based weaning foods

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ABSTRACT

Background: Protein-energy malnutrition is regarded as a public health problem in developing countries as a result of poor feeding practices due to poverty. This study, therefore, is aimed at protein evaluation of two samples (Cerelac based on rice with milk and Ghoncheh based on rice with milk) of commercial weaning food.

Methods: Biological evaluation of the formulas was conducted in 21-day-old weaning Wistar rats, compared to a control diet of casein. The nutrient quality of the weaning foods was monitored by measuring Protein Efficiency Ratio (PER), Net Protein Ratio (NPR), Relative Net Protein Ratio (RNPR), True Protein Digestibility (TPD), Apparent Digestibility (AD), and Food Efficiency Ratio (FER).

Results: The content of TPD for Casein, Cerelac, and Ghoncheh was 93.77, 93.71, and 78.23, respectively, and the results among groups was significant ($P < 0.0001$). NPR value of Casein, Cerelac, and Ghoncheh was 4.38, 3.45, and 2.93, respectively. Results among the groups were significant ($P < 0.0001$). PER value of Casein, Cerelac, and Ghoncheh was 3.05, 2.22, and 1.4, respectively. Results among the groups was significant ($P < 0.0001$).

Conclusions: Results indicate that TPD, NPR, and PER values of Cerelac were higher than Ghoncheh..

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1. Introduction

Protein-energy malnutrition continues to be a major public health problem among children throughout the developing world (1, 2). Poverty and poor feeding practices have been attributed as major factors responsible for this nutrition problem (1, 3, and 4). Recent reports from the World Health Organization show that about 60 percent of all deaths occurring among children under 5 years of age in developing countries can be attributed to malnutrition (5). It is also estimated that about 50.6 million children under the age of 5 are malnourished, 90 percent of whom are from developing countries (6, 7). On the other hand, optimum use of protein required for the body depends on digestibility and pattern of essential amino acids (8). Therefore, determining and assessing the protein quality of food consumed is biologically necessary in terms of nutritional planning (8, 9). Food processing affects the pattern and bioavailability of essential amino acids and ultimately the protein quality of the end product (10-12). Thus, the necessity of using accurate, sensitive, rapid, and applicable methods to determine the quality of protein is felt. These methods should be able to measure the true protein digestibility and the efficiency of protein being used (13-16).

In general, protein quality assessment methods include biological, microbiological, chemical, and integrated approaches. Among the available methods, True Protein Digestibility (TPD), Apparent Digestibility (AD), Net Protein Ratio (NPR), Protein Efficiency Ratio (PER), Food Efficiency Ratio (FER), Net Protein Utilization (NPU), Biological Value (BV), and Protein Digestibility-Corrected Amino Acid Score (PDCAAS) are suggested as suitable methods for determining protein quality (17-19). The content of Cerelac TPD and wheat flour + soybean mixture, 50:50 ratio, was reported as 90.8 (18) and 92 (8) by other researchers. The content of homemade food NPR (based on the appropriate cereal/legume mixture) was reported 2.25 (14) by other researchers. Content of Cerelac and homemade food PER (based on the appropriate cereal/legume mixture) was reported 2.1 (18) and 2.52

(14).Therefore, considering the importance of protein qualitative value of foods, especially in low-income families, the study and evaluation of the proposed methods regarding the accuracy and applicability in that country seem to be necessary and can be used as a measurement to control the quality of products in future cases. Thus, this study was designed to compare the protein value of two commercial baby foods (Cerelac based on rice with milk and Ghoncheh based on rice with milk) on rats in Qom and Kashan University of Medical Sciences in 2010.

2. Material and Methods

This experimental study was performed on 64 male wistar rats, at weaning age (21 days).The animals were purchased from the Pasteur Institute (Karaj Branch). At first, samples of Cerelac and Ghoncheh were analyzed in terms of moisture, fat, fiber, ash, and protein by laboratory methods (20) to be used in the experimental diets. In the study, TPD and AD were used in two test diets (Cerelac based on rice with milk from Nestle- Iran.com and Ghoncheh based on rice with milk from Ghoncheh Parvar food products.com), a standard diet (Casein + Methionine), and a basal diet (free-protein) and for the study NPR, PER, and FER tests (study conditions in PER and FER were similar to NPR, except for PER study period, which was 28 days and it was a protein-free diet) two experimental test diets, a standard and a basal diet, were used. Regarding the compounds of homemade foods, the amounts of food and main nutrients were adjusted for the experimental diet (Table 1).

After being transferred to the laboratory, the rats were freely fed commercial baby food for five days (acclimation period). Then the animals were divided randomly into eight experimental groups of eight rats in each group: each group including two blocks and each block including four rats (a total of 64 primary rats). For evaluation of TPD and AD, four groups: Casein + Methionine, free- protein, Cerelac, Ghoncheh and for NPR, PER and FER evaluation four groups: Casein + Methionine, free protein, Cerelac, and Ghoncheh. According to results of similar studies, division of rats into blocks was by difference between blocks in term weight in the range of 0.5 g (18, 19, and 21). The rats were individually housed in polypropylene cages with suspended-wire bottoms in an animal care room maintained at 22±2°C and 50-70 percent relative humidity with alternating 12-hour periods of light and darkness throughout the study.

The test lasted for nine days including four first days of the preliminary period and five final days of balance period. During the test period, the animals, food was limited to 15 grams per day (based on dry matter), but the rats had free access to water. At the end of the balance period, food was in the air for three days. Then the amount of nitrogen intake by each rat was calculated. Fecal samples were placed in glass containers for three days at 50 °C and the level of nitrogen were determined (8, 18, 19, 22-29).TPD calculation is performed through the following relationship:

Ni: Nitrogen intake by rats in tests group

$$TPD = \frac{Ni - NF1 - NF2}{Ni} \times 100$$

NF1: Nitrogen excreted in stools of test group

NF2: Nitrogen excreted in stools of without protein group

AD calculation is performed through the following relationship:

$$AD = \frac{Ni - NF1}{Ni} \times 100$$

For the NPR determination, the animals were freely given food and water for 14 days. Food poured in each cage was collected separately and kept in plastic containers then kept at room temperature. Finally, protein intake by each rat was calculated and the NPR of each test and standard protein sources was reported for each rat (15, 18, 19, 21, and 27).

NPR = Weight gain of test group + weight loss of free- protein group/Weight of test protein consumed- protein intake of free- protein group

Relative NPR = (mean NPR of test protein) × 100/ mean NPR of reference protein

To determine the PER and FER, food and water were provided with no limitations for rats. Rats were fed under three diets (Casein, Cerelac and Ghoncheh) and finally were weighed after four weeks. Weight gain was recorded during this period then PER was calculated through the following relationship (15, 17-19, 21, 22, 30, and 31):

PER = Gain in body weight (g)/ Protein consumed (g)

Also PER was calculated through the following relationship:

FER = Gain in body weight (g)/ Food consumed (g)

The content of NPR, TPD, AD, PER, and FER were determined in Casein + Methionine and commercial baby foods groups and was analyzed by ANOVA test together with Scheffe, Dunett's T3 tests to compare between the groups. In all tests, the content of $P < 0.05$ was considered significant.

3. Results

This study was done on 64 rats, divided in eight groups of eight rats.

Protein intake on the Ghoncheh diet for studying TPD was the highest (4.92 g per rat). Protein excretion on the Ghoncheh diet was the highest (1.15 g per rat) and it differed significantly ($p < 0.0001$) from that of other diets, in which protein excretion ranged from 0.42 to 0.46 g per rat (Table 2). The content of TPD on the Casein diet was the highest (93.77 %) and it differed significantly ($p < 0.0001$) from that of another diet Ghoncheh. Also the content of TPD of the Cerelac group was significantly higher than Ghoncheh group (Table 2).

Weight gain on the Casein diet for the study NPR was the highest (35.56 g per rat) and it differed significantly ($p < 0.0001$) from that of other diets, in which weight gain ranged from 15.76 to 21.98 g per rat. Food intake on the Casein diet was the highest (137.01 g per rat). Protein intake on the Casein diet was the highest (13.51 g per rat). The content of NPR on the Casein diet was the highest (4.38 per rat) and it differed significantly ($p < 0.0001$) from that of other diets, in which NPR ranged from 2.93 to 3.45 per rat. Also the content of NPR on the Cerelac diet was significantly higher than Ghoncheh diet (Table 3).

Table 1. Raw materials for preparing the experimental diets (g per 100 g)

Diet ingredients	Cerelac	Ghoncheh	Caseine+Methionine	Free -Protein
Caseine	0	0	10	0.2
Cerelac	66.7	0	0	0
Ghoncheh	0	66.7	0	0
Sugar	5	5	5	5
Corn oil [†]	3.3	3.3	10	10
Vitamins	1	1	1	1
Minerals	4	4	4	4
Fiber (cellulose) ^{††}	4.1	4.7	5	5
L-Methionine	0	0	0.3	0
Choline chloride	0.2	0.2	0.2	0.2
Corn starch	15.7	15.1	64.5	74.6

[†]Set based on existing fat, protein, and starch sources, to reach 10 percent fat level in final diet

^{††}Set based on existing non-soluble fiber, protein, and starch sources, to reach 5 percent fiber level in final diet

Table 2. Protein intake, protein excretion, TPD and AD commercial baby foods in rats for five days

Diets	Protein intake	Protein excretion	TPD in vivo	AD in vitro
Casein	4.55±0.92	0.42±0.1	93.77±3.04	90.12±3.62
Cerelac	4.72±0.89	0.46±0.13	93.71±2.85	90.21±2.36
Ghoncheh	4.92±1.32	1.15±0.56	78.23±12.58	74.76±13.12
P-value	P=0.78	P<0.0001 [†]	P=0.001 [†]	P=0.001 [†]

[†] significant different Casein group with Ghoncheh and Cerelac group with Ghoncheh used to ANOVA test together with Dennett's T3

Table 3. Weight gain, food intake, protein intake, NPR, and RNPR commercial baby foods in rats for 14 days

Diets	Weight gain	Food intake	Protein intake	NPR	RNPR
Casein	35.56±10.64	137.01±1.02	13.51±1.4	4.38±0.47	100
Cerelac	21.98±9.43	132.76±27.35	13.27±2.73	3.45±0.32	78.76
Ghoncheh	15.76±7.37	134.71±15.99	13.47±1.59	2.93±0.35	66.89
P-value	P=0.001 [†]	P=0.91	P=0.96	P<0.0001 ^{††}	-

[†] significant different Casein group with Ghoncheh and Cerelac groups used to ANOVA test together with Scheffe

^{††} significant different Casein group with Ghoncheh and Cerelac groups; Cerelac group with Ghoncheh group used to ANOVA test together with Scheffe

Weight gain on the Casein diet for studying PER and FER was the highest (90.06 g per rat) and it differed significantly ($p < 0.0001$) from that of other diets where weight gain ranged from 39.61 to 71.42 g per rat. Food intake on the Cerelac diet was the highest (331.01 g per rat) and it differed significantly ($p = 0.008$) from that of other diets, in which weight gain ranged from 279.83 to 297.13 g per rat. Protein intake on the Cerelac diet was the highest (31.85 g per rat). The content of PER on the Casein diet was the highest (3.05 per rat) and it differed significantly ($p < 0.0001$) from that of other diets, where PER ranged from 1.4 to 2.22 per rat. Also the content of PER on the Cerelac diet was significantly higher than the Ghoncheh diet. The content of FER on the Casein diet was the highest (0.3 per rat) and it differed significantly ($p < 0.0001$) from that of other diets, in which FER ranged from 0.14 to 0.21 per rat. Also the content of FER on the Cerelac diet was significantly higher than the Ghoncheh diet (Table 4).

Table 4. Weight gain, food intake, protein intake, PER, and FER commercial baby foods in rats for 28 days

Diets	Weight gain	Food intake	Protein intake	PER	FER
Casein	90.06±14.34	297.13±27.23	29.36±2.71	3.05±0.24	0.3±0.02
Cerelac	71.42±15.12	331.01±33.91	31.85±4.38	2.22±0.2	0.21±0.03
Ghoncheh	39.61±8.36	279.83±28.26	27.98±2.82	1.4±0.21	0.14±0.02
P-value	$P < 0.0001^{\dagger}$	$P = 0.008^{\dagger\dagger}$	$P = 0.09$	$P < 0.0001^{\dagger}$	$P < 0.0001^{\dagger}$

[†] Significant different Casein group with Cerelac and Ghoncheh groups; Cerelac group with Ghoncheh group used to ANOVA test together with Scheffe

^{††} Significant different Cerelac group with Ghoncheh group used to ANOVA test together with Scheffe

4. Discussions

After six months of life, infants need to take adequate energy and nutritious weaning foods to supplement breast milk (32). However, as scientific studies have revealed, in many parts of developing countries, most of the traditional weaning foods were made up cereals and tubers that are low in protein and other essential micronutrients, which are vital for the normal physical growth and cognitive development of a child (1, 32).

Results of this study showed that Cerelac commercial weaning food based on rice with milk has higher protein value than Ghoncheh based on rice with milk and is almost equal to Casein standard. The content of TPD for the protein of Cerelac and Ghoncheh in this study was obtained 93.71 and 78.23. While Essien, et al., reported 73.5 for Nutrend commercial weaning food (33), Al-othman, et al., 93-95 for Cerelac based on milk (21), Al-othman, et al., 94-95 for Cerelac based on milk with wheat (21) and Essien, et al., 87 for Cerelac based on milk with wheat (34) which was almost similar to Cerelac in our study. The content of TPD for the protein of Casein in our study was 93.77 whereas Al-othman, et al., reported it to be 96 (21), Koo, et al., 99 (35), and Gahlawat, et al., 92 (18), similar to this study. On the other hand, the main factors that caused the difference in the TPD content of casein and commercial weaning food is protein intake and excretion in experimental groups.

The content of NPR for the protein of Cerelac and Ghoncheh in this study was obtained 3.45 and 2.93, while Essien, et al., reported it to be 2.23 for Nutrend commercial weaning food (33), Asemi, et al., 4.3 for Cerelac based on milk (34), and Ijarotimi, et al., 1.29 for Nutrend commercial weaning food (1). The content of NPR for the protein of Casein in this study was 4.38 whereas Mensa-Wilmot, et al., reported it to be 3.5 (22), Kalra, et al., 3.65 (19) and Asemi, et al., 4.3 (34). In other words, the difference between Casein and NPR is related to the amount of food, protein intake, the quality of protein consumed (the main factors in calculating NPR includes test group weight gain, non-protein group weight loss, test group protein intake). The content of PER for the protein of Cerelac and Ghoncheh in this study was obtained 2.22 and 1.4 while Gahlawat, et al., reported 2.31 for Cerelac (18), Ijarotimi, et al., 2.09 for Nutrend commercial weaning food (1), and Essien, et al., 2.06 for Nutrend commercial weaning food (33). The content of PER for the protein of Casein in this study was 3.05 whereas Asemi, et al., reported it to be 3 (34), Mensa-Wilmot, et al., 3.5 (22), and Kalra, et al., 2.87 (19).

Overall, protein quality is affected by several factors, the most important of them include: 1: Type of protein: the digestion and absorption of plant proteins is less than animal proteins as they get trapped in the carbohydrate cell wall and are less accessible (8). 2: Food processing: Food processing may cause more destruction of amino acids and reduce their bioavailability. For example, in milk processing, the average temperature in the presence of reductive sugars (glucose and galactose), causes loss of availability of amino acid lysine, which it so-called brown or Millard reaction, and wastes lots of lysine in high temperatures (36, 37). 3: Low protein digestibility of the diet in developing countries can be because of less refined cereal/legume being used and this is true particularly for wheat (8). 4: The rating of commercial formula (Cerelac) compared to Ghoncheh in term of color, aroma, taste, mouth feel, and overall acceptability could be attributed to the incorporation of flavoring, sweetening, and other sensory enhancing agents to the formula during its formulation, therefore it causes higher food intake. 5:

Several findings have revealed that infant foods made of cereals or tubers were low in several nutrients including protein, vitamin A, zinc, and iron. These nutrients are of special importance due to their impact on physical and cognitive development (38, 39). Ghoncheh weaning food introduced in this study has lower content iron element than Cerelac and lacks a Mg element. Mg element is involved in glycolic and citric acid cycle (energy metabolism). 6: Several findings showed that Probiotics are involved in improving lactose tolerance (40-43). Existence of bifidobacterium lactis in Cerelac weaning food has led to a higher protein quality than Ghoncheh.

The results obtained by qualitative biological evaluation through TPD, NPR, and PER methods on Casein protein sources, Cerelac, and Ghoncheh were satisfactory and indicate the right formulation of diet and suitability of Wistar race for the study. Generally nutritional quality of protein is affected by three factors: 1: combination of amino acids 2: protein digestion 3: consumers' need for amino acids. Therefore, high-quality protein combined with amino acids is completely digested when the pattern of amino acids corresponds to the human and animal's needs (44).

5. Conclusion

The study suggested that the protein quality of Cerelac was higher than Ghoncheh weaning food. Therefore it is suggested:

- 1: Researchers conduct projects on the other supplements of cereal/legume to obtain the useful results.
- 2: It's recommended that standard rules apply for homemade food protein based on cereal/legume.
- 3: Considering almost lower actual protein digestibility of Ghoncheh in this study compared with similar foreign ones, it is necessary to take basic policies to increase the quality of proteins, including temperature control at the time of production.

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