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Blood Serum Amino Acid Pool of European Migratory Chiroptera *Vespertilio murinus* Linnaeus, 1758 and *Pipistrellus nathusii* Keyserling et Blasius, 1839 of the Ural Fauna

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Abstract—The pool of free amino acids (AAs) in the blood serum was studied in two European migratory bat species, *Vespertilio murinus* and *Pipistrellus nathusii*, of the Urals. Bats from this year's bloods were examined, and significant differences were observed in main metabolic groups of free AAs, including glycogenic (GGAAs), nonessential (NEAAs), essential (EAAs), and sulfur-containing (SCAAs) AAs ($p < 0.05$). Based on the percent content of the metabolic groups in the total AA pool, GGAAs (79.7%) and EAAs (49.4%) were found to predominate in *P. nathusii*, and GGAAs (74.9%) and NEAAs (58.4%), in *V. murinus.* No difference in AAA and BCAA contents was observed between *V. murinus* and *P. nathusii* ($p > 0.05$). The migratory species were shown to significantly differ in the metabolic groups of serum AAs from the resident species *Myotis dasycneme* ($p < 0.05$).

Keywords: bats, migratory and resident bat species, amino acids

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INTRODUCTION

The chiropteran faunistic complex of the Ural region includes the typical European species particolored bat *Vespertilio murinus* and Nathusius' pipistrelle *Pipistrellus nathusii*, which stay in the region with its continental climate over a short summer and migrate seasonally from their summer to winter habitats [1, 2]. Maternity roosts of *V. murinus* and *P. nathusii* are geographically associated with northeastern and eastern habitats, while migrating populations of the species move south and southwest to their wintering sites [3–6]. The migration distance can reach 3000–4000 km [7, 8]. Ecological and biogeographical factors are main drivers of migration, the set including seasonal changes, a spatiotemporal resource distribution, environmental factors due to global changes, predation, and competition [9]. Extreme climatic fluctuations alter the phenology of migratory behavior and greatly affect the survival and reproduction in bats [10]. The migration period is among the most important physiological challenges because higher energy consumption is required for a migration strategy in animals [11].

Studies of the ecological and physiological processes that sustain the migration strategy and stable adaptation of migratory bat species to biotic and abiotic environmental factors are of immense importance for solving the problems of biodiversity preservation and far-sighted use of animal resources, as well as for a long-term monitoring of the sizes and stability of bat populations. We have shown previously that responses to a broad range of environmental factors are characteristic of bats as well as of other vertebrates with a sufficient development of the circulatory and immune systems [12, 13]. The amino acid (AA) pool protects the body from damage and sustains energy and substance metabolism, and its optimal state is of special importance for maintaining homeostasis. In view of this, the objective of this work was to study the free AA pool of the blood in migratory bat species.

MATERIALS AND METHODS

Bats ($n = 34$) were captured and housed in the lab in compliance with the international principles of the Helsinki Declaration requiring that the welfare of animals used for experimental and scientific purposes be respected [14]. Protocols of studies with experimental animals were approved by the Ethics Committee at the Institute of Plant and Animal Ecology (protocol no. 11 dated April 29, 2022). Bats were captured with mist nets in the second decade of July (a brooding season)

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Table 1. Contents of AA metabolic groups (%) and concentrations of free AAs (μmol/L) in the blood serum in bats

AA, %	I. *V. murinus*	II. *P. nathusii*	III. *M. dasycneme*	Permutation ANOVA Permutation Tukey's		
	$\bar{X}_{boot} \pm SE_{boot}$ [95% CI_{boot}]			I–II	I–III	II–III
Glycogenic AAs (GGAAs)	74.89 ± 1.02 [72.85–76.79]	**79.69 ± 0.4*** **[78.95–80.5]**	64.01 ± 2.9*▲ [57.51–69.81]	$Pr(\|F_{ran}\| \geq 20.77) = 0.0001$		
				0.01	0.01	0.02
Essential AAs (EAAs)	**58.36 ± 1.34 [55.7–60.86]**	44.37 ± 2.24* [40.55–49.04]	47.93 ± 2.73* [41.84–53.43]	$Pr(\|F_{ran}\| \geq 14.07) = 0.001$		
				0.002	0.01	0.42
Nonessential AAs (NEAAs)	32.02 ± 1.65 [28.84–35.26]	**49.42 ± 2.97*** **[43.43–54.66]**	37.01 ± 0.97 [35.07–39.17]	$Pr(\|F_{ran}\| \geq 15.29) = 0.001$		
				0.002	0.15	0.03
Sulfur-containing AAs (SCAAs)	8.99 ± 0.53 [7.95–10.04]	6.02 ± 0.67* [4.56–7.17]	14.44 ± 1.81*▲ [10.75–18.46]	$Pr(\|F_{ran}\| \geq 14.43) = 0.0001$		
				0.01	0.02	0.02
Branched-chain AAs (BCAAs)	6.43 ± 1.01 [4.55–8.5]	8.51 ± 1.15 [4.97–12.02]	15.18 ± 0.56*▲ [14.05–16.42]	$Pr(\|F_{ran}\| \geq 12.13) = 0.002$		
				0.23	0.01	0.02
Aromatic AAs (AAAs)	4.75 ± 0.64 [3.53–6.02]	3.03 ± 0.46 [2.15–3.97]	7.92 ± 0.68*▲ [6.54–9.44]	$Pr(\|F_{ran}\| \geq 8.17) = 0.01$		
				0.11	0.05	0.02
Free AA pool, μmol/L	1488.5 ± 161.7 [1206.8–1834.3]	2701.3 ± 555.4 **[1614.7–3781.7]**	934.7 ± 67.7*▲ [790.4–1079.0]	$Pr(\|F_{ran}\| \geq 4.83) = 0.02$		
				0.05	0.05	0.04

Differences between (*) I and II or I and III or (▲) between II and III were significant at $p < 0.05$; $\bar{X}_{boot} \pm SE_{boot}$ are the arithmetic mean and error of the mean of the bootstrap distribution; [95% CI_{boot}] is the confidence interval of the bootstrap distribution.

on the banks of the Bol'shoi Kisegach Lake (Chelyabinsk oblast).

Our sample included bats from this year's bloods of two migratory species, the parti-colored bat *V. murinus* and Nathusius' pipistrelle *P. nathusii*, which are characterized by long-distance seasonal migrations. The latter is a migratory species that inhabits the forest and forest–steppe zones of the Southern Urals and is widespread in the region; *V. murinus* is also a migratory species and is widespread in the Urals [1]. Comparisons were performed with the pond bat *Myotis dasycneme* Boie, 1825, which is a residential species and form large brooding colonies in the Middle and Southern Urals [1]. Bats without signs of disease were transferred in individual container to the lab on the capture day.

Given the functional role of free AAs in the regulation of main metabolic processes involved in protein, nucleic acid, and hormone biosyntheses and immunological processes [15], evaluation of the AA pool in bat tissues makes it possible to understand the role that the AA pool plays in adaptive potential of migratory species exposed to continuous climatic fluctuations in their habitats. Sample preparation and free serum AA assays by ion exchange liquid chromatography run on an AAA-339M automated analyzer (Microtechna, Czech Republic) were carried out as described previously [16]. Total concentrations and percent contents were calculated for the essential AAs (EAAs) alanine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, serine, tyrosine, cysteine, and proline; nonessential AAs (NEAAs) threonine, valine, lysine, leucine, isoleucine, methionine, phenylalanine, arginine, histidine, and tryptophan; glycogenic AAs (GGAAs) glycine, threonine, glutamic acid, glutamine, alanine, arginine, histidine, serine, valine, asparagine, aspartic acid, cysteine, methionine, tryptophan, and proline; sulfur-containing AAs (SCAAs) cysteine, methionine, and taurine; branched-chain AAs (BCAAs) valine, leucine, and isoleucine; and aromatic AAs (AAAs) phenylalanine and tyrosine. In total, 384 amino acid samples were examined. The results were processed using the software package Statistica for Windows v. 10.0. Principal component analysis (PCA) was carried out in the R statistical environment (R 3.1.2, the Ade4 package) [17].

RESULTS AND DISCUSSION

Qualitatively, there were 22 AAs in the AA spectrum of the blood serum in the bat species under study. The total concentrations of EAAs and NEAAs did not significantly differ between subadult females and males ($p < 0.05$). The total free AA concentration of the blood serum in migratory *P. nathusii* was 2701.3 ± 555.4 μmol/L, 1.8 times higher than in *V. murinus* (1488.5 ± 161.7 μmol/L) and 2.9 times higher than in residential *M. dasycneme* (934.7 ± 67.7 μmol/L) (Table 1). A greater AA pool provides substrates for metabolic processes and plays a role in the formation of the immune system in bats migrating over long dis-

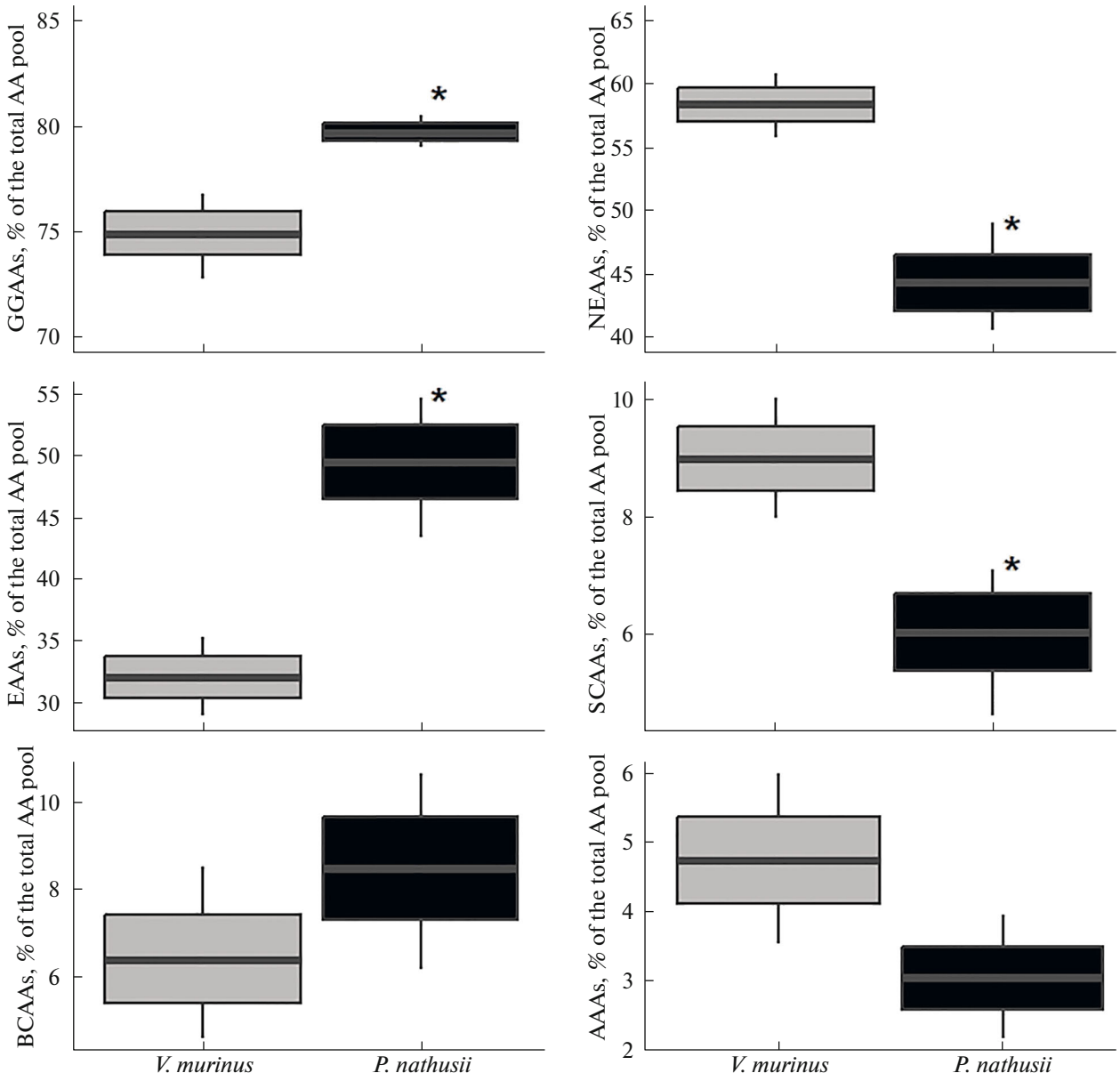

Fig. 1. Contents of the AA metabolic groups (% of the total AA pool) in the blood serum in the migratory bat species. The middle of a box shows the arithmetic mean, the boundaries show the error of the mean, and the whiskers show the confidence interval of the bootstrap distribution. (*) Differences between *V. murinus* and *P. nathusii* were significant.

tances. When the contents of the AA metabolic groups were calculated in percent of the total AA pool, GGAAs (79.7%) and EAAs (49.4%) were found to be the most prevalent in *P. nathusii* and GGAAs (74.9%) and NEAAs (58.4%), in *V. murinus* compared with *M. dasycneme* (Table 1).

The SCAA percent content also showed a significant interspecific difference between *P. nathusii* and *V. murinus* ($p = 0.01$) (Fig. 1). The BCAA ($p = 0.23$) and AAA ($p = 0.11$) serum contents did not significantly differ between the two species. These AAs provide an additional energy source, possess immunomodulatory properties, and act as energy substrates in syntheses of biologically active compounds and mediators. The finding reflects the similarity of specific metabolic pathways of migratory bats (Fig. 1). High concentrations of metabolically active GGAAs in *P. nathusii* (2147 μmol/L) and *V. murinus* (1114 μmol/L) reflect that energy substrates are necessary for migratory bats to maintain homeostasis, which ensures hormonal support of the growth, development, and reproductive functions in animals (Fig. 1). The GGAAs lysine and glycine are necessary for growth hormone stimulation and faster wound healing, and their total contents in the migratory species (323.3 μmol/L in *V. murinus* and 394.6 μmol/L in *P. nathusii*) were more

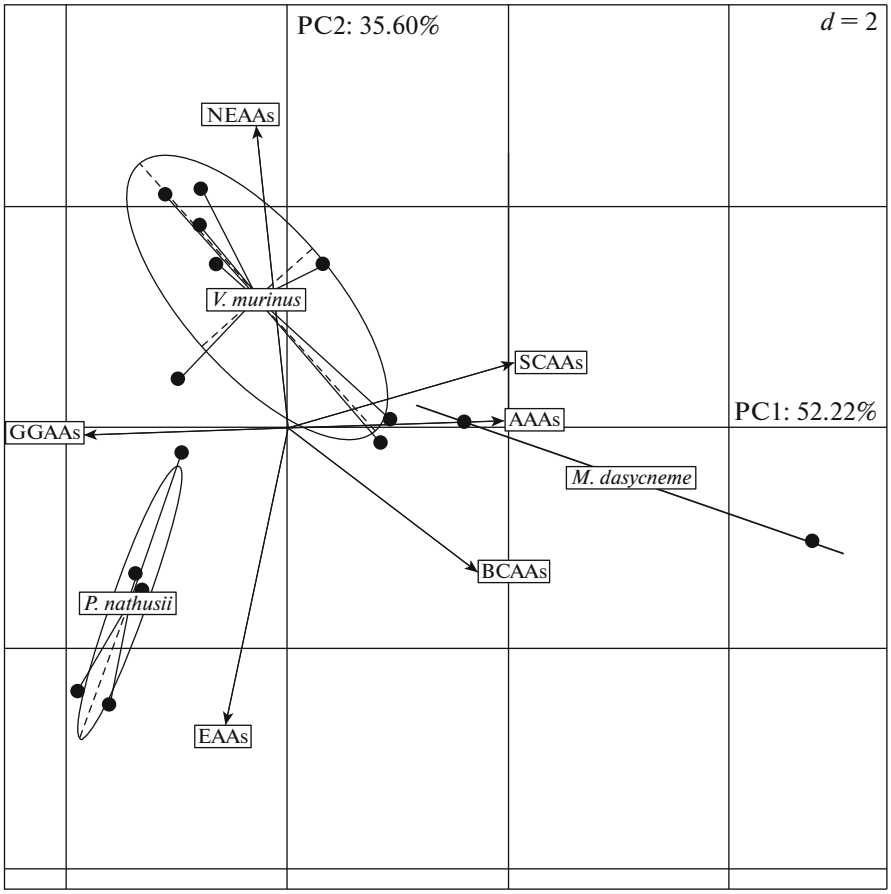

Fig. 2. Metabolic groups of serum AAs (% of the total AA pool) of the bats in the space of the two first principal components. PC1 and PC2 are the principal component axes, % is the percentage of data variance explained by the principal components; arrows reflect the correlations of the initial variables with PC1 and PC2; ellipses show the 95% confidence areas.

than twice higher than in residential *M. dasycneme* (163.7 μmol/L). Lysine acts as an antioxidant and is involved in the regulation of immune functions and detoxification of xenobiotics [15]. The tripeptide glutathione is involved in detoxification of metabolic products, and the total content of the three AAs (glycine, glutamic acid, and cysteine) involved in its synthesis in migratory bats has been found to be twice higher than in bond bat [18].

It should be noted that the full spectrum of functionally significant EAAs was observed in the bats. Compared with *V. murinus*, *P. nathusii* showed a 2.6 times higher isoleucine content, a 3.1 times higher tryptophan content, an 8.0 times higher arginine content, a 3.7 times higher valine content, and a 2.2 times higher leucine content. The BCAAs valine, isoleucine, and leucine are involved in protein synthesis, facilitate restoration of bone and muscle tissues, and sustain metabolic processes in muscles during long flights [15]. The serum threonine contents in the migratory species *V. murinus* (103.2 μmol/L) and *P. nathusii* (130.8 μmol/L) were 2.1–2.6 times higher than in *M. dasycneme* (49.4 μmol/L). Threonine is involved in collagen and elastin syntheses and thus provides for complex restoration of damaged muscle fibers. The serum arginine content in *V. murinus* (84.3 μmol/L) was six times higher than in *M. dasycneme* (14.4 μmol/L), and arginine (686.2 μmol/L) accounted for 25.4% of the total free AA pool in *P. nathusii*. The EAA arginine increases the muscle mass and reduces the fat volume, acting to normalize the connective tissue state in bats during preparation to long-distance seasonal migration [19]. A higher arginine content is accompanied by stimulation of phagocytic activity of neutrophils and production of certain cytokines [20]. Tryptophan similarly occurred at higher serum concentrations in *V. murinus* and *P. nathusii*. Tryptophan affects carbohydrate metabolism and modulates the immune functions by acting through several metabolites, including serotonin and melatonin [15, 19]. Along with the above similarities in metabolism of functionally important AAs in maintaining homeostasis, certain opposite changes were observed in the mobilization of emergency mechanisms that regulate the AA pool in the migratory and residential bat species. Based on the literature data and our findings, it is possible to assume that biotic and

abiotic environmental factors determine the formation of the pool of AAs and their derivatives in bats.

Multivariate analysis (PCA) of plasma parameters makes it possible to visualize the contents of free AA metabolic groups, which modify the main metabolic flows in the three bat species of the Ural fauna. PCA supported the above results (Fig. 2). With a 95% confidence interval, principal component 1 (PC1) was found to account for 52.2% of the total variance in the metabolic groups of the total AA pool found in the bat plasma, and principal component 2 (PC2), for 35.6% of the variance (Fig. 2). With the variables involved, PC1 and PC2 ensure significant spatial differentiation of the main AA metabolic groups in *V. murinus* and *P. nathusii* compared with *M. dasycneme*. PC1 separates a *M. dasycneme* cluster from a group of *V. murinus* and *P. nathusii*. High coefficients of correlation with PC1 and significant contributions to PC1 were demonstrated for GGAAs (31.44%), SCAAs (25.2%), AAAs (23.20%), and BCAAs (17.80%) (Fig. 2).

A high percent content of GGAAs was mostly observed in *V. murinus* and *P. nathusii* (Fig. 2). Significant contributions to PC2 were detected for NEAAs (44.36%) and EAAs (43.39%); their coefficients of correlation with PC2 were sufficiently high. PC2 distinctly separates the groups of *V. murinus* and *P. nathusii*. NEAAs were found to prevail in the blood serum in *V. murinus*; EAAs, in *P. nathusii* (Fig. 2).

Our study of genetically determined modulations in free AAs as a necessary energy and constructive pool in the blood serum of bats showed that substantial reserves are characteristic of nitrogen metabolism in the migratory species *V. murinus* and *P. nathusii*. GGAAs and EAAs proved to play a key role, facilitating a high resistance to seasonal variation that occurs during long-distance transcontinental migration as part of the annual life cycle.

It is possible to assume that the species-specific features of the free AA metabolic groups in the serum sustains the migration strategy in *V. murinus* and *P. nathusii*, facilitating their adaptive resistance and survival during long seasonal flights and promoting successful colonization of new habitats.

The results obtained in our study of the AA spectrum of the blood serum in *V. murinus* and *P. nathusii* can be used as a reference to monitor the populations of the migratory bat species and to use the bats as bioindicator species.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Animals were captured and kept in the lab in compliance with guidelines of the Declaration of Helsinki on the protection of animals used for experimental and scientific purposes [15]. Animal studies were approved by the Ethics Committee at the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences (protocol no. 11 dated April 29, 2022).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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