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Effect of Starvation on the Content of Free Amino Acids in Plasma of Different Breeds of Hen

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Summary. 'Leghorn', 'Cornish' and 'White Rock' hens were subjected to starvation. Free amino acids were determined in blood samples taken after 48, 72 and 96 h of starvation. A progressive decrease in concentration of the majority of amino acids was found. Changes in amino acid concentrations during starvation were dependent on the breed of hen.

Key words: Free Amino Acids - Plasma - Starvation - Hens

Introduction

Feeding animals with amino acid deficient feed brings about a diminution of weight gain and food consumption as well as histological changes (Sidransky and Baba 1960; Svendseid et al. 1966; Richardson et al. 1965; Kelly and Scott 1968). Several authors (Charkey et al. 1953, 1954; Gray et al. 1960; Hill and Olsen 1963a) have pointed out that in birds the level of free amino acids in plasma behaves differently, compared with other animals. An increase in the level of some amino acids has been found under conditions where their supply was limited. In our earlier paper (Gondko et al. 1977) we observed a dependence of the level of free amino acids on the breed of hen. It seemed of value therefore to examine the levels of free amino acids in different breeds during starvation in an attempt to shed light on differences in amino acid requirements among these breeds.

Materials and Methods

'Leghorn' (Lg), 'White Rock' (WR) and 'Cornish' (Cr) hens, one year old, bred under the same conditions, were fed the same food for at least one week ad libitum before feeding was stopped. Blood was taken 12 hr after stopping feeding and heparinized to obtain a control, (C). The hens were then starved and blood was taken from them in the morning, 48, 72 and 96 h after taking the control samples. Ten hens of each breed were used for the experiment.

Preparation of plasma amino acids

Heparinized blood was centrifuged to separate plasm from blood cells. A white layer of leukocytes was removed from the erythrocytes. Two ml of plasma were added to 18 ml absolute methanol (1:9) and the protein sediment was centrifuged at 30 000 × g. The supernatant was collected and the sediment was washed twice with 5 ml of 90% ethanol. Alcohol was evaporated at 40°C in an oven and the dry residue was dissolved in 0.01 N HCl supplemented with a standard norleucine solution.

Analysis of free amino acids

Free amino acids contained in the extracts were separated by the method of Stein and Moore (1954) using two chromatographic columns filled with cationic ion exchanger. Chromatographic separation was performed with an automatic amino acid analyzer JEOL JLC-6 AH. Amino acid content was calculated from integrator data and chromatographic profiles, and expressed as mg/100 ml plasma and per cent constitution. Sixteen amino acids were identified and determined quantitatively. The obtained results were subjected to statistical analysis. Total sum of amino acids, ΣAA, sum of basic amino acids, ΣBAA, sum of acidic amino aicds, ΣAAA, and the ratio of baisc to acidic amino acids, ΣBAA/ΣAAA, were also calculated.

Results and Discussion

The contents of free amino acids expressed as absolute values (μm/100 ml plasma) are presented in Table 1.

Table 3 shows statistically significant differences between the control and successive days of starvation, and Table 4 statistically significant differences between the three breeds.

Table 1. The content of free amino acids (μM/100 ml) in plasma of 'Leghorn', 'White Rock' and 'Cornish' hens during starvation

Amino acid	Breed	Control	Hours after Starvation		
			48 h	72 h	96 h
		m ± ci	m ± ci	m ± ci	m ± ci
Lysine	Lg	8.33 ± 3.96	13.49 ± 5.74	6.37 ± 1.96	4.26 ± 2.81
	WR	7.86 ± 3.69	10.81 ± 2.08	6.19 ± 2.44	4.90 ± 2.84
	Cr	6.16 ± 1.51	10.17 ± 2.31	4.74 ± 1.88	2.98 ± 1.30
Histidine	Lg	2.20 ± 0.90	5.71 ± 1.99	4.23 ± 2.14	7.32 ± 3.71
	WR	3.69 ± 1.58	4.71 ± 1.02	3.87 ± 0.55	4.38 ± 0.95
	Cr	2.64 ± 0.90	4.78 ± 1.38	5.48 ± 2.28	3.95 ± 1.84
Arginine	Lg	4.39 ± 2.49	9.03 ± 2.74	2.52 ± 0.87	2.47 ± 1.62
	WR	7.11 ± 4.39	7.65 ± 1.99	2.43 ± 0.60	4.13 ± 2.19
	Cr	4.13 ± 1.84	7.48 ± 1.78	2.73 ± 0.97	1.96 ± 0.34
Aspartic acid	Lg	3.60 ± 1.61	2.26 ± 0.92	3.11 ± 0.91	2.43 ± 0.69
	WR	5.51 ± 2.94	2.97 ± 0.93	3.56 ± 1.06	2.06 ± 0.68
	Cr	5.64 ± 1.87	1.99 ± 0.48	3.49 ± 0.88	2.11 ± 0.66
Threonine	Lg	35.94 ± 6.75	52.37 ± 15.01	37.38 ± 10.10	34.87 ± 13.10
	WR	44.64 ± 13.01	38.07 ± 3.17	34.78 ± 3.99	32.39 ± 6.06
	Cr	45.47 ± 7.96	37.98 ± 9.66	34.45 ± 4.55	30.72 ± 8.23
Serine	Lg	36.49 ± 8.32	26.39 ± 10.35	28.25 ± 10.56	19.18 ± 8.73
	WR	38.50 ± 9.43	33.88 ± 5.99	39.60 ± 12.51	28.33 ± 13.31
	Cr	35.09 ± 6.34	27.22 ± 7.03	27.13 ± 5.20	29.44 ± 3.79
Glutamic acid	Lg	8.31 ± 0.46	7.87 ± 2.74	7.79 ± 2.54	6.59 ± 2.06
	WR	11.57 ± 3.73	9.61 ± 2.26	9.25 ± 1.83	7.83 ± 1.06
	Cr	12.63 ± 3.30	8.78 ± 1.61	8.34 ± 1.52	6.61 ± 2.01
Proline	Lg	17.68 ± 6.40	10.20 ± 3.16	8.36 ± 2.14	5.15 ± 2.96
	WR	36.78 ± 9.98	9.70 ± 1.32	8.27 ± 2.19	6.81 ± 1.94
	Cr	33.18 ± 10.17	9.12 ± 1.38	7.79 ± 1.04	7.21 ± 1.42
Glycine	Lg	27.09 ± 5.22	24.11 ± 5.26	22.15 ± 1.84	17.62 ± 5.49
	WR	38.73 ± 10.17	22.32 ± 2.52	22.14 ± 4.28	22.07 ± 7.38
	Cr	39.01 ± 5.77	20.09 ± 4.31	20.78 ± 2.29	20.06 ± 4.61
Alanine	Lg	27.28 ± 7.41	28.41 ± 5.80	19.23 ± 3.27	11.61 ± 4.14
	WR	32.85 ± 3.41	27.06 ± 2.99	25.99 ± 5.54	24.25 ± 4.06
	Cr	29.17 ± 4.17	23.11 ± 1.84	19.47 ± 2.76	19.37 ± 4.56
Valine	Lg	19.04 ± 8.83	20.23 ± 3.64	18.72 ± 5.12	25.44 ± 6.88
	WR	22.96 ± 6.15	19.57 ± 4.03	16.00 ± 3.55	10.37 ± 0.66
	Cr	24.64 ± 3.27	19.98 ± 6.00	16.11 ± 5.88	13.23 ± 4.73
Methionine	Lg	1.18 ± 0.34	1.54 ± 0.60	-	-
	WR	1.23 ± 0.45	1.81 ± 0.48	0.17 ± 0.06	0.75 ± 0.33
	Cr	1.33 ± 0.46	1.45 ± 0.67	0.16 ± 0.11	0.37 ± 0.56
Isoleucine	Lg	7.37 ± 4.09	11.60 ± 3.07	9.35 ± 2.36	10.44 ± 2.98
	WR	8.96 ± 2.56	10.52 ± 2.19	7.04 ± 1.25	5.28 ± 1.25
	Cr	5.99 ± 1.75	10.49 ± 3.12	8.99 ± 3.21	5.47 ± 2.39
Leucine	Lg	12.15 ± 5.77	15.86 ± 2.29	14.32 ± 3.97	17.73 ± 4.70
	WR	14.32 ± 3.64	16.66 ± 2.26	9.79 ± 1.61	7.48 ± 1.42
	Cr	15.20 ± 1.91	15.50 ± 3.53	13.71 ± 5.00	9.44 ± 4.08
Tyrosine	Lg	5.92 ± 1.71	6.69 ± 1.84	5.21 ± 1.31	4.65 ± 1.11
	WR	6.81 ± 2.76	5.51 ± 1.33	4.07 ± 1.22	4.39 ± 0.97
	Cr	7.37 ± 1.34	5.00 ± 1.04	4.60 ± 0.86	4.36 ± 0.59
Phenyl-alanine	Lg	5.19 ± 1.84	6.27 ± 0.85	5.43 ± 1.12	5.71 ± 0.76
	WR	6.27 ± 1.63	6.65 ± 0.68	4.36 ± 0.51	3.73 ± 0.61
	Cr	6.55 ± 0.71	5.02 ± 0.69	5.30 ± 1.36	4.09 ± 0.62
ΣBAA	Lg	17.16 ± 9.67	30.29 ± 7.73	12.82 ± 3.59	13.71 ± 7.56
	WR	18.67 ± 9.29	23.19 ± 3.76	12.63 ± 3.55	15.51 ± 8.21
	Cr	12.93 ± 3.03	22.36 ± 4.11	12.66 ± 4.73	8.61 ± 3.38

Table 1 (Continued)

Amino acid	Breed	Control	Hours after Starvation 48 h	72 h	96 h
		m ± ci	m ± ci	m ± ci	m ± ci
ΣAAA	Lg	12.36 ± 2.72	10.14 ± 3.57	10.90 ± 3.29	9.02 ± 2.81
	WR	17.09 ± 6.71	12.58 ± 2.97	12.81 ± 2.84	9.90 ± 1.70
	Cr	18.27 ± 4.94	10.07 ± 1.52	11.84 ± 2.78	8.72 ± 2.67
ΣBAA/ΣAAA	Lg	1.32 ± 0.47	2.91 ± 0.53	1.10 ± 0.27	1.23 ± 0.46
	WR	1.09 ± 0.38	1.98 ± 0.44	0.90 ± 0.17	1.21 ± 0.37
	Cr	0.71 ± 0.51	2.13 ± 0.39	0.94 ± 0.29	0.80 ± 0.32
ΣAA	Lg	210.29 ± 28.43	240.85 ± 42.13	191.12 ± 27.99	175.18 ± 14.19
	WR	294.52 ± 82.95	230.98 ± 34.48	204.87 ± 75.41	177.32 ± 36.27
	Cr	280.53 ± 35.81	212.70 ± 41.16	180.69 ± 25.31	157.78 ± 27.02

m ± ci - mean ± confidence interval ($\alpha = 0.05$)
ΣBAA - sum of basic amino acids
ΣAAA - sum of acidic amino acids
ΣAA - sum of all amino acids
Lg - 'Leghorn' hens
WR - 'White Rock' hens
Cr - 'Cornish' hens

Prolonged starvation resulted in a decrease of the total amino acid content of hen plasma. In 'Cornish' hens the total sum of amino acids decreased by nearly one-half from 280 μM/100 ml, the control level, down to 157 μM/100 ml after 96 h of starvation. A smaller decrease, from 294 μM/100 ml to 177 μM/100 ml, was noted in the plasma of 'White Rock' hens. 'Leghorn' hens had the lowest content of amino acids in plasma of control blood, 210 μM/100 ml, and the smallest starvation-induced decrease of total amino acids, 175 μM/100 ml after 96 h. Amino acid totals after 96 h of starvation were similar for all three breeds.

The content of basic amino acids tended to increase. This increase was statistically significant in the case of lysine (Cr), histidine (Lg, Cr) and arginine (Lg, Cr) as shown in Table 3. However, after 72 h of starvation the content of these amino acids decreased, and was even below the control values for lysine and arginine.

Statistically significant decreases in acidic amino acids after 48 h starvation were noted only in plasma of 'Cornish' (asparatic acid and glutamic acid) and 'White Rock' hens (asparatic acid). These shifts in amino acid levels were reflected in changes of the ratio of basic to acidic amino acids. A statistically significant increase of this ratio was observed after 48 h of starvation when a decrease below the control value occurred. The greatest change in this ratio was noted in the case of 'Leghorn' hens, from 1.32 to 2.90.

Generally the level of most free amino acids in hen plasma decreased during starvation. This was evident in the case of glycine, alanine and proline where the pattern of changes was the same as in the sum of amino acids. A statistically significant decrease in the levels of threonine, serine, valine, tyrosine and phenylalanine was noted only in some breeds (Table 3). For example, the level of threonine decreased in 'White Rock' and 'Cornish' hens, while in 'Leghorn' hens a statistically significant increase after 48 h of starvation was followed by a decrease to the control level. In 'Leghorn' hens a statistically significant decrease in serine concentration was observed. The changes in concentrations of valine, leucine, and isoleucine were similar. Plasma levels of these amino acids decreased during starvation of 'White Rock' and 'Cornish' hens, while in 'Leghorn' hens a statistically insignificant increase of these amino acids took place after 96 h of starvation. The levels of phenylalanine and tyrosine decreased slightly during the course of the experiment in all the investigated breeds.

Percentage contributions of free amino acids to the total sum of plasma amino acids during starvation are shown in Table 2. Statistically significant differences are presented in Table 3 and 4.

Table 2. Composition (%) of the free amino acid pool of plasma of 'Leghorn', 'White Rock' and 'Cornish' hens during starvation

Amino acid	Breed	Control	Hours after Starvation		
			48 h	72 h	96 h
		m ± ci	m ± ci	m ± ci	m ± ci
Lysine	Lg	3.40 ± 1.03	5.28 ± 1.46	3.23 ± 0.81	1.79 ± 0.66
	WR	2.65 ± 0.04	4.46 ± 0.75	2.90 ± 0.90	2.47 ± 1.16
	Cr	2.56 ± 0.88	5.05 ± 1.34	2.48 ± 0.77	1.85 ± 0.71
Histidine	Lg	1.11 ± 0.39	2.92 ± 1.25	2.32 ± 1.06	4.03 ± 1.42
	WR	1.24 ± 0.50	1.94 ± 0.34	1.95 ± 0.32	2.46 ± 0.21
	Cr	1.16 ± 0.53	2.09 ± 0.31	2.88 ± 1.02	2.45 ± 1.11
Arginine	Lg	2.37 ± 1.10	4.11 ± 0.95	1.25 ± 0.43	1.29 ± 0.72
	WR	2.17 ± 0.90	3.16 ± 0.81	1.12 ± 0.18	2.16 ± 0.88
	Cr	1.43 ± 0.59	3.61 ± 0.58	1.40 ± 0.37	1.18 ± 0.20
Aspartic acid	Lg	1.48 ± 0.47	0.92 ± 0.30	1.59 ± 0.42	1.47 ± 0.34
	WR	1.68 ± 0.50	1.22 ± 0.36	1.68 ± 0.29	1.18 ± 0.32
	Cr	2.01 ± 0.60	0.98 ± 0.21	1.89 ± 0.41	1.32 ± 0.33
Threonine	Lg	15.35 ± 1.15	21.16 ± 3.01	18.93 ± 2.73	19.17 ± 4.22
	WR	15.17 ± 1.70	16.61 ± 3.37	19.06 ± 2.80	18.88 ± 4.20
	Cr	16.09 ± 1.57	18.03 ± 1.20	19.04 ± 1.70	19.13 ± 2.42
Serine	Lg	16.23 ± 5.10	18.87 ± 3.90	12.52 ± 2.92	10.60 ± 3.06
	WR	13.22 ± 0.51	14.00 ± 2.10	10.19 ± 2.38	15.04 ± 5.80
	Cr	12.58 ± 1.99	13.14 ± 2.26	15.18 ± 2.87	7.22 ± 4.76
Glutamic acid	Lg	3.78 ± 0.47	2.87 ± 0.45	3.95 ± 0.83	3.73 ± 0.76
	WR	3.82 ± 0.45	3.96 ± 0.77	4.52 ± 0.54	4.60 ± 0.85
	Cr	4.41 ± 0.79	4.36 ± 0.83	4.60 ± 0.75	4.20 ± 1.11
Proline	Lg	9.32 ± 3.14	4.47 ± 0.50	4.25 ± 0.79	2.79 ± 1.42
	WR	12.68 ± 1.67	4.05 ± 0.69	4.00 ± 0.61	3.90 ± 0.88
	Cr	11.55 ± 2.84	4.47 ± 0.44	4.14 ± 0.39	4.63 ± 0.92
Glycine	Lg	13.27 ± 1.04	9.00 ± 1.04	10.93 ± 0.60	9.03 ± 1.30
	WR	14.41 ± 0.65	9.93 ± 1.45	10.30 ± 0.95	12.19 ± 2.43
	Cr	13.94 ± 1.41	9.65 ± 0.46	11.32 ± 0.21	11.09 ± 0.64
Alanine	Lg	11.69 ± 2.50	8.43 ± 1.46	10.02 ± 1.18	5.82 ± 0.91
	WR	11.47 ± 1.33	11.28 ± 1.40	13.39 ± 1.04	14.63 ± 0.85
	Cr	10.45 ± 1.13	12.33 ± 1.92	10.06 ± 1.67	12.30 ± 2.18
Valine	Lg	6.74 ± 1.46	10.09 ± 3.93	10.16 ± 3.40	15.04 ± 5.26
	WR	7.95 ± 0.81	8.05 ± 1.31	7.30 ± 1.29	6.03 ± 1.14
	Cr	8.82 ± 0.83	9.58 ± 2.26	8.56 ± 2.08	7.12 ± 1.32
Methionine	Lg	0.52 ± 0.17	0.62 ± 0.21	-	-
	WR	0.42 ± 0.09	0.76 ± 0.23	0.08 ± 0.03	0.51 ± 0.23
	Cr	0.46 ± 0.14	0.74 ± 0.42	0.07 ± 0.09	0.20 ± 0.31
Isoleucine	Lg	2.60 ± 0.11	5.05 ± 1.82	5.02 ± 1.57	6.32 ± 2.25
	WR	3.06 ± 0.45	4.35 ± 0.84	3.43 ± 0.30	3.52 ± 0.95
	Cr	3.41 ± 0.44	4.92 ± 0.79	4.67 ± 1.08	2.83 ± 0.83
Leucine	Lg	4.22 ± 0.67	7.81 ± 1.23	7.72 ± 2.59	10.72 ± 3.58
	WR	4.98 ± 0.59	6.94 ± 0.99	4.76 ± 0.28	4.77 ± 0.81
	Cr	5.45 ± 0.55	7.44 ± 0.92	7.19 ± 1.70	4.98 ± 1.45
Tyrosine	Lg	2.53 ± 0.71	2.71 ± 0.18	2.68 ± 0.49	2.62 ± 0.31
	WR	2.26 ± 0.61	2.29 ± 0.54	1.91 ± 0.30	3.09 ± 1.11
	Cr	2.66 ± 0.53	2.43 ± 0.32	2.52 ± 0.32	2.51 ± 0.36
Phenyl-alanine	Lg	2.18 ± 0.64	2.70 ± 0.60	2.90 ± 0.69	3.34 ± 0.47
	WR	2.24 ± 0.41	2.36 ± 0.34	2.18 ± 0.23	2.38 ± 0.51
	Cr	2.48 ± 0.46	2.47 ± 0.30	2.85 ± 0.41	2.47 ± 0.20

m ± ci - mean ± confidence interval ($\alpha = 0.05$)
Lg - 'Leghorn' hens
WR - 'White Rock' hens
Cr - 'Cornish' hens

Table 3. Statistically significant differences in the level of free amino acids in plasma during starvation

Amino acid	c-48 h			c-72 h			c-96 h			48 h-72 h			48 h-96 h			72 h-96 h		
	Lg	WR	Cr	Lg	WR	Cr	Lg	WR	Cr	Lg	WR	Cr	Lg	WR	Cr	Lg	WR	Cr
Absolute Concentrations (μM/100 ml)																		
Lysine			++						++	+	++	++	++	++	+++			
Histidine	++		++			+	+											
Arginine	++		++						+	+++	+++	+++	+++	+	+++			
Aspartic acid			++					+	++			++					+	+
Threonine						++			++									
Serine							++											
Glutamic acid			+			++			++									
Proline	+	+++	+++	++	+++	+++	++	+++	+++				+	++				
Glycine		++	+++	+	++	+++	+	++	+++									
Alanine	+	++	++	+		+++	++	++	++			+	++			++		
Valine						++		++	+++					++			++	
Methionine					++	+					++			++			+	
Isoleucine								+	++		++			++	++			
Leucine					+			++	++		+++			+++	+		+	
Tyrosine			++			++			++									
Phenylalanine			++		+			++	+++		++			+++				
ΣBAA	+		++							+++	+++	++	++		+++			
ΣAAA			++			++		+	++									
ΣBAA/ΣAAA	+++	++	++							+++	+++	+++	+++	+	+++			
ΣAA			++		+	+++		++	+++	+			++	+	+			
Per cent Concentrations																		
Lysine		++	++				++			++	++	++	++	++	+++	++		
Histidine	+	+	++	+	++	++	+++	+						++			++	
Arginine	+		+++	+	+					+++	+++	+++	+++		+++		+	
Aspartic acid	+		++							++		++	+				+	+
Threonine	++			+	+	++		+										
Serine					+++						+++							
Glutamic acid	++									+								
Proline	++	+++	+++	++	+++	+++	++	+++	+++				+					
Glycine	+	+++	+++	++	+++	++	++					+++			+++			++
Alanine	+				+		+++	++			+		++	++		+++		
Valine							++	++	+					+				
Methionine		++			++	+					++							
Isoleucine		++	++	+		+	+								++			+
Leucine		++	++	+			++				+++		++	++				
Tyrosine																	+	
Phenylalanine							++											

Lg - 'Leghorn' hens WR - 'White Rock' hens Cr - 'Cornish' hens
c-48 - difference between control and 48 h of starvation
c-72 - difference between control and 72 h of starvation
c-96 - difference between control and 96 h of starvation
48 h-72 h - difference between 48 and 72 h of starvation
48 h-96 h - difference between 48 and 96 h of starvation
72 h-96 h - difference between 72 and 96 h of starvation
ΣBAA - sum of basic amino acids
ΣAAA - sum of acidic amino acids
ΣAA - sum of all amino acids
Statistically significant "+"-$0.05 \geqslant \alpha > 0.02$
Statistically significant "++"-$0.02 \geqslant \alpha > 0.01$
Statistically significant "+++"-$0.001 \geqslant \alpha$

Changes in percentage contribution of basic amino acids were similar to changes in absolute values during starvation but an increased contribution by histidine was noticeable for all breeds. Percentage contributions by acidic amino acids were very stable during the starvation period, as were the percentages of methionine, tyrosine and phenylalanine. The most significant change in per cent contribution was visible in the case of proline which decreased in all the breeds by about 70 % . Percentages of valine, leucine and isoleucine increased significantly in 'Leghorn' hens and decreased in 'Cornish' and 'White Rock' hens. The remaining amino acids (glycine, alanine, serine and threonine) showed diverse, strain-dependent patterns of change.

The number of interbreed differences after starvation was also subject to change. For example, interbreed differences in absolute concentrations oc-

Table 4. Statistically significant differences in the level of free amino acids in plasma during starvation

	Absolute concentrations (μM/100 ml)																	
	Lg-WR				Lg-Cr				WR-Cr				Lg-WR				Lg-Cr				WR-Cr			
	c	48h	72h	96c	c	48h	72h	96h	c	48h	72h	96h	c	48h	72h	96h	c	48h	72h	96h	c	48h	72h	96h
Lysine																								
Histidine																+								
Arginine																								
Aspartic acid																								
Threonine																								
Serine								+							+++					+			++	
Glutamic acid						+								+				++						
Proline		++				+							+							+				
Glycine						++														++				
Alanine		+	+	+++				++		+	+			++	+++	+++		+++					++	+
Valine				++				++								++	++							
Methionine																								
Isoleucine				++				++							+	+	+			++			+	
Leucine			+	+++				++							+	++	++			++			++	
Tyrosine															++								++	
Phenylalanine				+++				++							+	++				++			++	
ΣBAA																								
ΣAAA																								
ΣBAA/ΣAAA		++				++																		
ΣAA		++																						

Lg - WR - difference between 'Leghorn' and 'White Rock' hens
Lg - Cr - difference between 'Leghorn' and 'Cornish' hens
WR - Cr - difference between 'White Rock' and 'Cornish' hens
ΣBAA - sum of basic amino acids
ΣAAA - sum of acidic amino acids
ΣAA - sum of all amino acids

statistically significant "+" $0.05 \geqslant \alpha > 0.02$
statistically significant "++" $0.02 \geqslant \alpha > 0.001$
statistically significant "+++" $0.001 \geqslant \alpha$

curred in controls for three amino acids: glycine, glutamic acid and proline. After 96 h of starvation interbreed differences occurred for six amino acids: alanine, valine, serine, isoleucine, leucine and phenylalanine. Similar changes were noted for percentage contributions. Interbreed differences were exhibited by four amino acids while after 96 h interbreed differences concerned nine amino acids.

As shown by these data, changes in levels of free amino acids are dependent to a high degree on the breed of hen, which may explain the differences in level of some amino acids during starvation reported by various authors. For example, in 1953 Charkey found for the first time a considerable increase of lysine in plasma of starved chickens (Charkey et al. 1953). This increase was confirmed by other authors and found also in the case of threonine (Gray et al. 1960; Hill and Olsen 1963a,b). In 1963 Hill and Osen found an increase in concentration of 6 amino acids, and a decrease of 8 amino acids, in plasma of 21-day-old 'White Plymouth Rock' chickens in the course of starvation. Of glutamine, isoleucine, leucine, lysine, threonine and valine, the greatest increases were observed for lysine and threonine after 24 and 36 h of starvation (Hill and Olsen 1963b). These results were in line with the observations of Gray et al. (1960), who observed, however, a twofold lower increase of lysine in plasma of 4-week-old 'Barred Plymouth Rock' cockerels. It was found that the amino acids whose concentrations increased were endogenous. Feeding chickens with high energy food deficient in protein and amino acids brought about a decrease in the amino acid content of plasma. In the present investigations, the increase was confirmed only for lysine after 48 h of starvation. If one assumes that Hill and Olsen had finished their experiment after 48 h of starvation and that their studies were performed on another hen breed, the differences would be understandable.

Since we did not observe an increase in the concentration of any amino acid in plasma after 42 h of starvation, determination of the limiting amino acids

according to the method of Longenecker would seem possible, when applying only a longer starvation time (Longenecker and Kause 1959).

Elucidation of the reasons for the changes in concentration of individual amino acids during starvation is difficult at the present stage of investigation. Undoubtedly, a whole set of agents is concerned, and genetic factors could play a vital role. This would explain the decrease in glycine and alanine after 48 h of starvation and the subsequent maintenance of concentrations of these amino acids within rather strict limits in our investigations. Some authors (Charkey et al. 1953, 1954; Hill and Olsen 1963a,b) suggest that the increase of some amino acids is due to catabolism of proteins of the organism. Assuming this hypothesis to be correct, it is difficult to explain why levels of some amino acids decrease in the course of further starvation, sometimes even below the control level. It is also possible that the free amino acids of blood cells influence the changes in free amino acid concentrations in plasma. This question will be the subject of further investigations.

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