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APPRAISAL OF CURRENT PROTEIN SCORING SYSTEMS BY USE OF SELECTED IRANIAN DIETS[1]

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Abstract – The actual survey diets based on *per capita* per day consumption of food groups of four representative provinces of Iran were selected in order to evaluate the current protein scoring systems. The amino acid composition, net protein utilization (NPU), and the true limiting amino acid as revealed by the improvement of NPU following dietary amino acid supplementation were determined. The scoring systems tested included whole egg, human milk, whole rat carcass, FAO 1957 pattern and its modifications by Payne, and the FAO/WHO 1973 pattern.

All of the diets were shown to be limited by lysine. The second limiting amino acid was threonine in two diets and isoleucine and sulfur amino acids in the other two. The recommended pattern of FAO/WHO 1973 gave a better prediction of the true limiting amino acids than the other patterns, followed by whole rat carcass amino acids and the FAO 1957 modified pattern. As far as prediction of protein quality is concerned, once again the FAO/WHO 1973 pattern was superior to all other patterns.

INTRODUCTION

The value of dietary protein and its digestibility depends on its amino acid content. This is the basis for a scoring system. This nutritive value can be expressed as a single figure, protein or chemical score, which can be determined by comparison between the levels of each essential amino acid in a food protein and the content of the corresponding amino acid in the same quantity of a protein selected as a standard. The amino acid showing the lowest percentage is called the limiting amino acid and this percentage is the protein score [3].

The choice of the pattern of amino acid for any protein quality evaluation has been the subject of great controversy. Any reference pattern must be judged by its success in predicting the true limiting amino acid or at least the most frequently limiting amino acids in human diets, i.e. sulfur amino acids, lysine, threonine, tryptophan and isoleucine. The value of the pattern will be doubtful if it suggests any other amino acid as the limiting one.

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Block and Mitchell [3] scored different proteins against EAA of whole egg (B.V. 100%). György [12] proposed human milk as the reference pattern, but Synderman *et al.* [34] showed that in some diets, using these foods of high B.V., even the non-essential amino acids become limiting. Autret *et al.* [2] scored diets of 84 countries against egg and found about 1/2 of the diets were limited by isoleucine, due to high isoleucine content of egg.

McLaren and Pellett [20] found that very high levels of some amino acids in whole egg resulted in scores below the expected NPU values.

The FAO Committee [8] proposed a reference based on the pattern of requirements of human subjects, both adults and infants. Bressani *et al.* [4–6], Leverton and Steel [18] and Kirk *et al.* [17] found that the level of tryptophan was too high.

The proportion of sulfur amino acids in the FAO provisional pattern was also too high [6, 13, 25, 32, 33].

Howe *et al.* [14] observed that the level of lysine of the FAO 1957 pattern was adequate, but to meet the requirement of children a higher level of lysine in the pattern could be justified. This led Payne [27] to suggest a modification of FAO 1957 pattern, increasing lysine from 270 to 360 mg/g N, and reducing tryptophan from 90 to 70 mg/g N.

The sulfur:nitrogen ratio was said to be directly correlated to the protein value of the diet [22, 23], but Pellett *et al.* [30] found that this method was not applicable to the Middle Eastern diets.

The ratio of total essential amino acids in g per g of total nitrogen (E/T), and the ratio of each essential amino acid in mg per g of total essential amino acids (A/E) were suggested for the description of protein quality [10]. However, these scores were later found to be unsuitable for the prediction of biological values [7, 26].

The amino acid content of the body of the rat [29] was also suggested for a reference pattern provided the amino acid composition of the tissue did not alter with a change in diet.

Finally, a new scoring pattern was proposed in 1973 [11], by a Joint FAO/WHO Expert Committee, which gave due consideration to the needs of pre-school children and was adjusted in light of the experience obtained in the application of FAO 1957 reference pattern.

The present study tests the applicability of this proposed pattern to some actual diets.

MATERIALS AND METHODS

Diets

Representative diets of rural areas of four Iranian provinces (A, Kermanshahan; B, Khorasan; C, Kerman; D. Gorgan) were selected and prepared as detailed elsewhere [15].

Amino acid supplementation

For this purpose L-forms of amino acids were used whenever possible. If the L-form was unavailable, the DL-form was used in double quantities. L-Lysine-HCl, L-methionine, DL-isoleucine and DL-tryptophan were used in this study. The first limiting amino acid was added at increasing levels till a maximum value of NPU was obtained. In some cases a combination of amino acids was also used to find out the second limiting amino acid.

Table 1. The most frequently limiting amino acid content of the reference patterns used (mg amino acid/g N)

Amino acid	Human milk (FAO/WHO, 1965)	Whole egg (FAO, 1970)	Human milk (FAO, 1970)	Whole rat carcass	FAO 1957 pattern	FAO 1957 modified pat. [27]	FAO/WHO 1973
Lysine	402	436	428	447	270	360	340
Threonine	290	320	280	285	180	180	250
Methionine	140	210	101	130	144	144	
Cystine	134	152	84	115	126	126	
Total SAA	274	362	185	245	270	270	220
Valine	420	428	284	281	270	270	310
Isoleucine	411	393	254	224	270	270	250
Leucine	572	551	548	472	306	306	440
Tyrosine	355	260	205	219	180	180	
Phenylalanine	297	358	216	248	180	180	
Total aromatic	652	618	421	467	360	360	380
Tryptophan	106	93	105	73	90	70	60
Total EAA	3127	3201	2505	2494	2016	2086	2250

Table 2. Scores for diet A based on various reference patterns

Amino acid	Whole egg (FAO, 1970)	Human milk (FAO/WHO, 1965)	Human milk (FAO, 1970)	Whole rat carcass	FAO 1957 pattern	FAO 1957 modified pat. [27]	FAO/WHO 1973
Isoleucine	59(2)*	57(2)	92	104	86	86	93
Leucine	85	82	86	99	153	153	107
Lysine	39(1)	42(1)	49(1)	38(1)	63(1)	47(1)	50(1)
Total SAA	63	84	124	94	85	85(2)	104
Total aromatic	81	76	118	106	138	138	131
Threonine	60	67	69	68(2)	107	107	77(2)
Tryptophan	76	67	68(2)	97	79(2)	101	118
Valine	72	73	108	109	113	113	99

* Numbers in parentheses indicate first and second limiting amino acids.

Analysis of diets for amino acids

Samples of diets were hydrolysed for 24 hr according to the procedure recommended by the NAS/NRC [28]. To check against losses of amino acids a known quantity of nor-leucine was added before the process. The amino acid composition of hydrolysates was determined using a Phoenix Automatic Amino Acid Analyzer based on Spackman *et al.* [31] modified for new spherical results. Tryptophan was analysed by the alkaline hydrolysis procedure of Lunven [19].

Protein quality scores

The score was expressed as the level of the essential amino acid present in the diet in relation to that of the same amino acid present in the reference pattern. The reference patterns used were: FAO provisional pattern [8], whole egg [9], whole rat carcass amino acid composition [29], human milk, the FAO 1957 pattern modified [27] and that proposed by FAO/WHO 1973 [11], as shown in Table 1.

Net protein utilization

NPU of diets was determined according to Miller [21]. NPU_{op} and NPU_{st} were calculated according to Miller [21], and Miller and Payne [24], respectively. Nitrogen analyses of diets and carcasses were performed by the technique of AOAC [1].

RESULTS AND DISCUSSION

Table 2 shows the scores for diet A, when the various reference patterns are used. In this diet lysine was predicted to be the first limiting amino acid. This was confirmed by the addition of different limiting amimo acids and measurement of NPU increments (Table 3).

Table 3. NPU_{op} and NPU_{st} of diets A and B with amino acid supplementations

Amino acid additions g/100 g diet	Diet A		Diet B	
	NPU_{op}	NPU_{st}	NPU_{op}	NPU_{st}
None	43	48	41	47
0.25L	65	76	51	58
0.25M	43	47	39	43
0.25Th	44	48	41	46
0.25Tr	41	44	40	45
0.25I	43	47	42	47
0.15L	50	57	48	54
0.35L	66	77	54	62
0.35L + 0.25M	69	80	51	57
0.35L + 0.25Th	75	88	57	65
0.35L + 0.25Tr	68	78	50	57
0.35L + 0.25I	67	79	59	69

L, lysine; M, methionine; Th, threonine; Tr, tryptophan; I, isoleucine.

Table 4. Scores and the limiting amino acids of diet A after supplementation

Amino acid supplementation g/100 g diet	Whole egg (FAO, 1970)	Human milk (FAO/WHO, 1965)	Human milk (FAO, 1970)	Whole rat carcass	FAO 1957 pattern	FAO 1957 modified pat. [27]	FAO/WHO 1973
None	39(L)	42(L)	49(L)	38(L)	63(L)	47(L)	50(L)
0.15L	51(L)	55(L)	52(L)	50(L)	79(Tr)	62(L)	65(L)
0.25L	59(I)	57(I)	63(I)	60(L)	79(Tr)	75(L)	79(L)
0.35L	59(I)	57(I)	68(Tr)	68(Th)	79(Tr)	85(S)	78(Th)
0.35L + 0.25M	59(I)	57(I)	68(Tr)	68(Th)	79(Tr)	86(I)	78(Th)
0.35L + 0.25Th	59(I)	57(I)	68(Tr)	94(S)	79(Tr)	85(S)	93(L)
0.35L + 0.25Tr	59(I)	57(I)	86(Le)	86(Th)	85(S)	85(S)	78(Th)
0.35L + 0.25I	60(Th)	67(Th,Tr)	68(Tr)	68(Th)	79(Tr)	85(S)	85(Th)

L, lysine; M, methionine; Th, threonine; Tr, tryptophan; I, isoleucine; Le, leucine; S, sulfur amino acids. The limiting amino acids are indicated in parentheses.

Table 5. Scores for diet B based on various reference patterns

Amino acid	Whole egg (FAO, 1970)	Human milk (FAO/WHO, 1965)	Human milk (FAO, 1970)	Whole rat carcass	FAO 1957 pattern	FAO 1957 modified pat. [27]	FAO/WHO 1973
Isoleucine	57(2)*	54(2)	87	99	82	82	89
Leucine	77	74	78	90	139	139	97
Lysine	41(1)	44(1)	42(1)	39(1)	66(1)	49(1)	52(1)
Total SAA	59	77	115	87	79(2)	79(2)	96
Total aromatic	74	70	109	98	127	127	121
Threonine	67	74	76	75	119	119	86(2)
Tryptophan	81	71	71(2)	103	83	107	125
Valine	63	64	95	96	100	100	87

* Numbers in parentheses indicate first and second limiting amino acids.

The second limiting amino acid was isoleucine when the diet was scored against whole egg and human milk [9], tryptophan when scored against human milk [9] and FAO 1957 patterns; threonine when scored against whole rat carcass and the FAO/WHO 1973 pattern [11], and SAA when scored against FAO 1957 modified by Payne [27]. The scores and the limiting amino acids of diet A with and without different amino acid supplementations are shown in Table 4. In order to find out which scoring system was able to predict the second limiting amino acid correctly, the lysine-supplemented diet was fortified with the suspected amino acids. The experimental results (Table 3) showed that although all of the NPU values improved, by far the greatest improvement was achieved when the diet was supplemented with threonine. This indicates the superiority of the FAO/WHO 1973 pattern and the whole rat carcass over the other scoring systems.

Lysine was predicted as the first limiting amino acid of diet B, according to all of the scoring patterns (Table 5). In this experiment, supplementation with lysine increased the NPU value (Table 3). The addition of other amino acids did not produce this effect. Increasing the level of supplementation from 0.15 to 0.25 and 0.35 g% showed a constant increment in the NPU, which agrees well with the prediction made both by the modified FAO 1957 pattern and the FAO/WHO 1973 pattern [11]. The further addition of suspected second limiting amino acids produced an important increase only with isoleucine. Only scoring against egg and human milk [10] gave a prediction that was in accordance with the experimental findings (Table 6).

In diets C and D also all of the patterns used for scoring indicated that lysine was the first limiting amino acid. Animal experimentation agreed with these predictions, i.e. there were increases in the NPU values only upon supplementation with lysine. A further increment in NPU of diet C was shown at each higher level of lysine supplementation (from 61 to 78, NPU_{st}, for 0.15–0.40 g% lysine supplementation).

At the highest level of lysine addition in diet C, the experimental values indicated threonine to be the second limiting amino acid. The further addition of other amino acids did not change the NPU of this lysine-enriched diet. This is in disagreement with the FAO/WHO 1973 [11] and the modified FAO 1957 patterns both of which indicate that SAA should be the second limiting ones.

To determine the second limiting amino acid of diet D, further supplementation with other potentially limiting ones was tried. Surprisingly, all of those tested (methionine, threonine, tryptophan and isoleucine at 0.25 g% level, and their combination with 0.35 g% lysine) produced increments in the NPU values observed. This is not in agreement with accepted concepts of amino acid supplementation. However, only addition of methionine resulted in the highest NPU_{st} (from 56 to 80). This finding might be considered as an indication that methionine is the second limiting amino acid as predicted both from the original FAO 1957 pattern and its later modifications.

A review of the predictions of the true limiting amino acids by various scoring systems vs the experimental findings is made in Table 7. The results show that the best scoring system is that recommended by FAO/WHO 1973 [11]. This pattern was able to predict the actual first and second limiting amino acid 15 times out of 17. On this basis, the second best patterns were both that of Payne [27] and the whole rat carcass pattern. However, both of these patterns failed to indicate correctly the second limiting amino acid on nearly all occasions. Whole rat carcass pattern suffers from some important limitations. Its main disadvantage is that the amino acid composition of rat carcass reported by different authors is not uniform. Also, as in foods of high biological value,

Table 6. Scores and the limiting amino acids of diet B after supplementation

Amino acid supplementation g/100 g diet	Whole egg (FAO, 1970)	Human milk (FAO/WHO, 1965)	Human milk (FAO, 1970)	Whole rat carcass	FAO 1957 pattern	FAO 1957 modified pat. [27]	FAO/WHO 1973
None	41(L)	44(L)	42(L)	39(L)	66(L)	49(L)	52(L)
0.15L	53(L)	54(I)	54(L)	51(L)	79(S)	64(L)	68(L)
0.25L	57(I)	54(I)	61(L)	59(L)	79(S)	73(L)	77(L)
0.35L	57(I)	54(I)	71(Tr)	71(L)	79(S)	79(S)	79(L)
0.35L + 0.25M	57(I)	54(I)	71(Tr)	71(L)	82(I)	82(I)	85(Th)
0.35L + 0.25Th	57(I)	54(I)	71(Tr)	71(L)	79(S)	79(S)	86(V)
0.35L + 0.25Tr	57(I)	54(I)	76(Th)	71(L)	79(S)	79(S)	79(L)
0.35L + 0.25I	59(S)	64(V)	71(Tr)	71(L)	79(S)	79(S)	89(V)

L, lysine; I, isoleucine; V, valine; Tr, tryptophan; Le, leucine; Th, threonine; M, methionine; S, sulfur amino acids. The limiting amino acids are indicated in parentheses.

Table 7. The correctness of the prediction of the limiting amino acid using various reference patterns

Diets and amino acid additions g/100 g	Experimental limiting amino acid	Whole egg (FAO, 1970)	Human milk (FAO/WHO, 1965)	Human milk (FAO, 1970)	Whole rat carcass	FAO 1957 pattern	FAO 1957 modified pat. [27]	FAO/WHO 1973
A	L	+	+	+	+	+	+	+
A + 0.15L	L	+		+	+		+	+
A + 0.25L	L			+	+		+	+
A + 0.35L	S					+	+	
B	L	+	+	+	+	+	+	+
B + 0.15L	L	+		+	+		+	+
B + 0.25L	L			+	+		+	+
B + 0.35L	I	+	+					
C	L	+	+	+	+	+	+	+
C + 0.15L	L	+	+	+	+		+	+
C + 0.25L	L	+		+	+		+	+
C + 0.35L	L				+		+	+
C + 0.40L	Th							+
D	L	+	+	+	+	+	+	+
D + 0.15L	L	+	+	+	+		+	+
D + 0.25L	L			+	+		+	+
D + 0.35L	Th				+			+
Total No. of (+)		10	7	12	14	5	14	15

L, lysine; S, sulfur amino acids; I, isoleucine; Th, threonine.
The plus signs (+) indicate correct prediction.

Table 8. Correlation coefficients and regression equations relating NPU_{st} values to protein quality scores

Scoring system	r	p	Regression equations
Whole egg (FAO, 1970)	0.84	<0.001	$Y = 0.70\,X + 11.58$
Human milk (FAO/WHO, 1965)	0.77	<0.001	$Y = 0.48\,X + 25.68$
Human milk (FAO, 1970)	0.78	<0.001	$Y = 0.77\,X + 11.85$
Whole rat carcass	0.84	<0.001	$Y = 1.01\,X - 4.60$
FAO 1957 pattern	0.58	<0.01	$Y = 0.32\,X + 56.52$
FAO 1957 modified [27]	0.89	<0.001	$Y = 1.21\,X - 4.50$
FAO/WHO, 1973	0.85	<0.001	$Y = 1.17\,X + 0.03$

r, correlation coefficient; *X*, score; *Y*, NPU_{st}; *p*, the probability of observing a value greater than the calculated regression (*r*).

there is an apparent excess of some non-essential amino acids in the composition of rat carcass. This can lead to incorrect predictions both of the order of sequence and magnitude of limitations. The level of threonine in the FAO 1957 modified pattern was low which resulted in its failure in predicting the second limiting amino acid in most cases.

Human milk [9] was better than the remaining scoring systems but owing to its low levels of total SAA and threonine it failed to predict the second limiting amino acid in all of the diets.

The success of a given pattern can also be measured by the closeness of its prediction of the nutritive value of the protein with that of the biological value. To test this, correlation coefficients were calculated for the NPU_{st} values and the appropriate scores (Table 8). Correlation coefficients were highly significant ($p<0.001$) for all of the patterns, except for the FAO 1957 pattern ($p<0.01$). Yet regression equations (Table 8) indicated that FAO/WHO 1973 was still the best predictor of protein quality when compared with biological evaluation.

Our results, therefore, confirm the conclusion of Kaba and Pellett [16] concerning the superiority of the FAO/WHO 1973 pattern in predicting both the true limiting amino acids and the protein quality.

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