The Amino Acid Content of Hen's Egg in Relation to Dietary Protein Intake, Breed and Environment[1]

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In 1963 the Joint FAO/WHO Expert Group on Protein Requirements decided to adopt the essential amino acid pattern of either egg or human milk for reference purposes in preference to the FAO provisional pattern proposed in 1957.

The data, however, were not sufficiently precise to provide a basis for choosing between the essential amino acid pattern of these two protein sources.

The Group suggested therefore that further research be conducted in the field of protein requirements and indicated that more work was needed on the pattern of essential amino acids and, in general, on the quantity of amino acids in foods.

Since the publication of the FAO/WHO report on this meeting in 1965 (2), much progress has been made in the techniques of amino acid assay with the development of high-speed automatic amino acid analysers and with the introduction of spheric polystyrene sulfonated resins of high resolving power.

In spite of the extension of these new laboratory facilities, it is surprising to see how poor is the international scientific literature in recent analytical work on the amino acid composition of hen's egg.

Similarly, we have found very few references on the amino acid composition of hen's egg in relation to the protein content of the diet and to other factors such as breed and environmental conditions which may have an influence on the amino acid pattern of the egg and, therefore, introduce another variable in the assessment of protein quality of foods and in the study of protein requirements where whole hen's egg is used as reference protein.

An exhaustive compilation of available data carried out in the FAO Nutrition Division (3) on the amino acid content of foods shows that analyses of egg are small in number and the results often variable. Most of them have been made by microbiological methods which are known to be less accurate than ion-exchange chromatography and which, furthermore, are all previous to 1965.

For these reasons we have carried out a study of the hen's egg amino acid content by ion-exchange chromatography, using the most recent automatic equipment, including an electronic curve integrator.

Methodology

Two breeds of hen have been used: 40 pullets New Hampshire (N.H.) and 20 pullets White Leghorn var. Livornese (W.L.) which were fed on two isocaloric diets with a protein content (N × 6.25) respectively of 20% and 11% and a balanced amino acid pattern (see Annex 1). The experiment started with 3 month-old animals and the collection of eggs lasted one full year.

One group of animals was fed in individual cages and the other was free-running in an open-air enclosure in order to check possible differences in egg protein composition between animals bred under controlled conditions and those subject to climatic fluctuations. The number of eggs laid, their weight, relative weights of yolk, white and shell were recorded and the protein and amino acid content of whole egg, yolk and egg-white were determined taking into account the various parameters: protein intake, breed, enclosure or cage.

Analytical techniques

The analytical techniques used are as follows:

Total Nitrogen: microkjeldahl (4)

Amino acids ion-exchange chromatography (5)

- Beckman Model 120 C amino acid analyser

- Resin PA 28 for neutral and basic amino acids PA 35 for acid amino acids

Cystine as cysteic acid (6)

Tryptophan by ion-exchange chromatography after alkaline hydrolysis (7)

Experiment

The animals were distributed among 6 groups of 10 pullets (2 W.L., 4 N.H.). of these 6 groups, 3 were fed on the lower protein feed (11%) and 3 on the higher protein feed (20%). Two groups of N.H. were free-running birds. All animals were fed ad libitum and all eggs were identified with a number corresponding to each hen in special laying cages.

Ten eggs from each group were pooled at random every fortnight. These eggs were homogeneized with a turmix and lyophilized in 24 hours in an Edwards ELPTC freeze dryer. The egg powder was homogeneized once more and an aliquot was taken from the total sample used for the various assays.

Results

This study was carried out on about 2000 eggs. The samples were analyzed always in duplicate and often in triplicate for the individual determination of protein, amino acids, cystine and tryptophan respectively.

Protein content of egg

A significant difference was noted in both breeds as to the quantity of protein in egg, in relation to the protein level in the feeds (Table 1).

Table 1

Protein Content of Egg (in % - dry weight)

Breed	White Leghorn		New Hampshire	
Diet	11% Protein	20% Protein	11% Protein	20% Protein
Egg Protein (N × 6.25)	47.5	48.6	45.5	46.0

Hens given a high protein feed lay eggs with 1 to 2% more protein than in the other group. Moreover, as might be expected, the total number of eggs laid by the first group was higher than by the second.

In addition, it appears that breed has an influence on the protein content of the egg; although the W.L. hens lay eggs weighing about 5% less than the N.H., their protein content, regardless of diet, is 5% higher.

This is probably due to the fact that the yolk in W.L. is relatively bigger than in N.H. and being richer in protein (16% versus 10.9%) than egg-white, it makes a significant difference to the comparative protein content of the whole egg.

These results were also found in the free-range group.

Amino acid content of egg

There is practically no difference between the amino acid patterns of hens fed both diets and between each breed. The remarkable stability of hen's egg amino acid content should therefore be underlined.

In general, our results show slightly lower values than previously published data for all the amino acids except for cystine, lysine and tryptophan. The cystine content, in particular, is much higher. It should also be noted that the isoleucine content is considerably (about 20%) lower than currently accepted figures.

Our data are compared to previously published data and are expressed respectively in mg per gram of total nitrogen (Table 2), in mg per gram of total essential amino acids (A/E ratio) (Table 3).

In Table 4 we have tabulated the amino acid composition of eggs produced by the two strains fed respectively the 11% and 20% protein diets over a period of one year. It is obvious from this table that the amino acid content of hen's egg is very stable regardless strain and protein intake. In addition, our detailed data, which do not appear in this report, indicate that there is no significant seasonal variation in hens under controlled conditions nor in those living in an open-air enclosure.

Table 2

Essential amino acids in egg protein and in human milk compared to 1957 FAO provisional pattern (mg per g of total nitrogen)

Amino acid	1957 FAO provisional Pattern 1	Human milk 1965 2	Hen's egg 1946 3	Hen's egg 1965 4	Hen's egg 1970 5	Hen's egg 1971 6
Isoleucine	270	411	500	415	393	331
Leucine	306	572	575	553	551	523
Lysine	270	402	450	403	436	445
Total "aromatic"	360	652	675	627	618	577
Phenylalanine	180	297	-	365	358	328
Tyrosine	180	355	-	262	260	249
Total "sulfur containing"	270	274	406	346	362	403
Cystine	126	134	-	149	152	208
Methionine	144	140	-	197	210	195
Threonine	180	290	306	317	320	294
Tryptophan	90	106	94	100	93	117
Valine	270	420	456	454	428	413
Total essential amino acids	2016	3127	3462	3215	3201	3103

1 Protein requirements - FAO Nutr. Studies No. 16, 1957

2 Protein requirements - FAO Nutr. Meetings Report Series No. 37, 1965

3 Mitchell, H.H., R.J. Block - J. Biol. Chem., 163, 599 - 620 (1946)

4 Protein requirements - FAO Nutr. Meetings Report Series No. 37, 1965

5 Amino acid content of foods - FAO Nutr. Studies No. 24, 1970

6 The amino acid composition of hen's egg in relation to the protein content of poultry feed - C. Le Clément de St. Marcq, E. Carnovale, A. Fratoni and P. Lunven (to be published)

Table 3

Selected essential amino acid patterns (A/E)

A/E ratio: mg per g of total essential amino acids

Amino acid	1957 FAO provisional pattern	Human milk 1965	Hen's egg 1965	Hen's egg 1971	Recommended levels 1965
Isoleucine	134	137	129	108	-
Leucine	152	184	172	170	-
Lysine	134	128	125	144	<134
Total "aromatic"	178	226	195	187	-
Phenylalanine	89	114	114	106	-
Tyrosine	89	112	81	81	-
Total "sulfur containing"	133	87	107	130	94-110
Cystine	62	43	46	68	-
Methionine	71	44	61	63	-
Threonine	89	99	99	95	-
Tryptophan	45	34	31	38	30-35
Valine	134	147	141	134	-

Table 4

Amino acid content of hen's egg (mg/gN) in relation to dietary protein level and breed

Amino acid	White Leghorn		New Hampshire		Mean and range	Hen's egg 1970
	11% prot.	20% prot.	11% prot.	20% prot.		
Lysine	448	441	442	448	445 (395-502)	436
Histidine	137	138	140	137	138 (116-159)	152
Arginine	373	368	373	376	373 (329-396)	381
Aspartic Acid	610	609	612	610	610 (580-717)	601
Threonine	289	295	301	290	294 (261-324)	320
Serine	447	456	459	445	452 (396-505)	478
Glutamic Acid	822	831	820	818	823 (779-873)	796
Prcline	237	232	233	232	233 (195-259)	260
Glycine	197	196	197	197	197 (185-209)	207
Alanine	338	338	340	338	338 (307-363)	370
Cystine	212	207	208	206	208 (170-254)	152
Valine	412	413	410	419	413 (371-457)	428
Methionine	195	200	191	195	195 (164-249)	210
Isoleucine	333	328	327	336	331 (291-369)	393
Leucine	519	524	526	522	523 (480-551)	551
Tyrosine	250	249	251	248	249 (224-281)	260
Phenylalanine	332	329	326	325	328 (288-370)	358
Tryptophan	115	111	128	114	117 (83-145)	93

Discussion

The 1965 Joint FAO/WHO Expert Committee report on protein requirements describes, in p. 35 and following, the various considerations which led them to adopt the whole hen's egg as reference protein.

In particular:

- The Committee expressed the view that the FAO provisional pattern contained too much tryptophan, sulfur-containing amino acids and lysine. This view was supported by observations on man and experimental animals.

- The Committee therefore recommended that these amino acids be present in lesser quantities in the reference protein (see Table 3) and considering that the new levels assigned to them were closer to those of hen's egg amino acid pattern when expressed in terms of A/E ratio, decided to adopt the latter as reference protein (8).

(At this point, it might be interesting to note that the correlation between the chemical score calculated by the A/E ratio method and the data on biological value is poorer than when the score is expressed in terms of mg of individual essential amino acid per gram of total nitrogen (Mitchell and Block method)) (9).

- In the light of the new data provided in this report, it appears that the hen's egg pattern (1971) expressed in terms of A/E ratio resemble much more closely the 1957 FAO provisional pattern than the 1965 hen's egg and human milk amino acid patterns.

- One of the immediate implications of these new data on the composition of the reference protein is that the disparity between an improved reference amino acid pattern as suggested by the Committee in 1965 and the whole hen's egg amino acid patterns. becomes even greater.

- This disparity is greatest in the case of lysine, tryptophan and sulfur-containing amino acids when they are expressed in mg per g of total nitrogen but is still important when the essential amino acid patterns are calculated as A/E.

- It should also be noted that the isoleucine content of hen's egg is considerably lower than in previously published data. This is particularly important because the frequency of isoleucine as the limiting amino acid seems to have been over-estimated in chemical score determinations based on the 1965 hen's egg and human milk amino acid patterns.

The Committee therefore, taking into account all the works which have been done on man and on animals, might wish to take this opportunity to design a synthetic amino acid reference pattern which would give better correlation between the chemical score of dietary proteins and their biological assessment.

Summary

1. Protein intake and breed seem to have no effect on the amino acid composition of hen's egg although there are some small changes in total protein content.
2. Lysine, tryptophan and especially cystine are present in greater proportion than in previously published data whereas isoleucine is much lower than the currently accepted figures.
3. These results imply that the questions raised by the Joint FAO/WHO Expert Group on Protein Requirements in 1965 regarding the excess of tryptophan, sulfur-containing amino acids and lysine in the 1957 FAO provisional pattern are also valid for the whole egg protein which contains even higher quantities of these essential amino acids.

ANNEX 1

Composition of poultry feeds

	Diet 20% protein (N × 6.25)		Diet 11% protein (N × 6.25)	
	% of feed	Protein % of feed	% of feed	Protein % of feed
Maize flour	34	3.2	46	4.4
Wheat flour	18	2	21	2.4
Oats flour	12	1.4	12	1.4
Soya flour (defatted)	16	7.7	-	-
Wheat bran	-	-	8	1.1
Lucern	2	0.4	2	0.4
Dry skim milk	1.5	0.5	0.5	0.1
Fat	3.5	-	3.5	-
Fish meal	4	2.8	0.5	0.3
Powdered meat	4	2	0.5	0.2
Minerals and vitamins	5	-	5	-
DL Methionine	-	-	0.2	-
L Lysine	-	-	0.2	0.7
Gelatine (p. arginine)	-	-	0.6	-
Total	100.0	20.0	100.0	11.0

ANNEX 1 (Cont'd)

Amino acid composition of poultry feeds

Amino acid	Diet 11% protein (N × 6.25)		Diet 20% protein (N × 6.25)	
	% of protein	% of feed	% of protein	% of feed
Lysine	4.780	0.528	5.066	1.003
Histidine	2.229	0.246	2.288	0.453
Arginine	5.010	0.554	5.796	1.148
Aspartic Acid	6.098	0.674	8.049	1.594
Threonine	3.178	0.351	3.576	0.708
Serine	4.227	0.467	4.554	0.902
Glutamic Acid	20.056	2.216	19.108	3.783
Proline	8.106	0.896	6.647	1.316
Glycine	5.410	0.598	5.212	1.032
Alanine	5.366	0.593	5.057	1.001
Half cystine	2.483	0.274	2.237	0.443
Valine	4.495	0.497	4.526	0.896
Methionine	3.198	0.353	3.200	0.640
Isoleucine	3.394	0.375	3.899	0.772
Leucine	7.604	0.840	7.386	1.462
Tyrosine	2.627	0.290	3.176	0.629
Phenylalanine	4.096	0.453	4.474	0.886
Tryptophan	0.826	0.090	1.043	0.208
Ammonia	7.648	0.845	7.235	1.433

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(1) Research work carried out in the National Nutrition Institute, Rome, Italy. (Director, Prof. S. Visco).

(2) FAO. Protein Requirements. Report of a Joint FAO/WHO Expert Group. FAO Nutrition Meetings Report Series No. 37, 1965.

(3) FAO. Amino Acid Content of Foods. FAO Nutritional Studies No. 24, 1970.

(4) A.O.A.C. No. 2044 and 16001, 1960.

(5) Moore, S. and W.H. Stein. J. Biol. Chem., 192, 663, 1951.

(6) Schram, E., S. Moore and E.J. Bigwood. Biochem. J., 57, 33, 1954.

(7) Lunven, P. Thèse Fac. Pharm. Paris, 1968.

(8) FAO Nutrition Meetings Report Series No. 37. p. 38 (1965)

(9) Cresta, M., J. Périssé, M. Autret and E. Lombardo. Ann. Nutr. Alim. 1971 (in press).