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EVALUATION OF PROTEIN QUALITY OF SELECTED IRANIAN RURAL DIETS[1]

J. Jamalian, M.H. Saleh and R.I. Tannous[2]

Abstract — Prevalence of protein–calorie deficiency in infants and pre-school children has manifested itself in many parts of the world. Nutrition surveys in different parts of Iran have led investigators to believe that insufficiency of the right foods and/or imbalance of diets is the cause of nutritional diseases. From survey data it has been possible to assess the nutritional status in general of the different groups investigated. In this study it was intended to find the quality of the protein of the diets consumed by the groups as reported in the literature. Thus rural diets of four provinces were prepared according to survey data. They were analysed for their constituent amino acids and other components. They were also tested biologically by feeding them to rats and determining the net protein utilization (NPU) both before and after lysine supplementation. The results indicate that the protein quality of the diets is poor, as revealed by analytical and biological procedures. Insufficient lysine is found to be one of the main factors limiting the protein quality of such diets. Improvement is noted in the protein quality upon supplementation with 0.25 or 0.35% lysine. Adequacies of these diets to meet the allowances and/or requirements of subjects of different age groups are discussed.

INTRODUCTION

Protein–calorie malnutrition is the major nutritional problem in the world, especially for infants and pre-school children [23]. Protein–calorie deficiency syndrome constitutes a spectrum ranging from pure protein deficiency [13], like the kwashiorkor type which is prevalent in countries with a very low protein staple, to mainly caloric deficiency as in the marasmic child.

Although dietary protein insufficiency can by itself be the dominating factor, its frequent combination with infections, and caloric and other essential nutrient inadequacies can further complicate the situation.

Surveys performed in Iran up till 1957 [31, 32, 33] have indicated the scarcity of

1. Contribution from the Department of Food Technology and Nutrition, Faculty of Agricultural Science, American University of Beirut, Lebanon, and the Department of Food Technology and Nutrition, College of Agriculture, Pahlavi University, Shiraz, Iran. Part of an M.Sc. thesis submitted by the second author.
2. Assistant Professor and former Instructor, Department of Food Technology and Nutrition, Pahlavi University, and Associate Professor, Department of Food Technology and Nutrition, American University of Beirut, respectively. The second author's present address: Industrial and Mining Development Bank of Iran, Tehran, Iran.

total protein, especially that of animal origin. In addition, an inadequate supply of energy for total population needs has also been stressed. It has been reported that out of 830 children under the age of 5 who were admitted to Massoudi Hospital in Tehran, nearly one third were suffering from malnutrition, with marasmic cases being about double the kwashiorkor ones. Fatalities were more common among the marasmic children than those with kwashiorkor. Malnutrition during the first year of life accounted for a high percentage of all cases, showing the importance of early malnutrition vs that in the pre-school children [7].

Further surveys of household food consumption in cities and villages of different provinces in the country [4–6, 8–10, 24–29] have shown that as a whole the caloric intakes are based mainly on cereals. Protein of animal origin constitutes a minor percentage of their total protein intake.

This investigation was undertaken to find out: (1) the protein quality of Iranian rural diets as normally prepared, using analytical and biological methods; and (2) the adequacy or inadequacy of such diets to meet the protein requirement and protein allowance of subjects in different age groups.

MATERIALS AND METHODS

Choice of diets and their preparation

Representative diets of rural regions of Kermanshahan, Khorasan, Kerman, and Gorgan were prepared on the basis of the household nutrition surveys of the Food and Nutrition Institute of Iran [10, 27–29] and designated as A, B, C and D, respectively. Their composition and their consumption *per capita* per day are shown in Table 1. These data have been used as the basis for formulating the diets for the study.

An attempt has been made to simulate these diets, as far as possible, to the actual diets consumed in the areas concerned. The legumes used were chickpeas and lentils for diets A, C and D. For diet B, however, 2/3 millet and 1/3 corn were used (because of their high consumption in the area). The fresh fruit used was either watermelon or apple according to the season. For miscellaneous food items, parboiled wheat and tomato paste were used. Spinach was the green vegetable, carrot was used for "other vegetables" and tea was not included. After appropriate processing, all of the ingredients were mixed and homogenized in a blender. Then they were dried in air current at 70°C for about 24 hr. The different samples were ground into powder suitable for feeding rats, and stored in plastic bags at −20°C until use. These diets were supplemented with either 0.25 or 0.35% L-lysine.

Amino acid analysis

The method of hydrolysis used was the acid hydrolysis recommended by the National Academy of Sciences/National Research Council [11, 19]. Hydrolysis time was 24 hr. As a check against loss of amino acids a known quantity of nor-leucine was added before the process.

To quantify the individual amino acids in the hydrolysates a Phoenix Automatic Amino Acid Analyzer (K-8000 Phoenix Precision Instrument Co., Philadelphia, Penn.) modified for new spherical resins was used. The technique was, in principle, based on the method devised by Spackman *et al.* [30]. Tryptophan is totally destroyed during 24 hr

Table 1. Percentage by gross weight of different food groups consumed per head per day

Food groups	Ingredients	Diet A (%)	Diet B (%)	Diet C (%)	Diet D (%)
Cereals	Bread	48.1	49.1	69.3	44.9
	Rice	4.8	1.1	1.9	19.2
	Others	0.2	0.9	0.0	0.0
Sugar		5.7	4.3	5.5	6.2
Potatoes		1.2	2.6	5.0	3.6
Legumes	Pulses	0.6	0.8	1.6	1.0
	Beans and nuts	0.0	0.2	0.0	0.0
Vegetables	Green leafy	0.1	0.1	2.4	4.5
	Tomato	5.4	2.0	0.2	0.1
	Onion	1.2	0.8	0.0	0.0
	Others	1.2	0.8	1.0	0.7
Fruits	Fresh	9.0	30.2	0.0	0.0
	Dried	1.4	0.1	2.0	0.2
Meat and poultry		2.9	2.1	0.6	3.1
Fish		0.0	0.0	0.1	1.7
Eggs		0.9	0.3	0.7	0.8
Milk and milk products	Milk and yoghurt	14.9	1.3	7.7	10.4
	Kashk	0.0	1.4	0.5	0.1
	Cheese	0.1	0.4	0.1	0.0
Fats and oils	Ghee and butter	2.0	0.5	0.9	0.8
	Shortening	0.3	0.7	0.3	1.7
Miscellaneous		0.0	0.6	0.0	0.3
Tea		0.0	0.0	0.3	0.3
Total		100	100	100	100

acid hydrolysis. Thus an alkaline hydrolysis was used for this amino acid [12].

Determination of protein value

NPU was determined using Sprague-Dawley rats and NPU_{op} was calculated according to Miller [14]. NPU_{st} was calculated from the equation of Miller and Payne [16] and Miller [14]. The micro-Kjeldahl technique of AOAC [1] was employed for determination of N content of diets and rat carcasses and a factor of 6.25 was used to convert %N to % protein. Protein quality scores were determined in reference to the recommended pattern of FAO/WHO 1973 [3] as detailed by Saleh *et al.* [22]. Metabolizable energy (M.E.) was determined using a Gallenkamp Ballistic Bomb Calorimeter [15], sucrose being a reference standard.

Net dietary protein as a percentage of total calories (NDpCal%) was obtained in two different ways as shown below:

(a) NDpCal% (determined) = NPU_{op} X PCal%, where PCal% is protein-calorie percentage, determined by the following formula:

$$PCal\% = \frac{P \times 4 \times 100}{M.E.}$$

where P = % protein in the diet and M.E. = metabolizable energy per 100 g.

(b) NDpCal% (calculated) was obtained by the equation of Miller and Payne [16]:

$$NDpCal\% = PCal\% \times score \times \frac{54 - PCal\%}{54 - \frac{400}{Score}}$$

RESULTS AND DISCUSSION

The amino acid compositions as well as the scores of the diets are shown in Table 2. Scores of the four diets as calculated by the FAO/WHO 1973 procedure are indicative of poor protein quality. It is also noted that all four diets are limiting in lysine. This is not unexpected because these diets are based mainly on cereals.

Table 3 summarizes the results of indices of protein quality and quantity. PCal% indicates the normal protein-calorie ratio in all four diets. However, it is important to note that by far the greatest portion of protein intake is of cereal origin. NPU_{op} and NPU_{st} indicate the poor quality of the proteins of the diets investigated. NDpCal% which

Table 2. Amino acid composition and protein quality scores of diets

Amino acid (mg/gN)	Diet A	Diet B	Diet C	Diet D
Lysine	169	178	195	186
Histidine	161	165	188	158
Arginine	338	316	368	320
Aspartic acid	395	372	422	435
Threonine	193	214	241	243
Serine	252	276	302	282
Glutamic acid	1696	1800	1961	1653
Proline	489	691	464	581
Glycine	265	254	271	266
Alanine	255	189	299	275
Cystine	114	108	125	91
Methionine	115	104	125	130
Total SAA	229	212	250	221
Valine	307	270	329	300
Isoleucine	233	222	258	231
Leucine	469	425	477	474
Tyrosine	213	181	223	262
Phenylalanine	285	277	298	312
Total aromatic	498	458	521	574
Tryptophan	71	75	69	77
Total N recovery %	93.0	93.0	93.2	93.1
Protein quality score (FAO/WHO 1973)	50(L)*	52(L)	57(L)	55(L)

* L, lysine, the most limiting amino acid.

Table 3. Indices of protein quality of diets

	Diet A	Diet B	Diet C	Diet D
N%	1.97	2.15	2.13	2.06
PCal%	12.50	13.60	13.00	12.50
M.E./100 g	394	396	410	411
NPU_{op}	43	41	41	49
NPU_{st}	48	47	46	56
NDpCal% (determined)	5.38	5.58	5.33	6.13

Table 4. Protein allowances in terms of NDpCal% from FAO/WHO 1957 report*

Subject	Age (yr)	Calories per day	Reference protein (g/day)	NDpCal% per day
Infant	0–1	–	–	8.0
Toddler	1–2	1230	24	7.8
Child	4–9	1970	29	5.9
Adolescent	–	3050	61	8.0
Adult	–	2960	34	4.6
Lactating mother	–	3200	76	9.5

* From Pellett and Jamalian [20]; originally prepared from Platt, Miller and Payne [21].

combines both the quality and the quantity of the dietary protein shows that, on the basis of protein allowance of Platt *et al.* [21] as reported by Pellett and Jamalian [20] (Table 4) diets A, B and C are inadequate for children, adolescents and lactating mothers, and can only meet the protein allowances for adults. Diet D, however, can meet the allowances for children (4–9 yr) and adults, but does not meet the allowance for other age groups.

In terms of protein requirements, however, on the basis of the data of Pellett and Jamalian [20] all these diets are adequate for children between 1/2–12 yr of age. Diets A, B and C are inadequate for adolescents and adults of both sexes, while D is just adequate for male adolescents and adults. As noted from the scores in Table 2, all these diets are limited in lysine.

The calculation of a simple ratio of average protein needs to average energy requirements has not been warranted by FAO/WHO 1973 [3]. Yet the Protein–Calorie Advisory Group of the United Nations [17] stress that "in examining the general quality of a diet, it is useful to express nutrients per unit of food energy".

Ever since the publication of FAO/WHO 1973 report [3], various procedures have been attempted to arrive at a "safe" protein–energy ratio in the diet [2, 18]. Such a ratio would be expected to meet the protein needs of almost all individuals provided their energy needs, as defined by FAO/WHO 1973, are met. Hence Beaton and Swiss [2] calculated certain "safe" PCal% ratios and predicted that needs of about 97.5% of all individuals (for which the ratios are meant) with moderate activity would be satisfied using such ratios.

Table 5. Safe levels of reference protein (egg or milk) in terms of NDpCal%

Age (yr)	"safe" PCal%*	NDpCal% (calculated)†
2–7	5.1	5.0
6–8	4.9	4.8
12–14	5.0	4.9
Adults	5.4	5.2

* From Beaton and Swiss [2].
† Calculated as in the text assuming a score of 100.

Table 6. Protein quality of the diets after lysine supplementation

Diets	NPU_{op}		NPU_{st}		NDpCal% (determined)	
	0.25%L*	0.35%L	0.25%L	0.35%L	0.25%L	0.35%L
A	65	66	76	77	8.13	8.25
B	51	54	58	62	6.94	7.34
C	55	59	65	70	7.15	7.67
D	55	56	65	65	6.88	7.00

* % Lysine added to the diets.

Table 5 shows these ratios and the corresponding NDpCal% values calculated from them assuming a score of 100 for the reference protein. According to the Protein-Calorie Advisory Group [17] these PCal% values should be taken as the lower limit of acceptable dietary protein concentration. Thus the calculated NDpCal% values should also be considered as the lower limits.

Comparison of data in Table 3 with those in Table 5 indicates that PCal% of all four diets (A, B, C and D) are relatively high. Yet these diets are of such a poor protein quality that their NDpCal% values are just above those required for subjects of 2–8 yr and 12–14 yr of age and adults (Table 5), provided that the energy intakes of the subjects are sufficient to meet their needs.

Table 6 shows the results of supplementation of the diets with 0.25 and 0.35% lysine. It is clear that there is a definite improvement in their quality upon supplementation (c.f. Table 3). The NDpCal% of supplemented diets indicate that diet A with 0.35% lysine supplementation can now meet the protein allowance for all age groups (Table 4), but is still inadequate for lactating mothers. However, it will cover the requirements of all age groups, including lactating mothers. Diets B, C and D with 0.35% supplementation are still inadequate to meet the protein allowances for infants, toddlers (1–2 yr), adolescents and lactating mothers, but they cover the requirements for subjects from the age of 6 months onwards, with the exception of diet D which cannot meet the requirement of pregnant women [20].

According to the reference data of Table 5, all four diets which seemed just adequate before supplementation can now meet a higher level of adequacy than the lower limits given in the table.

Assuming that the total energy intake of the subjects concerned is met and that there is proper food distribution within the family, it is clear that the quality of the diets can be improved significantly by the inclusion of more leguminous foods (provided properly processed) in the diets which will bring about sufficient improvement in their quality, through mutual supplementation, so that they can meet the nutritional needs of the different age groups in the areas investigated.

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