Amino acid composition of hen's egg

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1. Pooled samples of eggs from White Leghorn and New Hampshire hens given diets containing 110 and 200 g protein/kg were analysed for their content of essential amino acids.

2. The amino acid composition of the hen's egg protein was not significantly affected by variations in breed and diet.

3. The estimated values for lysine, total sulphur-containing amino acids and tryptophan in egg protein were higher by 8·9, 2·0 and 8%, respectively than those adopted for hen's egg by the FAO/WHO Expert Committee (FAO, 1965).

4. The isoleucine content of egg protein was found to be much lower (338 mg/g nitrogen) than that reported by other workers.

The FAO/WHO Expert Group on Protein Requirements (FAO, 1965) agreed to adopt the essential amino acid pattern of the hen's egg protein as a reference standard. In evaluating dietary proteins, several workers have observed significant differences in the chemical score calculated from the amino acid pattern and nutritional value determined by biological tests. However, different workers (Block & Mitchell, 1946–7; Rutgers University Bureau of Biological Research, 1950; Edwards, Carter & Outland, 1955) have used different patterns of amino acids as reference standards and this partly explains the differences observed in dietary protein evaluation. References are available only to a few publications on the essential amino acid composition of hen's egg protein associated with variation in breed, the quality and quantity of protein in the diet and the environment of the hens.

EXPERIMENTAL

Two-month-old White Leghorn and New Hampshire hens were used. They were fed *ad lib.* on diets containing 110 and 200 g protein/kg for a period of 12 months. The composition of the diet is shown in Table 1. The diets were supplemented with vitamins (Bird, 1947) and mineral salts (Briggs, Spivey, Keresztsesy & Silverman, 1952). Both diets contained the same amount of sulphur-containing amino acids (3·0 g), lysine (3·2 g), tryptophan (1·0 g) and arginine (4·5 g) per 16 g nitrogen.

The hens were divided into groups of ten and were housed either on deep litter or free range according to the following arrangement:

Breed …	White Leghorn		New Hampshire			
Environment …	Deep litter		Deep litter		Free range	
Protein content of diet (g/kg)	200	110	110	200	110	200
Group no.	6	8	7	5	10	3

Table 1. *Composition of experimental diets (g/kg)*

	Crude protein content (N × 6·25)	
	110	200
Maize flour	460	340
Wheat flour	210	180
Oat flour	120	120
Defatted soya-bean flour		160
Wheat bran	80	—
Lucerne	20	20
Skim-milk solids	5	15
Maize oil	35	35
Fish meal	5	40
Powdered meat	5	40
Vitamin–mineral mix*	50	50
DL-methionine	2	—
L-lysine hydrochloride	2	—
Gelatin	6	—

* According to Bird (1947) and Briggs *et al.* (1952).

The humidity and temperature varied from 51 to 100% relative humidity and from 3° to 34°, respectively.

The eggs were collected in trap cages, permitting the identification of the layer and the numbering of the eggs so that a statistically valid sample could be taken. The number and weights of the eggs and the quantity of diet consumed were recorded.

Every 2 weeks, six lots of ten eggs (one from each hen of each group) were selected from the eggs laid. The contents of the eggs from each lot were individually pooled, homogenized and freeze-dried.

The protein content and amino acid composition were determined for each sample and it was possible to carry out twenty-four determinations of protein content and seventeen determinations of amino acid content.

Total protein was determined by the Kjeldahl method (Association of Official Agricultural Chemists, 1965). The proteins were hydrolysed by conventional acid hydrolysis with 6 M-HCl in a sealed tube (Moore & Stein, 1951). One portion from each sample was hydrolysed separately for the determination of cystine as cysteic acid (Schram, Moore & Bigwood, 1954) and another was hydrolysed with 5 M-NaOH in a sealed tube for determination of tryptophan (Lunven, 1963). The amino acid content was determined with a Beckman-Spinco Model 120C Amino Acid Analyser equipped with a Beckman Model 125 Automatic Digital Integrator. Each analysis was done in duplicate. The amino acid compositions of the yolks and whites of eggs from hens on deep litter were determined. Ten eggs from each group, selected as described, were frozen and their shells were removed. The yolks and whites were separated, weighed, stirred separately, homogenized and freeze-dried. The protein content and amino acid composition were determined for each sample.

Table 2. *Amino acid composition (mg/g nitrogen) of eggs from two breeds of hen, housed either on deep litter or free range and given diets of protein content* 200 *g/kg (groups* 6, 5, 3) *or* 110 *g/kg (groups* 10, 8, 7)

	White Leghorn				New Hampshire									
	Deep litter				Deep litter				Free range					FAO/WHO egg pattern (FAO, 1965)
	Group 8		Group 6		Group 7		Group 5		Group 10		Group 3			
Amino acid	Value	Cv*	Value	Cv*	Value	Cv*	Value	Cv*	Value	Cv*	Value	Cv*	Mean value†	
Lysine	446	3·0	433	3·7	439	2·5	438	2·6	440	3·2	440	3·3	439	403
Histidine	143	5·9	139	4·3	143	5·9	140	4·2	146	5·9	139	5·2	142	—
Arginine	380	3·1	376	3·5	379	3·4	380	2·6	384	3·0	379	2·9	380	—
Aspartic acid	610	2·1	613	2·2	609	2·3	611	2·9	611	2·3	610	2·5	611	—
Threonine	290	3·1	296	4·4	302	3·6	297	4·3	298	3·2	293	3·6	296	317
Serine	447	3·6	458	4·2	466	3·8	457	4·8	454	3·8	450	3·7	455	—
Glutamic acid	823	2·1	833	1·4	821	2·6	823	1·5	824	2·1	827	1·8	825	—
Proline	242	4·2	233	2·9	244	4·9	237	4·3	239	3·7	237	4·3	239	—
Glycine	197	2·5	198	2·6	196	3·4	198	2·0	196	2·6	197	2·2	197	—
Alanine	341	1·8	344	2·0	342	2·3	340	1·1	340	2·0	341	2·2	341	—
Cystine	159	7·2	157	9·4	160	5·6	155	7·4	156	6·1	163	5·3	158	149
Valine	417	2·9	414	3·8	402	4·2	419	3·4	412	2·8	414	4·4	413	454
Methionine	196	4·2	198	5·5	192	5·2	191	4·9	196	4·1	199	6·8	195	197
Isoleucine	340	3·4	335	3·5	332	3·7	343	4·0	334	3·7	343	5·4	338	415
Leucine	532	1·9	535	2·7	538	2·1	536	2·4	534	2·2	534	2·4	535	553
Tyrosine	251	4·0	253	5·1	250	4·4	251	3·6	257	3·9	248	3·3	252	262
Phenylalanine	328	4·5	330	3·5	328	4·5	324	3·2	318	4·6	326	3·3	326	365
Tryptophan	108	9·0	105	8·6	107	8·4	110	9·4	111	8·2	110	7·7	108	100
Protein (mg/g dry matter)	464		474		441		450		435		453			

* Coefficient of variation.
† Mean values of duplicate determinations.

Table 3. *Amino acid composition (mg/g nitrogen) of egg white from two breeds of hen, given diets of protein content* 200 *g/kg (groups* 6 *and* 5) *or* 110 *g/kg (groups* 8 *and* 7)

	White Leghorn		New Hampshire				
Amino acid	Group 8	Group 6	Group 7	Group 5	Mean value	Coefficient of variation	FAO (1970)
Lysine	359	394	368	391	378	4·71	415
Histidine	119	137	135	137	132	5·84	147
Arginine	324	334	327	336	330	1·65	357
Aspartic acid	620	623	650	617	628	3·59	687
Threonine	269	275	278	265	272	2·26	299
Serine	456	409	441	410	429	5·08	456
Glutamic acid	874	879	855	871	869	1·55	937
Proline	216	194	207	220	209	7·70	228
Glycine	206	201	205	207	205	1·92	222
Alanine	359	351	358	362	357	1·50	380
Cystine	185	174	186	167	178	7·45	150
Valine	431	438	411	436	429	2·79	301
Methionine	236	240	253	230	240	4·10	248
Isoleucine	332	335	318	338	331	2·58	321
Leucine	517	526	517	525	521	1·56	518
Tyrosine	240	237	277	276	257	8·29	219
Phenylalanine	386	384	342	358	368	4·97	372
Tryptophan	121	117	122	106	116	6·15	—
Protein (mg/g dry matter)	845	852	840	829	841		

Table 4. *Amino acid composition (mg/g nitrogen) of egg yolk from two breeds of hen, given diets of protein content* 200 *g/kg (groups* 6 *and* 5) *or* 110 *g/kg (groups* 8 *and* 7)

Amino acid	White Leghorn		New Hampshire		Mean value	Coefficient of variation	FAO (1970)
	Group 8	Group 6	Group 7	Group 5			
Lysine	463	476	493	475	477	3·85	480
Histidine	139	154	142	156	148	5·50	158
Arginine	432	436	438	430	434	1·72	469
Aspartic acid	615	607	614	616	613	1·44	663
Threonine	313	307	309	325	313	4·03	346
Serine	509	488	487	511	499	4·90	564
Glutamic acid	772	803	788	756	780	2·88	872
Proline	264	239	225	261	247	7·27	269
Glycine	176	180	182	178	179	1·46	196
Alanine	315	318	312	307	313	2·13	344
Cystine	171	166	136	178	163	9·98	104
Valine	374	378	389	371	378	3·66	260
Methionine	187	165	176	172	175	6·53	161
Isoleucine	350	347	361	335	348	3·06	321
Leucine	543	554	558	537	548	2·19	533
Tyrosine	241	259	266	246	253	4·31	252
Phenylalanine	262	256	253	273	261	3·48	264
Tryptophan	123	117	121	122	121	6·76	—
Protein (mg/g dry matter)	315	312	285	307	305		

Table 5. *Essential amino acids in FAO* (1957) *provisional pattern and in milk and egg proteins (mg/g nitrogen)*

Amino acid	FAO Provisional pattern	Cow's milk (FAO, 1970)	Human milk (FAO, 1970)	Hen's egg (FAO, 1965)	Hen's egg (FAO, 1973)
Isoleucine	270	295	254	415	338
Leucine	306	596	548	553	535
Lysine	270	487	428	403	439
Total aromatic amino acids	360	633	421	627	578
Total sulphur amino acids	270	208	185	346	353
Threonine	180	278	280	317	296
Tryptophan	90	88	105	100	108
Valine	270	362	284	454	413

RESULTS

The protein content of the eggs varied from 407 to 495 g/kg dry matter. Table 2 shows the amino acid composition of proteins of eggs from the two breeds of hens.

The nitrogen recovery was measured for each amino acid determination and the mean value was 96 %. The results for the different diets, breeds and environments were compared and were statistically analysed by the Student's *t* test; no significant difference was found. The mean amino acid concentrations of egg protein showed little variation with the breed, environmental conditions or protein content of the diet. The coefficient of variation varied from 2·5 to 9·5 % for lysine, histidine, cystine, tyrosine and phenylalanine and from 7 to 10 % for tryptophan. Results for the amino acid composition of the egg white and the egg yolk are presented in Tables 3 and 4.

The concentrations of amino acids in the proteins of the whites and yolks showed a consistent pattern.

DISCUSSION

Previous work has not established whether or not differences in breed or in the quality or quantity of protein in the diet have a significant influence on the amino acid composition of egg protein. Giving hens diets deficient in methionine, tryptophan, cystine or lysine did not produce any significant changes in the quality of the egg protein (Evans, Davidson & Butts, 1950; Ingram, Cravens, Elvehjem & Halpin, 1951 *a*, *b*).

It has been reported (Smith, Wilson & Brown, 1954; Cunningham, Cottril & Funk, 1960; Coppock & Daniels, 1962) that changes in the environmental conditions can cause quantitative changes in the protein content and can alter the ratio of yolk to white of the egg but they do not affect the amino acid pattern.

The present study had a twofold purpose: (1) to verify whether or not the quality of the egg, as revealed by its essential amino acid composition, was independent of external and internal factors which are likely to bring about a physiological change in the animal, and (2) to remove the uncertainties about the amino acid composition of the hen's egg.

Our results have shown a consistency in the amino acid composition of hen's egg protein and also that the composition was not significantly affected by breed or by varying the quantity of dietary protein. However, the amino acid pattern of hen's egg protein was found to differ from that adopted by FAO/WHO (FAO, 1965); values obtained for lysine, total sulphur-containing amino acids and tryptophan were slightly higher (Table 5).

The measured isoleucine content of hen's egg protein (338 mg/g nitrogen) was significantly lower than that reported by other workers (Mitchell & Block, 1946; FAO, 1965; Cresta, Périssé, Autret & Lombardo, 1971) and is probably due to improved analytical procedures. The frequency with which this essential amino acid appears as the first or second limiting acid in practical diets consumed by populations in different parts of the world (Autret, Périssé, Sizaret & Cresta, 1968) is the result of calculations using the high value for the isoleucine content of egg protein reported in the early literature. In fact, the addition of isoleucine to diets in which protein efficiency is theoretically limited by a deficiency of this amino acid produces no significant improvement, except with diets based on millet or sorghum diets; these cereals have a high leucine content and the delaying effect of an excess of leucine on growth is balanced by the addition of isoleucine (Harper, 1958). On the other hand, when human milk or cow's milk is used as the reference standard, isoleucine is less frequently found to be limiting. The elimination of isoleucine as a common limiting amino acid in human diets in which it had appeared to be as limiting as the sulphur-containing amino acids is in agreement with biological evidence that the latter are the most commonly limiting amino acids in diets for man. This should therefore improve the correlation between the chemical score of proteins calculated from the amino acid pattern of egg protein and results obtained from the biological assay of proteins.

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