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The variation in amino acid levels in the blood plasma of breeding roosters during sexual maturation*

Summary

The paper provides an overview of results obtained in the study of variation in free amino acid levels in the blood plasma of breeding roosters (RIR 05 line) during sexual maturation (in the 10th, 15th, 20th and 25th weeks of age). The results prove that dynamic changes in free amino acid concentrations in blood plasma are connected with the sexual maturation of breeding roosters. During this period the concentrations of each particular free amino acid varied at a significance level of $P \leq 0.05$ and $P \leq 0.01$. Furthermore, in most cases the plasma concentrations of free amino acids in the 10th and 15th week were higher in comparison with those in the 20th and 25th week. Interestingly, the concentrations of individual free amino acids ranged over a wide interval (units, tens and hundreds of μmol/l). In this respect the free amino acids in plasma can be divided into three groups. The first group consisted of amino acids that occurred at low molar concentration (up to 100 μmol/l): cysteic acid, aspartic acid, α-aminoadipic acid, methionine, isoleucine, γ-aminobutyric acid, 3-methylhistidine, tryptophan and ornitine. The second group included amino acids occurring at medium to high molar concentration (100 – 300 μmol/l): taurine, glutamine, ½ cystine, valine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine. Finally, the third group contained threonine, serine, glutamic acid, proline, glycine and alanine that were present at high molar concentrations (above 300 μmol/l). Analyses of plasma in the period from the 10th to 25th week detected dynamic changes in levels of individual free amino acids and showed that the total content of these amino acids gradually decreased to the following mean values: 5 685.00 μmol/l in the 10th week, 5 076.21 μmol/l in the 15th week, 4 384.78 μmol/l in the 20th week and 4 793.30 μmol/l in the 25th week.

Key Words: rooster, sexual maturation, blood plasma, free amino acids

Zusammenfassung

Titel der Arbeit: **Aminosäurespektrum im Blutplasma von Zuchthähnen im Laufe ihrer Geschlechtsreife**

Es wird über Untersuchungen zum Spektrum freier Aminosäuren von Hähnen der Linie RIR 05 im Laufe der Geschlechtsreifung (10., 15., 20. und 25. Lebenswoche) berichtet. Die Ergebnisse weisen auf eine dynamische Entwicklung der einzelnen im Plasma vorhandenen freien Aminosäuren im Zusammenhang mit der Geschlechtsreifung von Zuchthähnen hin. Die Konzentrationen der einzelnen freien Aminosäuren variierten bei unterschiedlichem Signifikanzniveau. In den meisten Fällen wurden in der 10. und 15. Woche höhere Konzentrationen als in der 20. und 25 Woche nachgewiesen. Bemerkenswert ist, dass es im Laufe der Geschlechtsreifung im Blutplasma zu einer signifikanten Schwankung der freien Aminosäuren in Größenordnungen von Einern, Zehnern und Hundertsteln μmol/l Blutplasma kommt. Aus dieser Sicht kann das gesamte Spektrum von freien Aminosäuren im Blutplasma in drei Gruppen eingeteilt werden. Die erste Gruppe enthält freie Aminosäuren mit einer niedrigen molaren Konzentration von unter 100 μmol/l repräsentiert durch Zysteo-, Asparagin-, Aminoadip-, Methionin-, Isoleucin-, γ-Aminobuttersäure, 3-Methylhistidin, Tryptophan und Ornitin. Zur Gruppe der Aminosäuren mittel hoher molarer Konzentration von 100 bis 300 μmol/l zählen Taurin, Glutamin 1/2 Zystin, Valin, Leucin, Thyrosin, Phenylalanin, Histidin, Lysin und Arginin. Zur Gruppe mit einer molaren Konzentration von über 300 μmol/l zählen Threonin, Serin, Glutaminsäure, Prolin, Glycin und Alanin. Die Analysen beweisen, dass es im Laufe des Beobachtungszeitraumes außer einer dynamischen Veränderung bei den einzelnen freien Aminosäuren auch tendenziell zu einer schrittweisen Verringerung der Gesamtsumme der Aminosäuren im Blutplasma kommt. Die Durchschnittswerte der erfassten Aminosäurekonzentrationen betrugen in der 10. bis zur 20. Woche 5685,00, 5076,21, 4384,78 und 4793,30 μmol/l.

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Schlüsselwörter: Hahn, Geschlechtsreife, Blutplasma, freie Aminosäuren

Introduction

Saccharides, lipids and proteins are three basic components of human nutrition. Proteins are essential for living organisms as they enable anabolic processes and participate in the transformation of energy. However, diets are often deficient in proteins. Amino acids resulting from protein digestion are the major sources of nitrogen for an organism. Free amino acids and dipeptides are absorbed from the intestine. In blood they combine with amino acids released by degradation of tissue proteins or produced in protein biosynthesis to build a metabolic pool available for cells in all tissues.

The biological and nutrition values of poultry products are very high. Poultry meat represents an easily digestible nutrient with a high content of essential amino acids. The production of high-quality proteins depends upon comprehensive knowledge of the structures and effects of each particular amino acid. Undoubtedly, there are a large number of issues in this field to be addressed, for example the monitoring of amino acids levels in the blood plasma. Though a number of detailed studies have been carried out in large domestic animals, analogous data in poultry are sporadically reported. Thus, the majority of the basic physiological values in poultry are still unknown.

According to PROKOP (1991) a relatively high demand for feed by growing and highly productive poultry arises from enhanced protein biosynthesis, which is, from an energy point of view, the most demanding process in an organism. The metabolism in every organism requires all amino acids to be in a proper ratio. A disproportion in essential amino acids results in lowered utilization of other amino acids and leads to increasing demands for nitrogen substances in a feeding mixture. Such essential amino acids are termed “limiting” amino acids (ZELENKA et al., 1993).

PROKOP (1991) have reported that the current feeding mixtures for poultry are deficient in methionine. This amino acid participates not only in tissue building processes but also in the biosynthesis of antibodies. For example, it is capable of eliminating growth suppression caused by an excess of copper. Methionine deficiency results in increased intake of feedstuffs, inefficient utilization of nutrients and in inhibition of glycine absorption. Through its lipotropic effect, methionine affects physiological processes in the liver and controls the function of the thyroid gland.

Lysine is another essential amino acid. A deficiency of lysine in the diet causes growth suppression, the decrease of activity of transaminases and a high demand for oxygen by liver tissues (PROKOP,1991).

IORDÁNOVÁ (1980) studied variations in total protein levels, protein fractions and free amino acids in the blood plasma of chickens and pigs whose diet during growth was enriched in amino acids.

ZHORINA and STEPCHENKO (1991) monitored the content of free amino acids in the selected tissues of broilers which were fed with nitrogen humate.

The main aim of our research was to study the dynamics of free amino acids in the blood plasma of breeding roosters during sexual maturation in order to gain a deeper insight into the amino acid metabolism in this important period.

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Materials and Methods

The monitoring during the experiment was carried out in an approved animal enclosure at The Institute for Nutrition, Dietetics, Zoo-Hygiene and Food Crop Production at The University of Veterinary and Pharmaceutical Sciences in Brno.

10 breeding roosters (RIR 05 line) selected for the experiment were bedded in a cage with deep bedding. Over the whole monitoring period the respective zoohygienic and technological requirements for this breed of roosters were observed. The temperature was maintained from 15 to 18 °C and the light-day was adjusted according to the age of roosters as follows: 8 hours from 10th to 20th week, 9 hours in 20th week, 9.5 hours in 21st week, 10 hours in 22nd week, 10.5 hours in 23rd week, 11 hours in 24th week and 11.5 hours in 25th week. Tube plastic feeders and hanging drinkers (PLASSON MK II) were used for feeding. Roosters were fed ad libitum with a complex feeding mixture. According to our analyses the composition of the feeding mixture was as follows: 185.3 g of N-substances, 29.1 g of fat, 36.7 g of fiber, 55.9 g of ash, 12.45 MJ of metabolic energy, 11.3 g of Ca, 6.9 g of P and 1.9 g of Mg per kg dry matter.

The experimental monitoring of roosters was carried out in the period of their sexual maturation, i.e. from the 10th to the 25th weeks of age. In regular five-week intervals (between 7 and 8 a.m.) blood was taken from *vena basilica* and placed in test tubes coated with heparin. Blood plasma was immediately precipitated by the addition of 18% sulfosalicylic acid to remove proteins and prepare a sample for free amino acid analysis.

Free amino acids in the blood plasma of breeding roosters were determined by the widely known method of column chromatography using lithium citrate buffers and the AAA 339 (Mikrotechna Praha) automatic analyzer of amino acids.

Samples of blood plasma were assayed for the following amino acids (μmol/l): cysteic acid, taurine, aspartic acid, threonine, serine, glutamic acid, glutamine, α-aminoadipic, proline, glycine, alanine, ½ cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, γ-aminobutyric acid, histidine, 3-methylhistidine, tryptophan, ornithine, lysine, arginine.

The results were worked up using mathematical and statistical methods by VENČIKOV and VENČIKOV (1977). All the presented results in tables are mean values (with standard deviation). The results obtained were calculated statistically according to the Student's T-test $P \leq 0.05$* respectively $P \leq 0.01$**.

Results

The results of amino acid analyses are shown in Tables 1-2.

The mean concentrations of cysteic acid during this period ranged from 12.30 to 19.19 μmol/l with an inconclusive increase being detected from the 20th to the 25th week. The mean values for aspartic acid varied between 67.01 and 101.00 μmol/l. A statistically significant difference ($P \leq 0.05$) was found for mean values in 20th (77.37 μmol/l) and in the 25th week (67.01 μmol/l). An interesting trend in mean concentrations of α-aminoadipic acid was observed. The mean concentrations fell into a relatively wide interval spanning from 3.84 μmol/l (in 15th week) to 21.67 μmol/l (in 10th week). A statistically very significant ($P \leq 0.01$) decrease of the α-aminoadipic acid concentration was detected in the 15th week (3.84 μmol/l), while in the 20th week the concentration rose conclusively to 10.90 μmol/l. The mean concentration of methionine ranged from 49.02 to 65.19 μmol/l whereas in 25th week it increased

conclusively ($P \leq 0.05$) from 49.02 μmol/l to 59.87 μmol/l. Isoleucine concentrations increased conclusively ($P \leq 0.01$) to 100.33 μmol/l in the 25th week. The level of γ-aminobutyric acid increased significantly ($P \leq 0.01$) from 49.64 μmol/l in the 10th week to 73.64 μmol/l in the 15th week, which was followed by a conclusive decline ($P \leq 0.01$) to 44.66 μmol/l in the 20th week. Statistically significant differences ($P \leq 0.05$) in concentrations of plasmatic 3-methylhistidine were detected in the 15th week (31.29 μmol/l), 20th week (25.90 μmol/l) and 25th week (34.11 μmol/l). The increase of the mean concentrations of tryptophan from 60.69 μmol/l (10th week) to 65.65 μmol/l (20th

Table 1
Mean values of free amino acids with low molar concentrations (up to 100 μmol/l medium to high (100-300 μmol/l) and high molar concentrations (above 300 μmol/l) in the blood plasma of breeding roosters in the period of sexual matura (Mittelwerte von freien Aminosäuren mit niedriger (bis 100 μmol/l), mittlerer (100-300 μmol/l) und hoher (über 300 μmol/l) Mol-Konzentration im Blutplasma von Zuchthähnen während der Periode ihrer Geschlechtsreife)

Amino acid	10th week		15th week		20th week		25th week
	μmol/l	td	μmol/l	td	μmol/l	td	μmol/l
Low molar concentrations (up to 100 μmol/l)							
Cysteic A.	16.89 (7.79)	-	14.93 (7.22)	-	12.30 (6.94)	-	19.19 (18.95)
Aspartic A.	101.00 (21.27)	-	89.57 (18.54)	-	77.37 (9.52)	*	67.01 (10.67)
α-aminoadipic acid	21.67 (12.68)	**	3.84 (3.64)	**	10.90 (6.42)	-	12.49 (4.89)
Methionine	65.19 (13.74)	-	54.53 (9.86)	-	49.02 (14.83)	*	59.87 (8.57)
Isoleucine	94.09 (24.10)	-	78.11 (15.75)	-	67.61 (15.93)	**	100.33 (18.83)
γ-aminobutyric acid	49.64 (9.34)	**	73.64 (12.32)	**	44.66 (19.63)	-	48.05 (19.15)
3-methyl histidine	25.48 (6.42)	*	31.29 (5.04)	*	25.90 (5.28)	*	34.11 (7.95)
Tryptophan	60.69 (12.50)	-	65.79 (12.80)	-	65.65 (14.70)	**	50.44 (6.31)
Ornithine	51.48 (17.86)	**	27.90 (10.97)	**	33.39 (8.76)	*	79.67 (31.53)
Medium to high molar concentration (100-300 μmol/l)							
Taurine	158.06 (75.19)	*	99.69 (39.47)	-	87.72 (18.61)	-	112.60 (42.56)
Glutamine	120.54 (28.57)	-	152.19 (43.43)	-	123.16 (33.82)	**	205.48 (34.85)
½ cystine	112.81 (24.58)	-	124.96 (19.31)	-	121.94 (27.53)	-	144.39 (21.42)
Valine	210.95 (53.17)	*	165.60 (33.51)	-	151.67 (46.60)	**	247.58 (42.84)
Leucine	201.32 (32.28)	-	196.87 (45.34)	-	164.09 (30.82)	*	205.84 (36.41)
Tyrosine	156.78 (21.71)	*	204.31 (57.89)	**	137.30 (29.24)	-	129.02 (32.96)
Phenylalanine	104.98 (15.66)	**	128.70 (15.62)	**	81.62 (15.00)	-	87.58 (8.96)
Histidine	108.26 (9.47)	**	135.79 (36.91)	*	92.45 (31.00)	-	106.59 (17.56)
Lysine	295.21 (56.63)	**	86.88 (29.67)	**	177.59 (64.52)	*	288.02 (78.08)
Arginine	214.57 (46.05)	-	167.27 (89.61)	-	139.02 (68.33)	-	174.92 (90.00)

Table 1 (Continuation)

Amino acid	10th week		15th week		20th week		25th week
	µmol/l	Td	µmol/l	td	µmol/l	td	µmol/l
High molar concentration (above 300 µmol/l)							
Threonine	815.91 (134.49)	**	507.22 (171.90)	-	655.85 (203.37)	-	648.31 (146.38)
Serine	493.95 (93.42)	-	455.45 (113.05)	-	362.15 (92.12)	-	313.54 (42.74)
Glutamic acid	750.44 (83.20)	**	597.44 (117.03)	-	549.73 (58.95)	**	485.28 (41.15)
Proline	442.55 (93.78)	**	586.87 (210.62)	-	356.64 (99.02)	-	435.42 (97.42)
Glycine	491.38 (57.45)	-	488.04 (89.40)	-	432.06 (83.24)	-	467.84 (65.76)
Alanine	521.16 (84.76)	-	539.33 (144.95)	**	364.99 (67.42)	*	269.76 (84.69)

Table 2
Total content of free amino acids monitored in blood plasma of breeding roosters during the period of sexual maturation (Gesamtgehalt von freien Aminosäuren im Blutplasma von Hähnen während der Periode ihrer Geschlechtsreife)

Amino acids	10th week	15thweek	20th week	25th week
µmol/l	5 685.00	5 076.21	4 384.78	4 793.30
Percent	100	89.29	77.13	84.31

week) was statistically inconclusive, but it was followed by a statistically significant ($P \leq 0.01$) decrease to 50.44 µmol/l in 25th week. Ornithine levels varied between 27.90 µmol/l and 79.67 µmol/l over the whole period, reaching the maximum in the 25th week. The most significant ($P \leq 0.01$) difference was found between ornithine concentrations in the 10th week (51.48 µmol/l) and the 15th week (27.90 µmol/l) and between the 15th week (27.90 µmol/l) and the 20th week (33.39 µmol/l). In addition, the difference between the concentrations in the 20th (33.39 µmol/l) and 25th (79.67 µmol) weeks was also statistically significant ($P \leq 0.05$) (Table 1).

The mean concentrations of taurine ranged from 87.72 to 158.06 µmol/l. A statistically conclusive difference ($P \leq 0.05$) was found between taurine levels in the 10th and 15th weeks. The mean concentrations of glutamine during the period of sexual maturation varied in range from 120.54 to 205.48 µmol/l, exhibiting a significant ($P \leq 0.01$) increase 205.48 µmol/l in the 25th week. Statistically insignificant changes (112.81 - 144.39 µmol/l) were noticed for ½ cystine during the period of roosters' sexual maturation. The mean concentrations of valine ranged from 151.67 µmol/l in the 20th week to 247.58 µmol/l in the 25th week. A significant ($P \leq 0.05$) decrease of valine levels to 165.60 µmol/l in15th week and a very conclusive ($P \leq 0.01$) increase to 247.58 µmol/l in the 25th week was detected. Leucine concentrations increased conclusively ($P \leq 0.05$) from 164.09 µmol/l (20th week) to 205.84 µmol/l (25th week). Mean concentrations of tyrosine showed a statistically significant ($P \leq 0.05$) increase from 156.78 µmol/l in the 10th week to 204.31 µmol/l in the 15th week, followed by a statistically significant ($P \leq 0.01$) decline to 137.30 µmol/l in the 20th week. Mean concentrations of fenylalanine ranged from 81.62 µmol/l to 128.70 µmol/l. Between the 10th and 15th weeks a highly significant ($P \leq 0.01$) increase of its mean concentrations (from 104.98 µmol/l to 128.70 µmol/l) was observed, which was followed by a statistically significant ($P \leq 0.01$) decrease to 81.62 µmol/l in the 20th

week. Histidine concentrations showed a statistically significant ($P \leq 0.01$) variation from 108.26 μmol/l in the 10th week to 135.79 μmol/l in the 15th week, followed by a significant ($P \leq 0.05$) decrease to 92.45 μmol/l in the 20th week. Highly significant changes ($P \leq 0.01$) were detected between lysine concentrations in the 10th and 15th weeks (295.21-86.88 μmol/l) and in the 15th and 20th weeks (86.88-177.59 μmol/l). Furthermore, a statistically significant ($P \leq 0.05$) variation in lysine levels was found between the 20th and 25th weeks (177.59-288.02 μmol/l). In the case of arginine, a statistically inconclusive decrease of its mean concentration from 214.57 μmol/l (10th week) to 139.02 μmol/l (20th week) was detected, followed by a statistically insignificant increase to 174.92 μmol/l in the 25th week (Table 1).

The mean values of threonine in plasma were in the range from 507.22 to 815.91 μmol/l, showing a statistically significant ($P \leq 0.01$) decrease in the 15th week. Serine levels exhibited an inconclusive decrease from 493.95 μmol/l in the 10th week to 313.54 μmol/l in the 25th week. In the case of glutamic acid, a statistically significant decrease ($P \leq 0.01$) of its concentration to 597.44 μmol/l and 485.28 μmol/l was detected in the 15th week and in the 25th week, respectively. The mean concentrations of proline ranged between 356.64 and 586.87 μmol/l. The difference between its mean concentrations in the 10th week (442.55 μmol/l) and the 15th week (586.87 μmol/l) was statistically significant ($P \leq 0.01$). The mean concentrations of glycine (432.06 – 491.38 μmol/l) during sexual maturation varied inconclusively. The difference between alanine levels in the 15th (539.33 μmol/l) and in the 20th (364.99 μmol/l) week was statistically significant ($P \leq 0.01$). A significant ($P \leq 0.05$) difference was also detected between the concentration in the 20th week (364.99 μmol/l) and that in the 25th (269.76 μmol/l) week (Table 1).

Discussion

Until now no papers on the monitoring of free amino acid concentrations in the blood plasma of breeding roosters during sexual maturation have been published - either in Czech or foreign journals. Some authors have studied variations in amino acid levels in relation to a particular element or synthetic amino acids or in connection with parasitic diseases.

GERGELYIOVÁ (1983) reported concentration values for some of amino acids in the blood plasma of turkey (essential amino acids: „phenylalanine“- 0.5 mg/100 ml, „threonine“- 5.5 mg/100 ml; non-essential amino acids: „asparagine“ 0.8 mg/100 ml, „serine“: 6.5 mg/100 ml). STODOLA and ŘEHOUT (1988) monitored the dynamics of changes in molar concentrations of free plasmatic amino acids in calves over one year and demonstrated that free amino acids in the blood plasma of calves ranged from 6 to 300 μmol/l. According to our results, the mean concentrations of free plasmatic amino acids varied from 3.84 to 815.91 μmol/l. Thus, the amino acid levels in breeding roosters are higher than those determined in mammals. The variation in free plasmatic amino acids in sheep was studied by TOMÁŠ and MICHNOVÁ (1983).

Aminoanalysis of the blood plasma of breeding roosters during sexual maturation showed that in most cases the molar concentrations in roosters were higher than those reported for mammals.

One interesting finding is that amino acid levels in blood plasma not only varied conclusively but ranged over a very wide interval (in units, tens and hundreds of μmol/l). In this respect free plasmatic amino acids can be divided into three groups.

The first group consisted of amino acids that occurred at low molar concentrations (up to 100 μmol/l): cysteic acid, aspartic acid, α-aminoadipic acid, methionine, isoleucine, γ-aminobutyric acid, 3-methylhistidine, tryptophan and ornithine (Table 1). The second group included amino acids occurring at medium to high molar concentrations (100 – 300 μmol/l): taurine, glutamine, ½ cystine, valine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine (Table 1). Finally, the third group contained threonine, serine, glutamic acid, proline, glycine and alanine that were present at high molar concentrations (above 300 μmol/l) (Table 1).
Analyses of plasma in the period from the 10th to the 25th week detected dynamic changes in levels of individual free amino acids and showed that the total content of these amino acids gradually decreased to the following mean values: 5,685.00 μmol/l in 10th week, 5,076.21 μmol/l in 15th week, 4,384.78 μmol/l in 20th week and 4,793.30 μmol/l in 25th week (Table 2).
This paper provides new physiological data on domestic fowls and demonstrates the dynamic changes occurring in the blood plasma of breeding roosters during sexual maturation.

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