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THE PATTERN OF AMINO ACID REQUIREMENTS OF ALBINO RAT FOR OPTIMAL GROWTH

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The albino rat is being widely used for determining the nutritive value of the proteins of foodstuffs and human dietaries.[1,2] Among the food proteins, the proteins of egg have been found to possess the highest nutritive value for promoting growth in rats.[1,2] Egg proteins contain nearly the same amounts of lysine but greater amounts of sulphur amino acids as compared with human milk proteins.[3] Human milk proteins have been found to be adequate for promoting optimum growth in infants. The net protein utilisation of human milk proteins has, however, been found to be slightly less than that of egg proteins in experiments with albino rats.[4] Cow's milk proteins contain more lysine and lesser amounts of sulphur amino acids than human milk and egg proteins and also possess a lower nutritive value than egg proteins. Cox *et al.*[5] have reported that the sulphur amino acid requirements of albino rats for optimum growth are greater than those of infants. The essential amino acid requirements of rats for optimal growth as determined by different workers[6-9] differ in some respects from the amino acid pattern of the proteins of egg and of human milk. Mitchell[10] reported that fortification of a diet containing 9% egg proteins with lysine brought about a slight but significant increase in the growth of rats. Howard *et al.*[11] concluded from their studies that the lysine content of a 'complete' protein for promoting optimal growth in rats is 5.2%. Bender[12] reported that a 'target' protein containing about 20% less of all essential amino acids than egg proteins possessed a NPU of 92-99 and that egg proteins at 10% level contain excessive amounts of several amino acids than that required for obtaining a NPU of 100. Protein blends having an amino acid composition similar to FAO reference protein pattern have been found to possess lower protein efficiency ratio than milk proteins.[13,14]

An 'ideal' amino acid pattern for meeting the amino acid needs of infants and children suggested by one of us[15] differs in some respects from the amino acid patterns of egg and human milk The object of the present investigation is to determine the pattern of amino acids required in terms of egg and milk proteins and their blends for promoting maximum growth of albino rats at 10% level of protein intake.

Experimental

Plan of the experiment: The plan of the experiment was to determine the growth, protein efficiency ratio (PER) and net protein utilisation (NPU) of (1) egg protein at 10, 8·5 and 7 5 percent levels with or without fortification with l-lysine or dilution with l-glutamic acid to 10 percent protein level in the diet (2) a mixture of equal amounts of egg and milk protein (similar in amino acid composition to the proteins of human milk excepting for a slightly higher lysine content) and the same fortified with dl-methionine to simulate the 'ideal' protein pattern suggested by one of us[15] and (3) protein blends having an amino acid pattern similar to FAO reference protein.

Materials: A sample of spray-dried egg protein supplied by Egg Marketing Board, U.K and skim milk powder of good quality were used in the experiments.

Essential amino acid composition: The essential amino acid composition of the proteins of egg powder and skim milk powder used in the study was determined according to the methods of Krishna-

murthy *et al.*[16] The essential amino acid composition of the different blends calculated from these values is given in Table I.

Animal Experiments: *Protein efficiency ratio*: The PER was determined by the method of Osborne, Mendel and Ferry.[17] The composition of experimental diets was similar to that used by Tasker *et al.*[18] Groups of weanling albino rats (28 days old and weighing from 40-45g) were housed individually in raised wire screen bottom cages and were fed on the different diets for a period of 4 weeks Records of the daily food intake and the weekly increase in body weight were maintained. The results are given in Tables II and III.

Determination of net protein utilisation: The NPU of different proteins was determined according to the method of Miller and Bender[19] with slight modifications as described by Panemangalore *et al.*[20] Groups of weanling male albino rats (28 days old and weighing between 40-45g) were used. The animals were distributed according to a randomised block design to eight groups of 8 rats each. The first group received a nitrogen-free synthetic diet while the remaining were fed on the experimental diets. The animals were sacrificed at the end of the 10 day period. The contents of the gastro-intestinal tract were removed by squeezing and the whole carcass was minced and dried to constant weight in a hot-air drier at 90-95°C. The nitrogen content of the carcass was determined according to the micro-Kjeldahl method. The NPU values were calculated according to Miller and Bender[19] as follows:

$$\text{NPU} = \frac{\text{Body N of test group} - \text{Body N of the non-protein group} + \text{N consumed by the non-protein group}}{\text{N consumed by test group}}$$

NPU (Standard) values were calculated according to Miller and Payne[21] using the following formula:

Table I. *The amino acid pattern of the proteins of egg, human milk, cow's milk, ideal reference protein, FAO pattern and egg protein diluted with glutamic acid as compared with estimated amino acid requirements of albino rats for optimal growth*

Amino acid (g/16gN)	Egg	Human milk	Cow's milk	Egg (8.5%) +glutamic acid (2.5%)	Egg (7.5%) +glutamic acid (4.2%)	Egg (6.5%) +glutamic acid (5.9%)+dl-methionine (0.07%)	Egg (5%) +milk (5%)[a]	Sesame (7.5%) +milk (2.5%)[b]	FAO pattern	Ideal reference protein	Growth needs of albino rats			
											Ref 6	Ref 8	Ref 9	Ref 12
Arginine	6.6	4.1	3.7	5.6	5.0	4.3	5.2	7.5	—	6.5	—	—	3.8	—
Histidine	2.4	2.2	2.7	2.0	1.8	1.6	2.6	2.0	—	2.4	4.0	2.1	2.3	1.8
Lysine	6 6	6.6	7.9	5.6	5.0	4.3	7.3	4.2	4.2	7.5	10.1	9.0	7.2	5.2
Leucine	8.8	9.1	10.0	7.5	6.6	5.7	9.4	8.0	4.8	10.0	8.0	6.9	8.7	7.8
Isoleucine	6 6	5.5	6.5	5.6	5.0	4.3	6.6	4.9	4.2	6.5	5.0	5.5	6.3	4.3
Methionine	3.1	2.3	2 5	2.6	2.3	2.7	2.8	2.7	2,2	2.5	—	—	—	—
Cystine	2.3	2.0	0.9	2.0	1.7	1.5	1.6	1.6	—	2.0	—	—	—	—
Total sulphur amino acids	5.4	4.3	3.4	4.6	4.0	4.2	4.4	4.3	4.2	4.8	7.5	4.9	6.2	4.7
Phenylalanine	5 8	4.4	4.9	4.9	4.4	3.8	5.4	6.0	2.8	5.8	4.6	—	5.9	4.9
Tyrosine	5.0	5.5	5.1	4.3	3.8	3.3	5.1	4.2	2.8	5.0	—	—	3.3	—
Total aromatic amino acids	10.8	9.9	10.0	9.2	8.2	7.1	10.4	10.2	5.6	10.8	—	7.2	9.2	—
Threonine	5.0	4.5	4.7	4.3	3 8	3.3	4.9	3.6	2.8	5.0	5.0	5.1	4.5	4.1
Tryptophan	1 7	1.6	1.4	1.5	1.3	1 1	1.6	1.5	1.4	1.6	1.0	1.1	1.4	1.0
Valine	7.4	6 3	7.0	6.3	5.6	4.8	7.2	4.7	4.2	7.0	7.0	5.8	7.5	5.0

(a) Similar in composition to the proteins of human milk except for a slightly higher lysine content

(b) Similar in composition to FAO reference protein

Table II. *Protein efficiency ratio of egg protein supplemented with lysine and egg protein diluted with varying levels of glutamic acid*
(Period of experiment—4 weeks)

Group no	Source of protein in the diet	Initial body weight g	Gain in weight g	Protein intake g	PER	Gain in body weight g/day	Critical difference 5%	1%	0.1%	
Series I (8 male rats per group)					±0.07 (14 d.f.)	±0.19 (14 d.f.)				
1.	Egg, 10%	46.5	132.4	27.9	4.74	4.73	c 0.18	0.26	0.38	a
2.	Egg, 8.5%	46.8	105.7	22.4	4.74	3.78	d 0 22	0.30	0.42	
3.	Egg, 8.5% + l-glutamic acid, 2.5%	47.1	105.8	26.3	4.03	3 78	c 0.46	0.69	1.00	b
							d 0.56	0.78	1.09	
Series II (8 male rats per group)					±0.11 (35 d.f.)	±0.22 (35 d.f.)				
4.	Egg, 10%	43.8	130.3	27.8	4.68	4.65				
5.	Egg, 10% + l-lysine, 0.3%	43.9	133.1	28 9	4.59	4.75	c 0.27	0.38	0.53	a
6.	Egg, 7.5%	44.1	95.0	20.0	4.72	3.39	d 0.32	0.43	0.57	
7.	Egg, 7.5% + l-glutamic acid, 4.2%	44 4	101.6	28.7	3.53	3.63	c 0.53	0.77	1.05	b
8.	Egg, 7.5% + l-lysine, 0.22%	44.3	105.5	22.2	4.74	3.77	d 0.64	0.86	1.13	
9.	Egg, 7.5% + l-glutamic acid, 4.2% + l-lysine, 0.22%	44.4	98.6	27.7	3.55	3.52				
Series III (8 male rats per group)					±0.08 (21 d.f.)	±0.10 (21 d.f.)				
10.	Egg, 10%	46.0	131.6	28.0	4.71	4.70	c 0.20	0.29	0.40	
11.	Egg, 6.5%	46.1	90.9	19.6	4.64	3.25	d 0.24	0.32	0.43	a
12.	Egg, 6.5% + l-glutamic acid, 5 9%	46.0	90.4	28.9	3.12	3.23	c 0.23	0.34	0.48	
13.	Egg, 6 5% + l-glutamic acid, 5.9% + dl-methionine, 0.07% (FAO reference pattern)	46.0	91.4	29.0	3.16	3.26	d 0.28	0.38	0.51	b

a Critical difference for protein efficiency ratio
b Critical difference for gain in weight
c One tailed test
d Two tailed test

$$NPU_{(st)} = \frac{NPU \times 54}{54 - P} - 8$$

Where P= percent protein in the diet.

Net protein ratio: The net protein ratio (NPR) of the different foods was calculated according to Bender and Doell,[22] using the following formula:

$$NPR = \frac{\text{Gain in weight of the test group + loss in weight of the non-protein group}}{\text{Protein intake of the test group}}$$

The results are given in Tables IV and V.

Results

Growth rate of rats on different levels of egg proteins (with and without fortification with l-lysine amd dilution with l-glutamic acid): The mean daily growth rates of rats on different levels of egg proteins viz. 10, 8·5, 7·5 and 6·5% (with and without added lysine and dilution with glutamic acid to 10% protein level) are given in Table II. The results show that the maximum growth rate (4·65 to 4·73g/day) was observed in rats fed on 10% egg protein and the daily growth rate progressively fell on diets containing 8·5%, 7·5% and 6·5% egg protein from 3·78 to 3·25g. Addition of l-glutamic acid to maintain the protein content in the above diets at 10% level did not bring about any significant increase in the growth rates. Supplementation of diets containing 10 and 7·5% egg proteins with l-lysine did not cause any significant increase in the growth rate, indicating thereby that the lysine level of 6·6g/16gN in egg is adequate for promoting maximum growth of rats when the level of egg protein in the diet is 10% or 7·5%.

PER of different levels of egg protein (with and without fortification with l-lysine

Table III. *Protein efficiency ratio of protein blends having amino acid composition similar to that of 'ideal' reference protein and FAO reference protein and the effect of supplementation of FAO reference pattern with varying levels of lysine*
(Level of protein in the diet, 10% ; period of experiment, 4 weeks)

Group no	Source of protein in the diet	Initial body weight g	Gain in body weight g	Protein intake g	PER		Gain in body weight g/day		Critical difference 5%	1%	0.1%	
Series IV (8 male rats per group)												
14.	Egg, 5% +skim milk powder, 5% (similar to human milk proteins)	41.8	109.8	27.4	4.31	±0.11 (15 d.f.)	3.92	±0.20 (15 d.f.)	c 0.26	0.39	0.55	a
15.	Egg,5%+skim milk powder, 5%+ dl-methionine, 0.05% (ideal reference protein)	41.8	114.3	25.1	4.56		4.08		d 0.32	0.44	0.61	
16.	Sesame flour, 7.5% +skim milk powder, 2.5% (FAO reference protein)	41.7	70.8	27.8	2.54		2.53		c 0.49	0.73	1.05	b
17.	Sesame flour, 7.5% +skim milk powder, 2.5% +l-lysine, 0.4%	42.0	91.7	30.0	3.04		3.27		d 0.60	0.83	1.14	
Series V (8 male rats per group)												
18.	*Sesame flour, 7.5% +skim milk powder, 2.5% (FAO reference protein)	46.4	71.8	28.3	2.54	±0.05 (35 d.f.)	2.56	±0.10 (35 d.f.)	c 0.13	0.19	0.26	a
19.	* ,, +l-lysine, 0.1%	46.5	92.5	31.5	2.93		3.30		d 0.16	0.21	0.28	
20.	* ,, +l-lysine, 0.2%	46.6	108.4	31.3	3.46		3.87					
21.	* ,, +l-lysine, 0.3%	46.4	103.3	30.2	3.42		3.69		c 0.23	0.34	0.46	b
22.	* ,, +l-lysine, 0 4%	46.5	109.5	32.3	3.58		3.91		d 0.28	0.38	0.50	
23.	Skim milk powder, 10%	46.6	76.0	24.3	3.13		2.71					

* The lysine contents of the proteins in the diets were 4.2,3.2,6.2,7.2 and 8.2g per 16gN
a Critical difference for protein efficiency ratio
b Critical difference for gain in weight
c One tailed test
d Two tailed test

and dilution with l-glutamic acid): The PER's of egg protein at 10, 8·5, 7·5 and 6·5% levels were 4·74, 4·74, 4·72 and 4·64 respectively, there being no significant difference between these values. Supplementation with l-lysine did not bring about any significant improvement in the PER's. Dilution of egg protein with l-glutamic acid (to maintain the protein intake at 10% level) however, brought about a significant decrease in the PER's, the values on the different diets being as follows: 10% egg protein, 4·74; 8·5% egg protein + l-glutamic acid, 4.03; 7.5% egg protein + l-glutamic acid, 3.53; and 6.5% egg protein +l-glutamic acid, 3.12. Supplementation of the above blend with l-lysine did not bring about any increase in the PER.

NPU of different levels of egg proteins (with and without dilution with l-glutamic acid): The NPU of diets containing 10, 8.5, 7.5 and 6.5% of egg proteins were 96.7, 95.8, 97.2 and 96.3 respectively there being no significant difference between the values. The corresponding NPU(st) values were 110.7, 109.5, 111.2 and 110.4. Dilution of the above diets with l-glutamic acid (to maintain the protein levels in the diets at 10%) brought about a significant decrease in the NPU values.

Growth rate, PER and NPU on diets containing proteins similar to FAO pattern, 'ideal' protein pattern and human milk proteins: The mean daily growth rates (Table III) of rats fed diets containing a mixture of 5% egg protein and 5% milk proteins (similar in amino acid composition to human milk proteins) and the same fortified with dl-methionine ('ideal' reference pattern) were 3.92 and 4.08g and lower than that observed with 10% egg protein diet (4.65 to 4.73). The PER's (Table III) of the above diets were 4.31 and 4.56 as compared with values of 4.68 to 4.74 obtained for diets containing 10% egg proteins. The NPU values (Table IV) of

Table IV. *Net protein utilisation of protein blends having amino acid composition similar to that of 'ideal' reference protein and FAO reference protein as compared with that of the proteins of egg and milk*
(Level of protein in diet, 10% ; 8 male rats per group)

Group no	Source of protein in diet	Initial body weight g	Final body weight g	Nitrogen intake g	Final body nitrogen g	NPU	$NPU_{(ss)}$	NPR
1.	None	48.6	40.8	0.03	1.17	—	—	—
2.	Egg, 10%	48.8	86.0	1.23	2.35	97.9	112.1	5.85
3.	Egg, 5% + skim milk powder, 5% (similar to human milk proteins)	48.5	82.5	1 19	2 26	93.5	106.7	5,64
4.	Egg, 5% + skim milk powder, 5% + dl-methionine, 0.05% (ideal protein pattern)	48.6	83.0	1.18	2 26	94.7	108.1	5.72
5.	Skim milk powder, 10%	48 4	68.5	1.02	1.97	79 6	89.7	4.36
6.	Skim milk powder, 10% + dl-methionine, 0.2%	48.9	76.1	1.20	2.18	86.4	98.0	4.67
7.	Sesame flour, 7·5% + skim milk powder, 2.5%	48 6	67.7	1.15	1.89	64.5	71.2	3.74
8.	Sesame flour, 7.5% + skim milk powder, 2.5% + l-lysine 0.4%	48.6	73.6	1.30	2.14	76.6	86.0	4.02
	Standard error of the mean (42 d.f.)					±1.25	±1.54	±0.09

	NPU		$NPU_{(ss)}$		NPR	
	a	b	a	b	a	b
Critical difference at 5%	2.98	3.57	3.67	4.40	0 22	0.26
,, ,, 1%	4.28	4 78	5.28	5.89	0.31	0.36
,, ,, 0.1%	5.85	6.27	7.19	7.72	0.42	0.45

a—One tailed test b—Two tailed test

the above diets were 93.5 and 94.7 as compared with a value of 97.9 obtained for 10% egg protein diet.

The mean daily growth rates (Tables II-III) of rats fed on diets containing proteins simulating FAO reference pattern (2.56 and 3 26g) were very much less than those observed with egg proteins (4.65 to 4.73), the 'ideal' reference pattern (4.08) and human milk protein pattern (3.92). The PER's of the diets containing proteins simulating FAO pattern (2.54 and 3.16) were very much less than those of egg proteins (4.68 to 4·74), human milk protein pattern (4.31) and 'ideal' reference protein pattern (4.56). Fortification of the FAO pattern based on sesame and skim milk with l-lysine at a level of 2g lysine per 16gN brought about a considerable increase in the PER indicating thereby that the FAO pattern is deficient in lysine. The NPU value of the diets (64.5 and 76.6) were also significantly lower than the NPU values of egg proteins (97.9), human milk protein pattern (93.5) and 'ideal' reference protein pattern (94.7).

Discussion

The results obtained in the present study have shown that the amino acid requirements of albino rats for maximum growth are provided by 10% egg proteins. Contrary to the results of Mitchell,[10] supplementation with l-lysine did not cause any further improvement in the growth rate or PER of egg proteins. The growth rate of rats fed diets containing 8.5% egg protein was significantly less than that observed with 10% egg protein diet, even though the PER and NPU values of the former were the same as those of the latter. A diet containing a mixture of 8.5% egg protein and 2.5% l-glutamic acid promoted significantly less growth and possessed significantly lower PER and NPU than those of 10% egg proteins. These results are at variance with those of Bender[12] who reported a NPU value of 99 for a blend of 8.5% egg proteins and 1.5% nonessential amino acids. Rama Rao *et al.*[9] reported the lysine requirement of rats (in diets containing 10% protein) for maximum growth to be 9.0g/16gN. The results of the present study, however,

Table V. *Net protein utilisation of egg protein diluted with varying levels of glutamic acid and FAO reference pattern based on egg protein*
(Level of protein in diet, 10% ; 8 male rats per group)

Group no.	Source of protein in the diet	Initial body weight g	Final body weight g	Nitrogen intake g	Final body nitrogen g	NPU	$NPU_{(st)}$	NPR
1.	None	45.1	36.4	0.04	1.08	—	—	—
2.	Egg, 10%	44.1	90.6	1.52	2.50	96.7	110 7	5.84
3.	Egg, 8.5%	44.5	83.1	1.18	2.17	95.8	109.5	6.44
4.	Egg, 8 5% + l-glutamic acid, 2.5%	44.1	81.1	1 34	2.24	89.0	101.4	5.49
5.	Egg, 7.5%	45.5	80.9	1.06	2.07	97.2	111.2	6.66
6.	Egg, 7.5% + l-glutamic acid, 4.2%	45.5	72.8	1.22	2.00	78.8	88.6	5.08
7.	Egg, 6.5%	44.8	75 6	0.97	1.98	96.3	110.4	6.44
8.	Egg, 6.5% + l-glutamic acid, 5.9% + dl-methionine, 0.07%	44.1	66.0	1.17	1.93	76.4	85.8	4.45
	Standard error of the mean (42 d.f.)					±1.0	±1.25	±0.13
						a b	a b	a b
	Critical difference at 5% level					2.4 2 8	3.0 3.6	0.31 0.37
	,, ,, 1% level					3 4 3.8	4.3 4.8	0.44 0.49
	,, ,, 0.1% level					4.7 5.0	5.8 6.3	0 60 0.65

a–One tailed test b–Two tailed test

indicate that a lysine content of 6.6g/16gN (same as that present in egg proteins) is adequate for promoting maximum growth at a 10% level of protein intake. The results obtained in this study, however, show that the sulphur amino acid content of 4.8g/16gN as that present in 'ideal' reference pattern is not adequate for promoting maximum growth in albino rats. These results are not in agreement with those of Rama Rao *et al*[8] who found that a total sulphur amino acid content of 4.9g/16gN was adequate for promoting maximum growth in albino rats on diets containing 10% protein and adequate amounts of other amino acids.

Since maximum growth was observed in rats fed on a diet containing 10% egg protein, it may be concluded from the results of this experiment that egg proteins provide the ideal amino acid pattern for promoting maximum growth in rats.

Summary

(1) The mean daily growth rates of albino rats on diets containing different levels (6,5% to 10%) of egg proteins were determined over a period of 4 weeks. A maximum growth rate of 4.65 to 4.73g/day was observed on diets containing 10% egg protein. The growth rates progressively decreased as the level of protein was lowered viz. 8.5% protein, 3.78g; 7.5% protein, 3. 39g and 6.5% protein, 3.25g. Addition of l-glutamic acid to maintain the protein content in the above diets at 10% level did not bring about any significant increase in the growth rates.

(2) No significant difference was observed in the protein efficiency ratios (4.64 to 4.74) or in the net protein utilisation (95.8 to 97.2) of diets containing 10, 8.5, 7.5 and 6.5% of egg proteins. On the other hand, when l-glutamic acid was added to maintain the protein at 10% level in all the diets, the PER and NPU progressively decreased from 4.74 to 3.12 and from 96.7 to 76.4 respectively, thus indicating that egg proteins at 10% level do not contain excessive amounts of essential amino acids over and above that required for promoting maximum PER and NPU.

(3) Supplementation of diets containing 10 and 7.5% egg proteins with l-lysine so as to raise the lysine content to 9.6g/16gN did not bring about any increase in the growth rate or in the PER thus indicating

that egg proteins contain adequate amounts of lysine.

(4) The mean daily growth rate of rats fed a diet containing a mixture of 5% egg proteins and 5% milk proteins (similar in amino acid composition to human milk proteins) was 3.92 and significantly lower than that observed with 10% egg protein. It may be concluded from the results that egg proteins provide the ideal amino acid pattern required for promoting maximum growth of albino rats, at 10% level of protein intake.

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