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The Level of Free Amino Acids in Erythrocytes of Different Breeds of Hen

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Summary. The content of free amino acids was determined in erythrocytes of adult 'Leghorn' (Lg, 'White Rock' (WR) and 'Cornish' (Cr) hens, bred under identical conditions. The concentration of total amino acids was twice as high in the erythrocytes as in plasma, amounting to 396 μm/100 ml, 424 μm/100 ml and 475 μm/100 ml in 'White Rock', 'Cornish' and 'Leghorn' hens, respectively.

Significant differences were found in the ratio of basic amino acids to acidic amino acids. These values were 0.76, 1.75 and 3.19 in 'White Rick', 'Leghorn' and 'Cornish' hens, respectively; in the plasma of all 3 breeds the ratio was 1. Statistically significant interbreed differences were expressed more distinctly in erythrocyte than in plasma amino acid concentrations. For absolute concentrations the differences were significant in the case of 9 amino acids.

Key words: Free Amino Acids - Erythrocytes - Hens

Introduction

Previous studies of the level of free amino acids in hen blood have been concerned mainly with plasma (Dean and Scott 1966; Longenecker and Kause 1959; Squibb 1966; Zimmerman and Scott 1965; Tasaki and Ohno 1971; Hewitt and Lewis 1972; Hill and Olsen 1963) and the levels in erythrocytes have not been investigated in more detail. It was established on the basis of a large amount of data that the composition of the free amino acid pool of hen plasma is influenced by the free amino acid composition of food (Gray et al. 1960). On the other hand, the results of Desmarais and Pare (1972) are evidence that the levels of free amino acids in hen plasma are conditioned genetically. It seemed therefore of value to determine the levels of free amino acids in plasma (Gondko et al. 1977) and erythrocytes of adult hens of different breeds and to study the effect of prolonged starvation on their values.

The present paper deals with the determination of free amino acids in erythrocytes of adult hens of three breeds: 'Leghorn' (Lg), 'White Rock' (WR) and 'Cornish' (Cr).

Materials and Methods

All the birds were derived from stock established at the Institute some years ago and maintained to avoid inbreeding. The specimens were chosen for the experiments at a random but excluding any relationships. All the hens were 12 months old. They were not laying and had just been eliminated from the flock due to the lack of egg production.

Since the examined hens belonged to three different breeds, they seemed to constitute good material for studies on the genetic variability of chosen biochemical parameters.

The hens were bred under identical conditions, fed ad libitum with a solid food and unlimited water supply. After a 12 h break in feeding, blood was taken from the wing vein and separated into erythrocytes and plasma. The content of the free amino acids was determined in erythrocytes and plasma (Gondko et al. 1977).

Erythrocytes were washed with 0.9 % NaCl until the yellow colour of plasma disappeared. After determination of hematocrit, 1 ml of the cell suspension was hemolysed with an equal amount of water with additional freezing and thawing. Details of the produce and separation of free amino acids in the automatic amino acid analyzer "JEOL" have been described elsewhere (Gondko et al. 1977).

Concentrations of amino acids were expressed as μm/100 ml cells and as a percentage assuming the sum of the concentrations of 16 amino acids to be 100 %. The results were subjected to statistical analysis by calculating mean values, $\bar{x}$, confidence levels, the Dixon Q test and comparison of differences between means by the Student t test.

Results and Discussion

Concentrations of free amino acids in erythrocytes of the examined breeds (Table 1) show considerable

Table 1. Absolute content (μM/100 ml) and percentage of free amino acids in red blood cells

Amino acid	Absolute content (μM/100 ml)			Percentage		
	'Leghorn'	'White Rock'	'Cornish'	'Leghorn'	'White Rock'	'Cornish'
	m ± ci	m ± ci	m ± ci	m ± ci	m ± ci	m ± ci
Lysine	34.35 ± 20.26	18.10 ± 8.12	36.42 ± 14.44	7.00 ± 3.20	4.53 ± 1.67	8.33 ± 2.32
Histidine	58.76 ± 19.16	15.34 ± 8.99	73.35 ± 13.80	12.96 ± 8.22	3.82 ± 2.10	17.54 ± 3.63
Arginine	7.82 ± 3.65	2.73 ± 0.87	8.08 ± 4.28	1.44 ± 0.72	0.62 ± 0.27	1.84 ± 0.81
Aspartic acid	40.95 ± 16.88	36.24 ± 9.35	32.69 ± 15.02	8.56 ± 1.53	10.38 ± 2.19	7.54 ± 3.30
Glutamic acid	21.37 ± 9.76	18.68 ± 7.22	10.98 ± 3.89	4.24 ± 0.91	4.96 ± 1.65	2.63 ± 0.92
Threonine	8.91 ± 6.97	6.04 ± 0.87	4.22 ± 1.03	1.09 ± 0.20	1.45 ± 0.37	0.99 ± 0.18
Serine	69.76 ± 11.26	55.33 ± 17.12	48.22 ± 8.07	13.03 ± 1.73	13.84 ± 2.59	11.32 ± 0.61
Proline	trace	trace	trace	trace	trace	trace
Glycine	92.62 ± 37.54	79.76 ± 8.32	87.61 ± 28.90	19.09 ± 3.76	20.85 ± 3.43	18.96 ± 2.57
Alanine	44.27 ± 15.22	49.59 ± 6.62	37.58 ± 8.56	8.47 ± 0.99	12.81 ± 1.69	8.76 ± 1.22
Valine	33.35 ± 11.50	28.37 ± 4.09	24.83 ± 9.20	7.05 ± 0.42	7.47 ± 1.61	5.70 ± 1.81
Methionine	trace	trace	trace	trace	trace	trace
Isoleucine	14.88 ± 3.19	12.08 ± 1.04	12.13 ± 1.66	2.73 ± 0.54	3.18 ± 0.56	2.90 ± 0.44
Leucine	22.89 ± 9.40	19.62 ± 1.66	20.77 ± 3.03	4.72 ± 1.15	5.15 ± 0.86	4.95 ± 0.70
Tyrosine	26.84 ± 4.34	19.68 ± 1.32	20.24 ± 2.40	4.94 ± 0.44	5.19 ± 0.94	4.80 ± 0.45
Phenylalanise	7.97 ± 2.37	7.33 ± 0.66	7.61 ± 1.08	1.85 ± 0.49	2.04 ± 0.20	1.82 ± 0.30
ΣBAA	100.93 ± 54.84	32.57 ± 11.94	117.86 ± 23.49			
ΣAAA	62.33 ± 24.57	54.94 ± 15.20	43.68 ± 17.20			
ΣBAA/ΣAAA	1.75 ± 0.72	0.76 ± 0.55	3.19 ± 1.21			
ΣAA	473.54 ± 165.58	396.30 ± 71.51	424.51 ± 62.71			

m ± ci - mean ± confidence interval (α = 0.05)
ΣBAA - Sum of basic amino acids
ΣAAA - Sum of acidic amino acids
ΣAA - Sum of all amino acids

differences compared with the amino acid composition of plasma (Gondko et al. 1977).

Glycine proved to be the dominant erythrocyte amino acid, constituting 19.09 %, 20.85 % and 18.96 % of the total pool of free amino acids of red cells in 'Leghorn', 'White Rock' and 'Cornish' hens. Serine constituted the second most abundant amino acid, amounting to 13.03 %, 13.84 % and 11.32 % in erythrocytes of 'Leghorn', 'White Rock' and 'Cornish' hens, respectively.

Histidine and asparatic acid also formed a significant portion of the overall sum of amino acids in 'Leghorn' hens (12.96 % and 8.56 % respectively), as did alanine and asparatic acid in 'White Rock' hens (12.81 % and 10.38 %, respectively) and histidine and alanine in 'Cornish' hens (17.54 % and 8.76 %, respectivley).

Methionine and proline were only trace amino acids in all three breeds. The other amino acids were present in small amounts: threonine (Lg - 1.09 %, Cr - 0.99 %, WR - 1.45 %) and arginine (Wr - 0.62 %, Lg - 1.44 %, Cr - 1.84 %).

Comparisons of per cent contributions of the amino acids revealed statistically significant differences concerning lysine, arginine, asparatic acid, glutamic acid, threonine, and alanine between 'White Rock' and 'Cornish' hens, asparatic acid and glutamic acid between 'Leghorn' and 'Cornish' hens, and histidine, arginine and alanine between 'Leghorn' and 'White Rock' hens.

Statistically significant differences in absolute concentrations (μm/100 ml) were found for 9 amino acids: Lys, His, Arg, Glu, Thr, Ser. Ala, Ileu, and Tyr. As shown by data grouped in Table 2, the largest number of statistically significant differences were found between 'Cornish' and 'White Rock' hens.

Red blood cells of 'White Rock' hens were characterized by the lowest content of alkaline amino acids, compared with the other two hen breeds. This was reflected in the ratios of basic amino acids to acidic amino acids: 0.76 in 'White Rock' hens, 1.75 in 'Leghorn' hens and 3.19 in 'Cornish' hens. Statistically significant differences were found between these breeds.

Table 2. Statistically significant differences ($p < 0.05$) in the content of erythrocyte free amino acids between 'Leghorn', 'White Rock' and 'Cornish' hens

Amino acid	Absolute content	Percentage
Lysine	x	x
Histidine	x	+
Arginine	x	x +
Aspartic acid		x 0
Glutamic	0	x 0
Threonine	x	x
Serine	0	
Proline		
Glycine		
Alanine	x	x +
Valine		
Methionine		
Isoleucine	+	
Leucine		
Tyrosine	+ 0	
Phenylalanine		
ΣBAA	x +	
ΣAAA		
ΣBAA/ΣAAA	x + 0	
ΣAA		

x - difference between 'Cornish' and 'White Rock' hens
+ - difference between 'Leghorn' and 'White Rock' hens
0 - difference between 'Leghorn' and 'Cornish' hens
ΣBAA - sum of basic amino acids
ΣAAA - sum of acidic amino acids
ΣAA - sum of all amino acids

The 9 statistically significant differences in amino acid concentrations between 'White Rock' and 'Cornish' hens concerned lysine, histidine, arginine, threonine and alanine (Table 2). Erythrocytes of 'Leghorn' and 'Cornish' hens exhibited differences in serine and glutamic acid levels, while erythrocytes of 'Leghorn' and 'White Rock' hens differed in isoleucine and tyrosine levels.

Erythrocytes were characterized by an almost two-fold higher concentration of the sum of 16 free amino acids, as compared to plasma. The sum of free amino acids in erythrocytes amounted to 396 μM/100 ml in 'White Rock' hens, 424 μM/100 ml in 'Cornish' hens and 475 μM/100 ml in 'Leghorn' hens.

Comparisons of free amino acids in plasma and red blood cells distinguished 3 groups of amino acids (Table 3). The first group comprises those with higher concentrations in erythrocytes than in plasma. A reverse relationship is characteristic of the second group. Concentrations of amino acids be-

Table 3. A comparison of the levels of free amino acids in erythrocytes and plasma of three breeds of hens

Breed	Amino acids with higher level in erythrocytes	Amino acids with higher level in plasma	Amino acids with equivalent levels in plasma and erythrocytes
Lg	Lys, Asp, Ser, Glu, Gly, Ile, Tyr	Thr, Pro, Met.	His, Arg, Val, Phe, Ala, Leu
WR	Lys, His, Asp, Gly, Ala, Ile, Leu, Tyr	Thr, Pro, Met	Arg, Val, Phe, Glu, Ser
Cr	Lys, His, Asp, Ser, Gly, Ile, Leu, Tyr	Thr, Pro, Met	Arg, Val, Phe, Glu, Ala

longing to the third group do not exhibit statistically significant differences between plasma and erythrocytes.

As shown in Table 3 interbreed differences occurred in this respect as well in the case of serine, histidine, alanine, leucine and glutamic acid. One may suggest, therefore, that genetic factors significantly influence the concentrations of free amino acids in erythrocytes as well as in plasma.

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