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AGE DYNAMICS OF FREE AMINO ACIDS IN THE TISSUES OF THE MEDICINAL LEECH *Hirudo verbana* Carena, 1820 UNDER ARTIFICIAL REPRODUCTION IN AQUACULTURE

L.A. KOVALCHUK[1] ✉, L.V. CHERNAYA[1], N.V. MIKSHEVICH[2]

[1]*Institute of Plant and Animal Ecology, Ural Branch RAS,* 202, ul. 8 Marta, Ekaterinburg, 620144 Russia, e-mail kovalchuk@ipae.uran.ru (✉ corresponding author), Chernaya_LV@mail.ru;
[2]*Ural State Pedagogical University*, 26, pr. Kosmonavtov, Yekaterinburg, 620017 Russia, e-mail mikshevich@gmail.com
ORCID:
Kovalchuk L.A. orcid.org/0000-0003-0467-1461 Mikshevich N.V. orcid.org/0000-0003-2388-4278
Chernaya L.V. orcid.org/0000-0002-3386-9824
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Abstract

Medicinal leeches (*Hirudo verbana* Carena, 1820) used in veterinary medicine, human medicine and pharmacology, are currently bred artificially at bio-factories. The technology of leech culture as a component of aquaculture biotechnics is based on the method of accelerated growth of leeches due to intensive feeding, which allows you to get high-quality products in a fairly short time (8-10 months). Nevertheless, the problem of assessing the physiological status of raised individuals upon artificial reproduction remains not fully addressed. The findings we present herein for the first time allow us to suggest that the age-specific amino acid composition of tissues can be used to assess the physiological state and well-being of medicinal leeches in nature and in aquaculture. The aim of the work is to study the age-dependent content of the free amino acids in the tissues of medicinal leeches artificially bred in aquaculture. Studies were conducted in 2012 on the leech (*Hirudo verbana* Carena, 1820) individuals without signs of diseases which were purchased at the biofactory International Center for Medicinal Leeches (Moscow Province, p. Udelnaya). Leeches were kept in the laboratory in glass vessels with dechlorinated water at 20-22 ºC and fed once a month with fresh bovine blood from healthy animals. The experimental specimens of *H. verbana* was: 5 days (newborn filaments, 0+), 1, 3, 5, 7 and 9 months old, with an average body weight of 0.029, 0.09, 0.25, 0.61, 1.33 and 1.81 g, respectively. Newborn individuals served as a control. The concentration of free amino acids (AA) in the skin and muscle tissue was determined on the analyzer AAA-339M (Mikrotechna, Czech Republic). A total of 105 leech specimens were used, 30 bioassays were prepared, and 660 element determinations were performed. The amino acid composition of the tissues of medicinal leeches *H. verbana* was represented by 23 AA and their derivatives. The dominant AA with an antioxidant orientation, regardless of age, were glutamine and glutamic acid, alanine, valine, leucine, and glycine, the total content of which was 65 % in filaments, and 58 % in 9-month — old individuals of the total fund AA. In the tissues of medicinal leeches, as well as in warm-blooded animals, the full composition of essential amino acids (EAA) was revealed: threonine, valine, lysine, leucine, isoleucine, histidine, arginine, methionine, phenylalanine, tryptophan. With age, the total concentrations of free amino acids decreased ($r = -0.98$ at $p = 0.000$), due to a sharp decrease in the content of arginine (24.0-fold), proline (13.4-fold), isoleucine (12.2- fold), glycine (8.5- fold), lysine (6.8- fold), histidine (6.6-fold), leucine (5.2-fold), ornithine (4.0-fold), glutamic acid and glutamine (3.8-fold), and alanine (2.9-fold). At the age of 5, 7 and 9 months, leeches had a trace content of secondary metabolites: taurine, citrulline and tryptophan ($p < 0.001$). However, the amino acid balance of nitrogen and protein metabolism was not disturbed, since the ratio of essential / non-essential AA did not change significantly and was 0.60 in newborn filaments, 0.73 — in 9-month-old leeches. It was found that the growth and development of *H. verbana* was accompanied by a significant decrease

in the indicator of maturity (glycine/alanine) from 0.75 (newborn filaments) to 0.25 (9 months). The information available in the literature and the data of our research allow us to put forward the concept of using functional amino acids as biomarkers in the development of the scientific bases of technologies for the industrial breeding of these amphibionts.

Keywords: medicinal leeches, leech culture, free amino acids, age, physiological status

In advanced aquaculture, technologies should suit to physiological features and requirements of aquatic organisms during their ontogenesis. The well-being of artificially reproduced species is closely related to their adaptability to environmental conditions. Reliable assessing physiological parameters of animals and their habitat, e.g., using ecological and physiological biomarkers, are necessary to quickly estimate the ecological safety and to create conditions for the sustainable development of aquatic organisms [1, 2]. Currently, work in this area is focused on the search and practical use of biomarkers of immunological, histopathological and physiological parameters of artificially reproduced aquatic organisms.

Available scholar publications and our findings draw us to the concept that functional amino acids (AA) which have a wide spectrum of metabolic activity and play an important role in energy and plastic metabolism in animals can serve as biomarkers [3-5]. Comparing the biochemical composition of 182 freshwater and marine zooplankton species showed the freshwater species to have lower levels of ATP and free amino acids and a higher RNA [6]. In many research papers, the metabolism of copepods, planktonic copepods, and daphnia is investigated [7, 8]. The biological role of free amino acids and their derivatives in marine invertebrates has been shown for muscle proteins of bivalve molluscs [9]. Enhanced amino acid metabolism which provides adaptive resistance to new environmental conditions was revealed in Black Sea mollusk *Anadara kagoshimensis* [10]. Trophic specificity of the amino acid composition of freshwater leech tissues and the key role of essential amino acids in adaptation of blood-sucking hirudinids to extreme habitat conditions are discussed [11, 12].

The age dynamics of free AAs and their derivatives in animal tissues can serve as an informative physiological indicator. This is especially important for mass reproduction when cultivation density is high [13-15]. Changes in the amino acid profile reflecting the role of free AAs occur during larval development of the plaice *Platichthys stellatus* [16]. In early ontogenesis of freshwater neotropical fish *Piaractus mesopotamicus*, high levels of essential amino acids were characteristic. Moreover, these findings indicated the role of taurine in the regulation of fluid osmolality [15]. In mackerel inhabiting the Chinese Sea, metabolically inert taurine in the muscles was seasonally variable [17]. The amino acid composition of commercial fish was influenced by fish nutrition [18]. Modifications of nitrogen metabolism and the role of free amino acids in low-temperature adaptation were studied on the example of an eurythermal pond fish *Perccottus glehni* [19]. These data confirm the importance of free amino acids for regulation of key metabolic processes ensuring the well-being of freshwater organisms in the natural and anthropogenic ecosystems.

Leeches for medical, veterinary and pharmaceutical use are cultivated in biofactories under controlled conditions [20, 21]. The current technology of leech aquaculture (hirudoculture) is based on the methodology developed in Russian back in the middle of the 20th century. Its main stages are the mating of the brood stock leeches, collection of juveniles from mature cocoons and their feeding with bovine blood to the final size [22, 23]. The growth rate of leeches in hirudoculture is exclusively due to growing conditions, e.g., the frequency of water changes, high

cultivation density, spatial limitation, etc., which either positively affect the physiological parameters or, in contrast, cause the high mortality rate of juveniles. However, assessing the well-being of reared leeches remains not fully addressed and the amino acid metabolism have not been given due attention. The ratio of vital free AAs in the tissues as a well-being criterion can contribute to the effective reproduction of medicinal leeches in hirudoculture.

Here, we have presented the first report on the age-specific amino acid profiles as a tool to assess the physiological state and well-being of the medicinal leech both in nature and in aquaculture.

The aim of this work was to determine the free amino acid profile in tissues of cultivated medicinal leeches as dependent on age.

Materials and methods. During cultivation (2012), the pharmacy leech (*Hirudo verbana* Carena, 1820) (purchased at the biofactory "International Center for Medical Leech", Moscow Province, Udelnaya settlement) without signs of diseases were kept in the laboratory in glass vessels with dechlorinated water at 20-22 °C and fed once a month with fresh bovine blood from healthy animals. The free amino acid dynamics was assessed in 5-day-old individuals (newborn filaments, 0+), 1-, 3-, 5-, 7- and 9-month-old leeches with the average body weight 0.029, 0.09, 0.25, 0.61, 1.33, and 1.81 g, respectively. Newborn leeches served as control. The studies were guided by the recommendations of the European convention on protection of the vertebrate animals used for experiment or in other scientific purposes [24, 25].

Free AAs were measured in the musculocutaneous tissue (an AAA-339M analyzer, Mikrotechna, Czech Republic). A 1 g portion of raw biomaterial was homogenized in 3 ml of phosphate buffer (pH 7.4). After two-stage centrifugation (a K-23D refrigerated centrifuge, MLW, Germany; 10,000 rpm for 15 min and 8,000 rpm for 30 min), 0.1 ml of 30 % sulfosalicylic acid was added for deproteinization and neutralized with 0.2 ml of 7 % lithium hydroxide. Norleucine (0.1 ml) was used as an internal standard. The following metabolic groups of AAs were measured: nonessential AAs (NEAs) — alanine, aspartic acid, glycine, glutamic acid + glutamine, proline, serine, tyrosine, cysteine; essential AAs (EAs) — arginine, valine, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, phenylalanine; branched-chain AAs (BCAs) — valine, isoleucine, leucine; aromatic AAs (AAs) — tyrosine, phenylalanine; sulfur-containing AAs (SCAs) — methionine, cysteine, cysteic acid. A total of 105 leeches were tested, 30 biosamples were prepared, 660 biochemical analyses were performed.

Standard software packages, the Microsoft Excel 2007 and STATISTICA 7.0 (StatSoft, Inc., USA) were used for statistical processing. The means (M) and standard errors of the means (±SEM) were calculated. The data were converted into logarithmic form (μmol/100 g) or arcsine form (% of total AAs). To statistically compare multiple values, Fisher's F-test was applied; differences from control were assessed by Dunnett's test (ANOVA). The correlation coefficient r was calculated to evaluate relationships between the free AA levels in leeches and their age. The data were visualized by principal component analysis (PCA) using the statistical environment R (R 3.1.2, packages Vegan and Ade4) [26]. Differences were considered statistically significant at $p < 0.05$.

Results. In *H. verbana*, we detected 23 AAs and their derivatives. The leeches, like warm-blooded animals [1, 2], had a full set of functionally significant essential amino acids (threonine, valine, lysine, leucine, isoleucine, histidine, arginine, methionine, phenylalanine, and tryptophan). The dominant antioxidant AAs, regardless of age, were glutamine, glutamic acid, alanine, valine, leucine, and

glycine which in total constituted 65 % pool of AAs in control (filaments) and 58 % pool in 9-month-old leeches.

The highest rates of amino acid metabolism were characteristic of juvenile leeches at the earliest stages of development (Table 1). Thus, the total concentrations of free AAs in newborns and 1-month-old individuals did not show statistically significant differences ($p > 0.05$), despite the multiple increase in the body weight due to intensive feeding. There was a linear drop in the total concentrations of free AAs ($r = –0.98$ at $p = 0.000$). Due to accelerated growth, in adult leeches aged 9 months, the total AAs decreased 3-fold compared to control ($p < 0.001$).

At early ontogenesis, leeches had a high requirement for amino acids necessary to rapidly increase muscle mass (valine, alanine, ornithine, arginine), to provide reproductive development (glutamine, lysine, histidine, ornithine, lysine), to synthesize elastin and collagen, the main proteins of connective tissues (alanine, glycine, lysine, proline, valine, leucine, threonine), to provide metabolic detoxification (glycine, glutamic acid, cysteine, tryptophan), to normalize carbohydrate and lipid metabolism, and to develop immunity (alanine, taurine, histidine, leucine).

The most significant age-related differences ($F_{5, 24} > 147.74$ at $p < 0.001$) were revealed for amino acids which are key physiological stimulants. With age, amino acid levels declined. There were a 24.0-fold decrease in arginine, a 13.4-fold decrease in proline, a 12.2-fold decrease in isoleucine, an 8.5-fold decrease in glycine, a 6.8-fold decrease in lysine, a 6.6-fold decrease in histidine, a 5.2-fold decrease in leucine, a 4.0-fold decrease in ornithine, a 3.8-fold decrease in glutamic acid and glutamine, and a 2.9-fold decrease in alanine ($p < 0.001$) (see Table 1).

Importantly, in leeches aged 5, 7, and 9 months, the pool of secondary metabolites, namely taurine, citrulline, and tryptophan, was completely exhausted ($p < 0.001$). In hirudoculture, the ontogenesis of leeches was also due to the high supply with specific metabolic groups of AAs, i.e., the branched-chain AAs (valine, leucine, isoleucine) which protect muscle fibers from oxidation and destruction, aromatic AAs (phenylalanine, tyrosine) involved in the synthesis of biogenic amines and neurotransmitters, and sulfur-containing AAs (cysteic acid, cysteine, methionine) possessing immunomodulatory and detoxifying properties (see Table 1).

The balance of nitrogenous and protein metabolism characterized by the ratio of essential to nonessential AAs did not change significantly both in control (EAs/NEAs = 0.60) and in 9-month-old leeches (EAs/NEAs = 0.73) (see Table 1). It is important to note that the pattern we observed for the maturity indicator (MI — the ratio of glycine to alanine) was opposite, that is, the MI values decrease from 0.75 in filamentous leeches to 0.25 in 9-month-old leeches. It is known that in commercial species, an increase in alanine and a decrease in glycine in fish tissues during growth is a stable trait, therefore, the alanine to glycine ratio can be used as an indicator of maturity, namely, MI values of 1.2-1.4 correspond to immature individuals, of 0.3-0.6 to mature ones [27]. According to our data, the MI parameter proposed to determine sexual maturity of fish can also be effective in assessing the age characteristics of leeches in hirudoculture.

We found statistically significant correlations between the age of medicinal leeches and the percentage for both the majority of free AAs and the major metabolic groups (% of the total AAs) in tissues (Fig. 1).

1. Age-dependent profiles of free amino acids (μmol/100 g) **in the tissues of the medical leech *Hirudo verbana* Carena, 1820 grown in hirudoculture** ($n = 30$, $M \pm$SEM)

Amino acid	Age, months						ANOVA	
	0+, control	1	3	5	7	9	$F_{5, 24}$	p
Cysteic acid	49.24±0.49	79.64±0.64	54.48±1.44^{NS}	46.25±1.68^{NS}	41.28±1.24	31.98±1.02	140.67	0.000
Taurine	11.65±0.36	9.39±0.51	6.46±0.50	Trace	Trace	Trace	1201.90	0.000
Aspartic acid	9.95±0.41	126.62±1.92	85.01±4.27	16.07±1.07	4.65±0.44	4.86±0.47	521.48	0.000
Threonine	81.88±1.20	96.20±1.68	58.17±0.85	77.60±3.38^{NS}	98.23±3.66	73.40±2.56^{NS}	42.45	0.000
Serine	91.20±2.86	48.47±1.09	87.78±1.57^{NS}	94.49±3.50^{NS}	104.02±1.22	76.03±2.93	93.99	0.000
Glutamate + Glutamine	608.20±5.79	413.89±2.62	381.01±3.08	285.49±6.89	203.07±1.36	158.33±3.17	1201.5	0.000
Proline	70.74±1.06	28.62±1.00	14.12±0.71	9.48±0.97	7.07±0.43	5.28±0.56	206.85	0.000
Glycine	157.01±2.10	208.32±2.27	134.16±1.60^{NS}	77.47±3.24	27.11±0.58	18.47±1.32	147.75	0.000
Alanine	209.13±1.74	236.69±1.52	205.97±1.54^{NS}	130.04±3.38	97.86±1.61	73.27±2.62	569.51	0.000
Citrulline	9.15±0.42	9.37±0.42^{NS}	4.72±0.35	Trace	Trace	Trace	1189.60	0.000
Valine	93.31±0.85	110.19±1.50	73.09±1.54	96.47±3.30^{NS}	121.77±1.46	91.32±2.12^{NS}	71.53	0.000
Cysteine	6.37±0.42	4.12±0.17	23.55±0.85	15.32±0.71	8.52±0.50	6.35±0.46^{NS}	142.80	0.000
Methionine	23.56±0.84	14.80±0.43	53.29±1.48	36.58±3.40	22.99±0.62^{NS}	18.95±0.56	93.91	0.000
Isoleucine	121.31±1.40	98.36±1.79^{NS}	34.51±0.68	23.90±2.99	13.14±0.72	9.98±0.67	263.72	0.000
Leucine	180.72±2.49	154.04±1.39	130.88±2.61	86.36±3.67	46.1±0.95	34.67±0.62	840.56	0.000
Tyrosine	15.44±0.76	8.80±0.71	27.51±1.12	20.41±1.73^{NS}	16.11±0.60^{NS}	11.77±0.80^{NS}	40.30	0.000
Phenylalanine	30.87±1.40	34.9±0.54^{NS}	56.34±1.11	41.87±2.62	23.76±0.44	17.83±0.81	107.67	0.000

Продолжение таблицы 1

Tryptophan	2.82±0.19	22.71±0.77	5.53±0.33	Trace	Trace	Trace	1659.40	0.000
Ornithine	27.43±0.89	27.12±1.01^{NS}	29.20±0.49^{NS}	18.48±1.00	9.15±0.51	6.82±0.49	176.12	0.000
Lysine	70.39±1.49	79.41±1.63^{NS}	79.29±1.01^{NS}	45.46±2.09	13.96±0.54	10.35±0.82	479.28	0.000
Histidine	1.19±0.02	4.68±0.42	11.77±0.43	3.86±0.07	0.89±0.06^{NS}	0.18±0.01	572.90	0.000
Arginine	46.98±1.08	31.17±0.77	22.93±0.66	14.43±1.09	7.72±0.51	1.96±0.07	551.75	0.000
AA pool	1918.51±26.98	1847.58±23.72^{NS}	1579.74±26.41	1140.04±42.57	867.37±13.74	650.80±12.60	418.82	0.000
EAs	653.02±10.66	646.52±10.54^{NS}	525.80±10.71	426.53±19.98	348.56±8.96	258.64±8.24	203.22	0.000
NEAs	1168.02±21.23	1075.54±10.83^{NS}	959.10±14.01	648.79±20.97	468.37±6.50	354.36±7.29	611.56	0.000
BCAs	395.33±4.66	362.59±4.51	238.48±4.83	206.73±9.20	181.02±2.93	135.97±2.09	316.78	0.000
AAs	49.13±2.32	66.49±1.96	89.38±2.52	62.29±3.84	39.83±1.01	29.61±0.48	101.66	0.000
SCAs	90.83±1.45	107.94±1.65	137.78±4.20	98.15±5.58^{NS}	72.79±2.35	56.28±0.31	99.47	0.000
EAs/NEAs	0.60	0.60	0.60	0.66	0.74	0.73		
MI (Glycine/Alanine)	0.75	0.88	0.65	0.60	0.28	0.25		

N o t e. AAs — amino acids, EAs — essential amino acids, NEAs — nonessential amino acids, BCAs — branched-chain amino acids, AAs —aromatic amino acids, SCAs — sulphur-containing amino acids, MI — иmaturity indicator.

^{NS} No statistically significant differences from control (at $p > 0.05$).

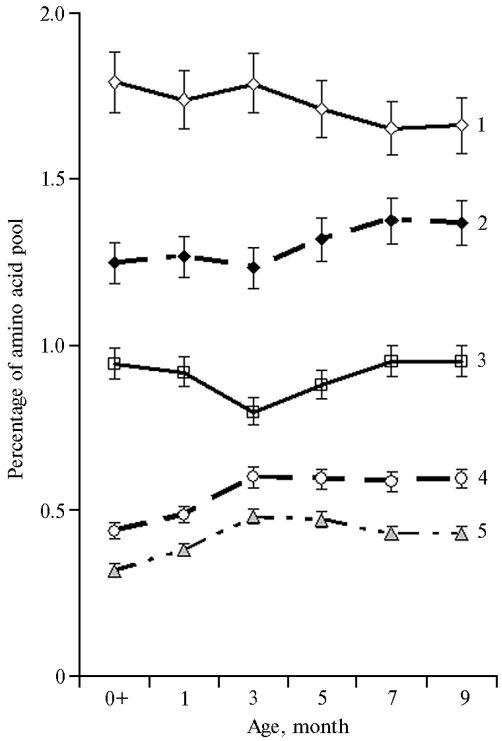

Fig. 1. Age-dependent levels of metabolic groups of amino acids in the tissues of medical leech *Hirudo verbana* Carena, 1820 grown in hirudoculture: 1 — nonessential amino acids, 2 — essential amino acids, 3 — branched-chain amino acids, 4 — aromatic amino acids, 5 — sulfur-containing amino acids ($n = 30$, $M \pm$SEM).

The growth and development of *H. verbana* for 9 months were accompanied by a significant increase in the percentage of tissue valine, methionine, serine, tyrosine, threonine, cysteic acid, cysteine, phenylalanine ($r = 0.55$-0.90), total EAs ($r = 0.86$), BCAs ($r = 0.60$) and SCAs ($r = 0.83$) ($p < 0.05$). As leeches grew, there was a significant decrease in the percentage of tissue arginine, aspartic acid, glycine, glutamate and glutamine, isoleucine, leucine, lysine, ornithine, proline, taurine, tryptophan, and citrulline (r from –0.38 to –0.96) and NEAs pool ($r = -0.85$) ($p < 0.05$). The age specificity of the amino acid content (% of the pool) in the tissues of *H. verbana* was clearly detected using the method of principal component analysis (Fig. 2, Table 2). Figure 2 shows the spatial differentiation of the studied age groups of medicinal leeches according to their physiological needs for individual amino acids. For 7- and 9-month-old leeches, there was an obvious identity of the amino acid composition of tissues.

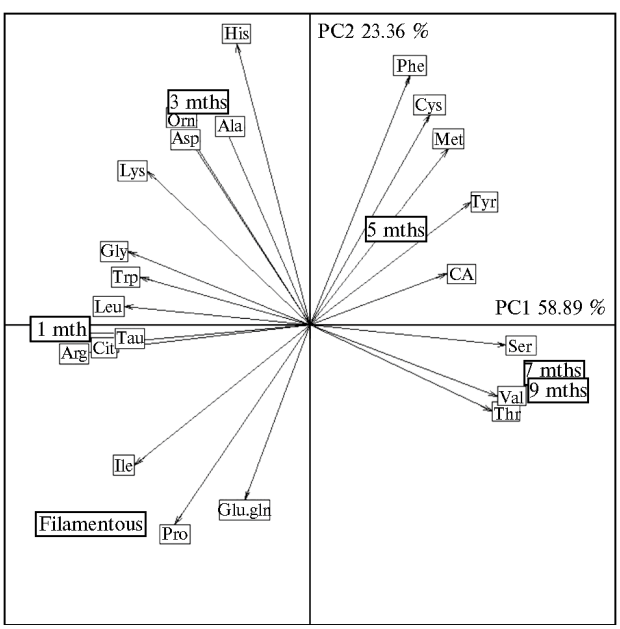

Fig. 2. The PCA analysis of age-dependent amino acid profiles in tissues of medical leech *Hirudo verbana* Carena, 1820 grown in hirudoculture: CA — Cysteic acid, Tau — taurine, Asp — aspartic acid, Thr —

threonine, Ser — serine, Glu.gln — glutamate + glutamine, Pro — proline, Gly — glycine, Ala — alanine, Cit — citrulline, Val — valine, Cys — cysteine, Met — methionine, Ile — isoleucine, Leu — leucine, Tyr — tyrosine, Phe — phenylalanine, Trp — tryptophan, Orn — ornithine, Lys — lysine, His — histidine, Arg — arginine. The arrows indicate the correlations of amino acids with the principal components (n = 30).

The first principal component (PC1, 58.89 % of the total variance) was closely related to amino acids which contribute over 6.02 % of the total variance to age differences, namely, taurine, threonine, serine, glycine, citrulline, valine, leucine, and arginine ($p < 0.001$). The largest contribution to the second principal component (PC2, 23.36 % of the total variance) was made by aspartic acid, proline, alanine, cysteine, phenylalanine, ornithine, and histidine ($p < 0.001$) (see Fig. 2, Table 2), the percentage of which in the leech tissues showed a low age-related variability.

2. Component analysis of the age-dependent concentration of free amino acids (% of the total pool) **in tissues of medical leech *Hirudo verbana* Carena, 1820 grown in hirudoculture** (n = 30)

Amino acid (i = 22)	Loadings, a_{ij}		Contribution = $(a^2_{ij} \times 100)/\lambda_j$, %	
	1	2	1	2
Cysteic acid	0.70***	0.16	3.81	0.52
Taurine	–0.95***	–0.05	6.98	0.04
Aspartic acid	–0.58***	0.63***	2.63	7.74
Threonine	0.91***	–0.29	6.41	1.61
Serine	0.97***	–0.08	7.29	0.12
Glutamate + Glutamine	–0.35	–0.57**	0.93	6.33
Proline	–0.69***	–0.64***	3.67	8.00
Glycine	–0.90***	0.25	6.31	1.18
Alanin	–0.44*	0.70***	1.49	9.65
Citrulline	–0.96***	–0.06	7.09	0.07
Valine	0.94***	–0.24	6.79	1.10
Cysteine	0.59***	0.67***	2.72	8.68
Methionine	0.69***	0.56**	3.64	6.05
Isoleucine	–0.88***	–0.44*	5.98	3.84
Leucine	–0.93***	0.07	6.72	0.09
Tyrosine	0.80***	0.38*	4.90	2.87
Phenylalanine	0.50**	0.80***	1.95	12.37
Tryptophan	–0.83***	0.17	5.35	0.56
Ornithine	–0.64***	0.64***	3.19	7.95
Lysine	–0.81***	0.50**	5.10	4.94
Histidine	–0.36	0.91***	1.02	16.10
Arginine	–0.88***	–0.09	6.03	0.17

N o t e. 1 — PC1, 2 — PC1 (Principal Components; j = 1, 2). Eigenvalues (λ_j) PC1 = 12.95, PC2 = 5.14. PC1 explains 58.89 % of the variance, PC2 explains 23.36 % of the variance.
*, **, *** Statistically significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Our findings disclosed the role of an increased pool of amino acids involved in the regulation of antioxidant metabolic processes due to which the body resistance to hypoxia increases. The antitoxic effect lasts for a 5-month period of growth. High amino acid metabolism during early ontogenesis was also established for other aquatic organisms, e.g., for brackishwater clam *Corbicula japonica*, starry flounder *Platichthys stellatus*, and small-scaled pacu *Piaractus mesopotamicus* [9, 15, 16].

Noteworthy is the high variability in nonessential and essential amino acid levels which is due to the increased requirements for carbohydrate, lipid metabolism and protein synthesis during active growth and development of leeches. This is consistent with the available data both for medicinal leeches and for planktonic species of copepods in which, with age, the tissue amino acid profile changes significantly showing the elevated level of tyrosine involved in the synthesis of hormones [8]. A high requirement for essential amino acids and taurine was found in fry of small-scaled pacu *Piaractus mesopotamicus* [15]. In the larvae of starry

flounder *Platichthys stellatus*, the leucine and isoleucine levels were higher compared to mature fish [16). In mollusks *Corbicula japonica* used for the industrial production of biologically active food additives, the pool of free amino acids depends significantly on the stage of development and, like in medicinal leeches, glutamic acid playing a key role in nitrogen metabolism is the dominant tissue amino acid during the whole life cycle [9].

Our findings revealed a balanced set of essential AAs in *H. verbana*, indicative of the suitability of habitat conditions at different periods of the leech life cycle. A peculiarity of the studied hirudinids is the optimal amino acid balance, as evidenced by the stable EAs/NEAs values during the early ontogenesis (EAs/NEAs = 0.60) and in 9-month-old leeches (EAs/NEAs = 0.73).

In accordance with the available publications, the age-related variability of the amino acid metabolism of aquatic organisms is due to their needs for priority nutrients, and the amino acid composition of tissues and organs objectively reflects the physiological state of individuals at different stages of the life cycle [1, 6, 10], as it is also found out in our research.

Thus, there is a close relationship between the quantitative and qualitative indicators of free amino acids (AAs) in the tissues of the medical leeches *Hirudo verbana* Carena, 1820 and their growth and development, which is due to the multifunctional role and the involvement of AAs in the metabolic processes at different stages of ontogenesis. The informativeness and adequacy of the biochemical parameters in assessing physiological state and adaptive capabilities of medical leeches in aquaculture allows us to suggest these indicators for assessing the physiological status of the leeches during cultivation, in final product of hydroculture, and in the natural environment. This draws us to put forward the concept of using functional AAs as biomarkers in the development of industrial technologies for cultivation of aquaculture objects. Experimental confirmation of the complete balanced set of free AAs characteristic of the tissues of medicinal leeches serves as an additional indication for the use of leech homogenates in medicine, pharmaceuticals and veterinary medicine.

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