

## The Content of Free Amino Acids in Plasma of Hens of Different Strains

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**Summary.** Free amino acids were estimated in the plasma of Leghorn, Cornish and White Rock hens, bred under identical conditions. It was found that the plasma of Leghorn hens had a lower content of amino acids. The differences were especially pronounced for proline, glutamic acid and glycine. It was established that a lower percentage of valine, leucine and isoleucine was typical of Leghorn hens in comparison with Cornish hens. The obtained results indicate that the level of free amino acids in blood plasma is genetically controlled.

### Introduction

The finding that the amino acid composition of food (diet) influences the free amino acid concentration in hen blood plasma led to investigations on the relationship between the kind of diet and the level of amino acids in plasma (Hill and Olsen 1963a, b; Dean and Scott 1965, 1966; Tasaki and Ohno 1971).

It has been generally assumed that a decrease or an increase of the content of an amino acid in the diet brings about a change in concentration of this amino acid in the plasma. A lack of an amino acid in the diet was always accompanied by a decrease in its level in plasma, and simultaneously, a decrease or an increase of the concentrations of other amino acids (Tasaki and Ohno 1971). This phenomenon is accompanied by a decrease in food consumption and of weight gain in hens in most cases. Apart from exogenous amino acids, other amino acids have been proved to be growth-limiting factors in chickens, glycine being the best example (Hill and Olsen 1963b). Further papers aimed to establish optimal concentrations of particular amino acids in food and their effects on food consumption and weight gain in hens (Hewitt and Lewis 1972a, b). Similar changes in concentration of some amino acids have been observed in the course of starvation (Hill and Olsen 1963a, b).

It seems from the data of Desmarais and Pare (1974) that the concentration of free amino acids in hen plasma may be genetically controlled. These authors found differences in the free amino acid con-

tent of plasma of different lines of Leghorn hen. A similar dependence has also been established for other animals (Combs et al. 1959; Tuba 1953). It was recently found that a whole set of biochemical parameters related to some productive features is genetically controlled, involving, among others, the blood glutathione level, the level of free amino acids in blood, and egg output (Stutts et al. 1956; Desmarais and Pare 1974).

Previous studies on the amino acid pool of hen plasma have concentrated on the relationship between the concentration of free amino acids in plasma and the composition of food (diet). However examinations of the content of free amino acids in plasma should take into account a number of other factors, such as: a) age (Malcew and Szibułowa 1973); b) physiological status of an animal (Judina and Andreeva 1971); c) sex; d) time period between blood collection and analyses (Hewitt and Lewis 1972c); e) way of feeding (Tasaki and Ohno 1971). The content of amino acids in plasma may be typical only for a given strain and may be subject to changes characteristic of this strain under different physiological conditions.

Taking this into account, it seemed worthwhile to resume investigations of the content of amino acids in the plasma of different strains of hen.

### Materials and Methods

Leghorn, Cornish and White Rock hens were bred at the farm of the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences in Jastrzebiec,

Table 1. Absolute content ( $\mu\text{M}/100\text{ ml}$ ) and percentage of free amino acids in plasma

Amino acid	Absolute content ( $\mu\text{M}/100\text{ ml}$ )			Percentage		
	Leghorn	White Rock	Cornish	Leghorn	White Rock	Cornish
Lysine	8.33 $\pm$ 3.96	7.86 $\pm$ 3.69	6.16 $\pm$ 1.51	3.40 $\pm$ 1.03	2.65 $\pm$ 0.84	2.56 $\pm$ 0.88
Histidine	2.20 $\pm$ 0.90	3.69 $\pm$ 1.58	2.64 $\pm$ 0.90	1.11 $\pm$ 0.39	1.24 $\pm$ 0.50	1.16 $\pm$ 0.53
Arginine	4.39 $\pm$ 2.49	7.11 $\pm$ 4.39	4.13 $\pm$ 1.84	2.37 $\pm$ 1.10	2.17 $\pm$ 0.90	1.43 $\pm$ 0.59
Asparatic acid	3.60 $\pm$ 1.61	5.51 $\pm$ 2.94	5.64 $\pm$ 1.87	1.48 $\pm$ 0.47	1.68 $\pm$ 0.50	2.01 $\pm$ 0.60
Glutamic acid	8.31 $\pm$ 0.46	11.57 $\pm$ 3.73	12.63 $\pm$ 3.30	3.78 $\pm$ 0.47	3.82 $\pm$ 0.45	4.41 $\pm$ 0.79
Threonine	35.94 $\pm$ 6.75	44.64 $\pm$ 13.01	45.47 $\pm$ 7.96	15.35 $\pm$ 1.15	15.17 $\pm$ 1.70	16.09 $\pm$ 1.57
Serine	36.49 $\pm$ 8.32	38.50 $\pm$ 9.43	35.09 $\pm$ 6.34	16.23 $\pm$ 5.10	13.22 $\pm$ 0.51	12.58 $\pm$ 1.99
Proline	17.68 $\pm$ 6.40	36.78 $\pm$ 9.98	33.18 $\pm$ 10.17	9.32 $\pm$ 3.14	12.68 $\pm$ 1.67	11.55 $\pm$ 2.84
Glycine	27.09 $\pm$ 5.22	38.73 $\pm$ 10.17	39.01 $\pm$ 5.77	13.27 $\pm$ 1.84	14.91 $\pm$ 0.65	13.94 $\pm$ 1.41
Alanine	27.28 $\pm$ 7.41	32.85 $\pm$ 3.41	29.17 $\pm$ 4.17	11.69 $\pm$ 2.50	11.47 $\pm$ 1.33	10.45 $\pm$ 1.13
Valine	19.04 $\pm$ 8.83	22.96 $\pm$ 6.15	24.64 $\pm$ 3.27	6.76 $\pm$ 1.46	7.95 $\pm$ 0.81	8.82 $\pm$ 0.83
Methionine	1.18 $\pm$ 0.34	1.23 $\pm$ 0.45	1.33 $\pm$ 0.46	0.52 $\pm$ 0.17	0.42 $\pm$ 0.09	0.46 $\pm$ 0.14
Isoleucine	7.37 $\pm$ 4.09	8.96 $\pm$ 2.56	5.99 $\pm$ 1.75	2.60 $\pm$ 0.11	3.06 $\pm$ 0.45	3.41 $\pm$ 0.44
Leucine	12.15 $\pm$ 5.77	14.32 $\pm$ 3.64	15.20 $\pm$ 1.91	4.22 $\pm$ 0.67	4.98 $\pm$ 0.59	5.45 $\pm$ 0.55
Tyrosine	5.92 $\pm$ 1.71	6.81 $\pm$ 2.76	7.37 $\pm$ 1.34	2.53 $\pm$ 0.71	2.26 $\pm$ 0.61	2.66 $\pm$ 0.53
Phenylalanine	5.19 $\pm$ 1.84	6.27 $\pm$ 1.63	6.55 $\pm$ 0.71	2.18 $\pm$ 0.64	2.24 $\pm$ 0.41	2.40 $\pm$ 0.46
$\Sigma\text{BAA}$	17.16 $\pm$ 9.67	18.67 $\pm$ 9.29	12.93 $\pm$ 3.03			
$\Sigma\text{AAA}$	12.36 $\pm$ 2.72	17.09 $\pm$ 6.71	18.27 $\pm$ 4.94			
$\Sigma\text{BAA}/\Sigma\text{AAA}$	1.39 $\pm$ 0.47	1.09 $\pm$ 0.38	0.71 $\pm$ 0.51			
$\Sigma\text{AA}$	210.29 $\pm$ 28.43	294.52 $\pm$ 82.95	280.53 $\pm$ 35.81			

$\Sigma\text{BAA}$  - A sum of basic amino acids,  $\Sigma\text{AAA}$  - A sum of acidic amino acids,  $\Sigma\text{AA}$  - A sum of all amino acids

The values given represent means from 8-10 specimens  $\pm 0.05$  confidence interval. Means from individual amino acids do not contain values rejected by the Dixon Q test, while the values of  $\Sigma\text{BAA}$ ,  $\Sigma\text{AAA}$  and  $\Sigma\text{BAA}/\Sigma\text{AAA}$  and  $\Sigma\text{AA}$  for different strains represent means from values of these sums for individual specimens, without application of the Dixon Q test.

under identical food and breeding conditions. Identical diet and water were given ad libitum.

Blood collected in heparin from the wing vein in the morning, before feeding, was centrifuged at 5000 rpm. for 15 min., to separate plasma from blood cells. Plasma proteins were sedimented with cold ethanol up to a final concentration of 90% (1:9). The samples were spun at 30,000 g for 20 min. and the obtained sediments were washed twice with 90% ethanol. Supernatants were evaporated in an oven at 40°C. The residues were dissolved in 0.01N HCl with the addition of standard norleucine solution and used for determinations of free amino acid content.

Separation of amino acids was performed by ion-exchange chromatography, according to Stein and Moore (1954), with an automatic amino acid analyzer "Jeol JLC - 6AH". Statistical evaluation of results included mean value, confidence range, Dixon Q test and Student t test.

## Results and Discussion

Absolute values of amino acid concentrations and per cent amino acid composition of blood plasma of Leghorn, White Rock and Cornish hens (based on 16 analysed amino acids) are presented in Table 1.

Some amino acids exhibit significant statistical variability. This applies especially to arginine, valine

and isoleucine in Leghorn hens and arginine in Cornish hens.

The obtained data show that serine is the dominant amino acid in the blood plasma of Leghorn hens (16.23% of the amino acid pool). The next most abundant amino acids are threonine (15.35%), glycine (13.27%) and alanine (11.69%). Threonine is the prevailing amino acid of White Rock and Cornish hen blood plasma (15.17% and 16.09% respectively). White Rock hen blood plasma is also characterized by an increased content of glycine (14.91%), serine (13.22%) and proline (12.68%). The contributions of glycine, serine and proline to the amino acid pool of Cornish hens amount to 13.94%, 12.58%, and 11.55%, respectively. Methionine is the amino acid with the lowest content in plasma in all the investigated strains (0.52% for Leghorn hens, 0.42% for White Rock hens and 0.46% for Cornish hens). Histidine is also low (1.11%, 1.24% and 1.16% for Leghorn, White Rock and Cornish hens, respectively). Asparatic acid too is a minor amino acid constituent of plasma in Leghorn (1.48%) and White Rock hens (1.68%), while arginine is so in Cornish hens (1.43%).

Table 2. Interstrain statistically significant differences in absolute content and percentage of free amino acids in plasma

Amino acid	Absolute content	Percentage
Lysine		
Histidine		
Arginine		
Asparatic acid		
Glutamic acid	0	
Threonine		
Serine		
Proline	0 +	+
Glycine	0	
Alanine		
Valine		0
Methionine		
Isoleucine		0
Leucine		0
Tyrosine		
Phenylalanine		
$\Sigma$ AA	0	
$\Sigma$ AA A sum of amino acids		
+	a difference between Leghorn and White Rock hens	
0	a difference between Leghorn and Cornish hens	
x	a difference between Cornish and White Rock hens	

The lowest content amino acid sum is typical of plasma of Leghorn hens (210  $\mu$ mole/100 ml); in the two remaining strains this value amounts to 294  $\mu$ mole/100 ml (White Rock hens) and 280  $\mu$ mole/100 ml (Cornish hens). This difference is statistically significant for Leghorn and Cornish hens (Table 2). No difference was found in the ratio of basic amino acids to acidic amino acids, which was about 1.0 for the examined strains (Table 2).

Leghorn hens have the lowest content of almost all amino acids. This difference is especially evident in the case of proline (the differences for the remaining strains are statistically significant), glutamic acid and glycine (where the difference between Leghorn and Cornish hens is statistically significant). Moreover, some differences in percent amino acid composition were found, e.g. Leghorn hens possess a lower percentage of valine, leucine and isoleucine than do Cornish hens. One can suggest that the content of individual amino acids in hen plasma is genetically controlled. This opinion is in accord with the results of Hrubrant (1965) and Desmarais and Pare (1974). Determination of the degree to which the amino acid level is genetically controlled, and how much it is influenced by the served diet and its utilization, would undoubtedly be a difficult task.

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