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Free amino acid concentration in serum and trapezius muscle from male and female silver foxes (*Vulpes vulpes*)

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Abstract: Serum and muscle concentrations of 29 amino acids were determined in Silver fox. Serum concentrations of proline, alanine, tyrosine and aromatic amino acids were significantly higher in males than in females (all $P = 0.05$). Taurine and glycine concentrations in skeletal muscles were significantly higher in males than in females ($P < 0.01$). Muscle concentrations of cysteic acid, taurine, aspartate, threonine, serine, glycine, alanine, citruline, valine, leucine, gamma-amino-butyrate, ethanoloamine, lysine and histidine were significantly higher than in serum in both sexes ($P < 0.05$). In females, the concentrations of glutamate, glutamine, cystathionine, isoleucine, tyrosine, phenylalanine, arginine and amino-adipic acid were significantly higher in muscles than in serum ($P < 0.05$). Tryptophan concentration was significantly higher in serum from males than in muscles ($P = 0.01$). The concentration of branched-chain amino acids in skeletal muscles was approximately two times higher than in serum in both groups of foxes ($P \leq 0.01$). Similar differences were obtained for aromatic amino acids in females ($P = 0.04$). The elaborated experimental model may serve for further studies focused on amino acid metabolism regulation in *Canide* and other monogastric mammals, especially with the use of environmental, dietary, pharmacological and toxicological factors. The elaborated experimental model may be an attractive alternative to replace some experiments on dogs.

Keywords: amino acids; animal model; ion-exchange chromatography; Carnivora; predator

The order Carnivora includes 274 animal species in 11 families (Van Valkenburgh 2007). Carnivorans first appeared in the Paleocene, about 63 million years ago and diverged into two major branches, the Caniformia and the Feliformia (Flynn 1998). The Carnivora order is characterized by the presence of carnassial teeth, a blade-like upper fourth premolar that occludes with a partially bladed lower first molar in a scissor-like action. The anatomical structure of teeth in Carnivora ensures effective hunting skills, biting of the other animal species and their tissue fragmentation. The Caniformia includes the canids represented by dogs, wolves, foxes, coyotes and jackals (Van Valkenburgh 2007). Silver fox (*Vulpes vulpes*) is a domesticated animal kept mainly for valuable fur. Its final quality depends on nutrition ensuring proper systemic growth and development and health status of animals. Proteins are considered as basic nutrients and are built of 10 essential (exogenous) and 10 non-essential (endogenous) amino acids in mammals. Animal-origin food preferred by Carnivora provides rich source of proteins and amino acids in comparison to plant-origin food. The species belonging to the Carnivora order occupy a high position in the food chain pyramid and accumulate ingested substances

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in the body (Kidawa and Kowalczyk 2011; Lanocha et al. 2012). The silver fox anatomy and physiology is similar to that of the dog in many characteristics. Silver fox is a monogastric predator and it may be utilized in many experimental studies as an alternative animal species to companion animals (dogs and cats), and a more appropriate experimental model than studies on cell cultures (*in vitro*), invertebrates and polygastric animals including sheep, goats and cows (Zhan et al. 1991; Tatara et al. 2014, 2018). Experimentations on foxes may provide numerous advantages in comparison to other animal models. In rodents, difficulties of serial blood collection, small size of blood vessels and whole body, and existing differences in gastrointestinal tract anatomy, digestion and absorption processes make these experimental models less attractive (Takeshita et al. 1997; Lu et al. 2010; Jing et al. 2012). Moreover, data interpretation from metabolic and nutritional studies in rodents cannot be simply interpolated to monogastric humans, consuming different type of food. Sexual and skeletal maturity in silver fox is reached at the age of 7–8 months, i.e. earlier than in polar foxes (*Alopex lagopus*) reaching final somatic maturation at the age of 9–11 months (Piotrowska et al. 2008). There is very limited information available concerning free amino acid concentration in different tissue compartments in Silver fox.

Thus, the aim of the current study was to determine physiological free amino acid concentrations in serum and skeletal muscle compartments in 8-month-old silver foxes. Moreover, the existence of possible sex-related differences of free amino acid concentrations as well as the differences of the amino acid concentrations between both these compartments were evaluated at this developmental stage in silver foxes.

MATERIAL AND METHODS

The experimental procedures used in this study were approved (permission number 20/2015) by the Local Ethics Committee on Animal Experimentation of the University of Life Sciences in Lublin, Poland.

Experimental design and sampling procedure. The study was performed on 8-month-old silver foxes (*Vulpes vulpes*) including males (n = 7) and females (n = 8). The animals were kept in pens under standard rearing conditions on commercial fox farm. The weaning procedure was performed at the age of 10 weeks of life. Two foxes were kept in single cage (1.0 × 1.1 m). All animals were provided with drinking water *ad libitum* and fed twice a day a standard diet. The diet was identical for males and females and it was based mainly on animal-origin waste products enriched with vitamin-mineral premix (Tatara et al. 2018). At the age of 8 months of life, the animals were sacrificed using electrical shock to remove skull on cervical spine level. Before electrical stunning, the blood samples were collected for serum using venipuncture of the *vena saphena lateralis* and after centrifugation, the obtained serum samples were kept frozen in 1.5 ml tubes at –25°C. Moreover, trapezius muscle samples (approximately 2 g of tissue without *fascia muscularis*) were collected for analysis after skin and subcutaneous tissue removal from skull and stored in Eppendorf tubes frozen at –25°C.

Determination of amino acid concentration in serum and skeletal muscles. Determination of free amino acid concentration in serum and skeletal muscles was performed using the ion-exchange chromatography method on an INGOS AAA-400 apparatus for automatic analysis of amino acids (Ingos Corp., Czech Republic). 1 ml serum samples from male and female foxes were homogenized in 1.0 ml of 6.0% sulphosalicylic acid and buffered to pH 2.9. The obtained samples were centrifuged for 15 min at 12 000 rpm and the resulting supernatants were used for automatic determination of the concentration of free amino acids (Tatara et al. 2014). To perform evaluation of free amino acid concentration in muscles, the trapezius muscle samples from each fox were prepared as 1.0 g samples and homogenized in 10 ml of 6.0% sulphosalicylic acid and buffered to pH 2.9. The homogenized skeletal muscle samples were centrifuged 15 min at 12 000 rpm. The obtained supernatant was used for the determination of free amino acid concentration (Golynski et al. 2016). An analytic column OSTION LG FA 3 mm × 200 mm was used for the separation of amino acids together with five lithium cytrate buffers (pH 2.90, 3.10, 3.35, 4.05 and 4.90, respectively). The amino acids were derivatized with ninhydrin and their identification using photocell combined with a computer was performed on the basis of retention time in comparison to the standards supplied by Ingos Corp. The original software MIKRO Version 1.8.0 (INGOS Corp.) was used for amino acid evaluation. Tyrosine (Tyr), phenylalanine (Phe) and tryptophan (Trp) were

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evaluated as aromatic amino acids (AAAs) while valine (Val), isoleucine (Ile) and leucine (Leu) as branched-chain amino acids (BCAAs). Moreover, the values of the muscle : serum ratio of amino acid concentrations were determined.

Statistical analysis. All data are presented as means ± SEM. Statistical analysis of the results was performed using STATISTICA software (Version 6.0). The comparison of mean values of the investigated variables between males and females was performed using non-paired Student's *t*-test for non-dependent variables. The comparison of mean values of the investigated amino acids between serum and skeletal muscle compartments within each sex group was performed using paired Student's *t*-test for dependent variables. *P*-value ≤ 0.05 was considered as statistically significant for all comparisons.

RESULTS

Body weight in male foxes reached 8.9 ± 0.2 kg and was significantly higher when compared

Table 1. Free amino acid concentration in serum (nmol/ml) from 8-month-old female and male silver foxes

Amino acid	Females	Males	*P*-value
Cysteic acid	9.62 ± 1.89	14.71 ± 3.25	0.206
Taurine	330 ± 83	245 ± 39	0.375
Aspartate	37.5 ± 4.85	36.29 ± 6.29	0.881
Threonine	156 ± 7.0	188 ± 28	0.299
Serine	196 ± 16	263 ± 35	0.118
Asparagine	19.0 ± 10.9	20.00 ± 9.3	0.945
Glutamate	120 ± 34	–	–
Glutamine	1040 ± 63	–	–
Proline	118 ± 38	462* ± 145	0.056
Glycine	364 ± 19	569 ± 143	0.203
Alanine	431 ± 49	575* ± 50	0.058
Citruline	29.0 ± 4.9	40.1 ± 5.6	0.163
Cystathionine	4.14 ± 0.82	9.67 ± 4.68	0.295
Cystine	5.50 ± 2.64	18.25 ± 9.62^	0.280
Methionine	50.4 ± 7.7	67.1 ± 14.4	0.329
Valine	187 ± 13	197 ± 26	0.737
Isoleucine	67.6 ± 10.5	94.1 ± 20.9	0.286
Leucine	122 ± 16	151 ± 21	0.282
Tyrosine	59.4 ± 8.6	83.6* ± 7.6	0.054
Phenylalanine	68.9 ± 9.0	87.6 ± 9.9	0.186
Gamma-amino-butyrate	3.63 ± 1.32	7.20 ± 2.26	0.216
Ethanoloamine	58.5 ± 11.4	48.3 ± 11.4	0.555
Ornithine	75.6 ± 26.5	73.4 ± 21.6	0.949
Lysine	107 ± 7.4	132 ± 19.3	0.252
Histidine	67.6 ± 6.1	84.7 ± 11.9	0.232
Arginine	100 ± 12	157 ± 32	0.140
Hydroxyproline	54.5 ± 22.0	238.5 ± 109.1	0.155
Amino-adipic acid	10.43 ± 2.65	9.17 ± 2.85	0.752
Alpha-amino-butyrate	46.9 ± 8.4	56.6 ± 8.6	0.433
Tryptophan	34.4 ± 8.5	71.7 ± 17.1	0.083

*$P \leq 0.05$

^less than $n = 6$

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Table 2. Free amino acid concentration (nmol/g) in trapezius muscle from 8-month-old female and male silver foxes

Amino acid	Females	Males	*P*-value
Cysteic acid	227 ± 18	227 ± 13	0.980
Taurine	6 196 ± 1 302	23 137*** ± 318	< 0.001
Aspartate	1 047 ± 76	1 079 ± 66	0.756
Threonine	525 ± 32	523 ± 45	0.971
Serine	921 ± 70	1 086 ± 77	0.139
Asparagine	71 ± 49	60 ± 27	0.849
Glutamate	2 822 ± 490	3 695 ± 216	0.158
Glutamine	18 724 ± 1 913	24 562 ± 2 353	0.083
Proline	443 ± 222	–	–
Glycine	2 532 ± 252	3 950** ± 346	0.006
Alanine	5 371 ± 426	5 944 ± 263	0.278
Citruline	212 ± 25	225 ± 33	0.747
Cystathionine	21.0 ± 8.84^	6.66 ± 2.48	0.183
Cystine	45.0 ± 27.6^	16.2 ± 4.01^	0.374
Methionine	78.7 ± 23.1	83.5 ± 17.1	0.870
Valine	313 ± 48	326 ± 20	0.810
Isoleucine	144 ± 31	115 ± 24	0.471
Leucine	305 ± 73	298 ± 38	0.930
Tyrosine	134 ± 28	131 ± 23	0.938
Phenylalanine	152 ± 39	146 ± 40	0.908
Gamma-amino-butyrate	52.4 ± 13.7	44.6 ± 14.1	0.697
Ethanoloamine	520 ± 97	392 ± 28	0.246
Ornithine	62.2 ± 15.0	28.3 ± 6.6	0.078
Lysine	342 ± 48	276 ± 31	0.273
Histidine	276 ± 32	222 ± 57	0.429
Arginine	292 ± 76^	211 ± 40	0.397
Hydroxyproline	–	–	–
Amino-adipic acid	231 ± 89	15* ± 6^	0.050
Alpha-amino-butyrate	48 ± 11	60 ± 24	0.649
Tryptophan	24 ± 11	16 ± 5	0.502

*$P \leq 0.05$, **$P < 0.01$, ***$P < 0.001$

^less than $n = 6$

to females where 7.4 ± 0.4 kg was obtained ($P = 0.003$). Results of serum concentration of free amino acids in female and male foxes are presented in Table 1. Serum concentration of proline (Pro) was significantly higher in males by 292% when compared to females ($P = 0.056$). Serum concentration of alanine (Ala) was significantly higher in males by 33% when compared to females ($P = 0.058$). Serum concentration of Tyr was significantly higher in males by 41% when

Table 3. Free amino acid concentration (nmol/ml) in serum from 8-month-old female and male silver foxes

Amino acid group	Females	Males	*P*-value
Branched-chain amino acids	376 ± 36	442 ± 64	0.392
Aromatic amino acids	167 ± 15	243* ± 30	0.050

*$P \leq 0.05$

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Table 4. Free amino acid concentration (nmol/g) in trapezius muscle from 8-month-old female and male silver foxes

Amino acid group	Females	Males	*P*-value
Branched-chain amino acids	763 ± 125	739 ± 69	0.876
Aromatic amino acids	331 ± 76	300 ± 75	0.776

compared to females ($P = 0.054$). Results of muscle concentration of free amino acids in females and males are presented in Table 2. The concentrations of taurine (Tau) and glycine (Gly) in skeletal muscles were significantly higher in males than in females by 273% and 56%, respectively (all $P < 0.01$). Significantly higher muscle concentration of amino-adipic acid (Adi) in females was stated

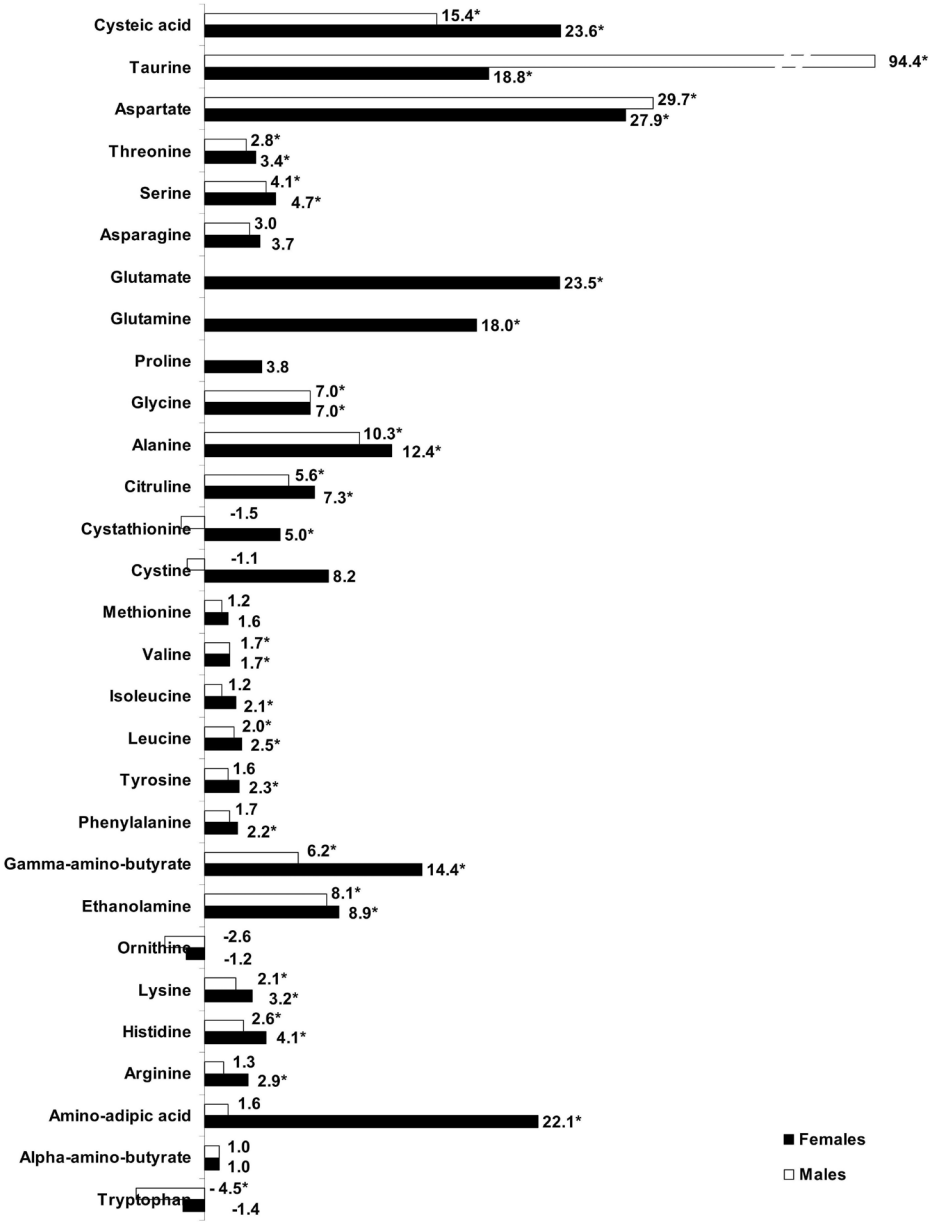

Figure 1. Values of the muscle to serum ratio of the evaluated amino acids in 8-month-old male and female silver foxes. Minus sign indicates higher amino acid concentration in serum compartment in comparison to muscles (bars on the left of the middle axis)

*statistically significant differences between skeletal muscle and serum compartments for $P < 0.05$

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when compared to males (P = 0.05), however Adi concentration was determined only in 4 samples in males. The concentrations of cysteic acid (Cya), Tau, aspartate (Asp), threonine (Thr), serine (Ser), glutamate (Glu), glutamine (Gln), Gly, Ala, citruline (Cit), cystathionine (Cth), Val, Ile, Leu, Tyr, Phe, gamma-amino-butyrate (GABA), ethanolamine (Eta), lysine (Lys), histidine (His), arginine (Arg) and Adi in trapezius muscle in females were significantly higher when compared to their concentrations in serum (all $P < 0.05$). The concentrations of Cya, Tau, Asp, Thr, Ser, Gly, Ala, Cit, Val, Leu, GABA, Eta, Lys and His in trapezius muscle in males were significantly higher when compared to those in serum (all $P \leq 0.05$). Muscle concentration of Trp in males was significantly lower than in serum (P = 0.014). The values of the muscle : serum ratio of amino acid concentrations in foxes are shown in Figure 1. The results of serum concentrations of BCAAs and AAAs are shown in Table 3. The concentration of AAAs was significantly higher in males by 46% when compared to females (P = 0.050). No significant difference of serum concentration of BCAAs between the groups of females and males was stated (P = 0.392). The results of concentrations of BCAAs and AAAs in trapezius muscle are shown in Table 4. The obtained results did not show significant differences of the concentrations of BCAAs and AAAs between the groups of males and females ($P > 0.05$). The concentrations of BCAAs and AAAs in females were significantly higher in skeletal muscles when compared to serum ($P < 0.05$). The concentration of BCAAs in males was significantly higher in skeletal muscles when compared to serum (P = 0.008). The concentration of AAAs in males did not significantly differ in skeletal muscles and serum ($P > 0.05$).

DISCUSSION

Physiological role of free amino acids. Amino acids are defined as organic substances containing both the amino and acidic groups connected with the carbon skeleton. Analytical studies have shown the existence of over 300 various amino acids in nature; however, only 20 alpha-amino acids serve as structural elements of proteins in animals and humans. Considering relatively poor efficiency of energy transfer from amino acids to adenosine triphosphate (ATP) (between 29% for methionine (Met) and 59% for Ile), mitochondrial oxidation of glucose and free fatty acids is the main energetic source for physiological processes in the body. Mitochondrial oxidation of amino acids may result from carbohydrate and fatty reserves utilization in the body and require carbon skeleton conversion to acetyl-CoA (Wu 2009). Behind structural function of amino acids in proteins and polypeptides, their physiological roles in the body concern also regulatory functions in metabolic processes determining growth, immunity, reproduction, homeostasis maintenance, protein turnover and health status. To the most important non-structural amino acids in animals and humans belong ornithine (Orn), Cit and homocysteine (Curis et al. 2007; Perla-Kajan et al. 2007; Sikorska et al. 2010). Free amino acids are utilized as precursors for protein synthesis and determination of their concentration provides evidence on particular amino acids presence, metabolic processes rate of the proteins and the total amino acid pool. The distribution of free amino acids varies in several body compartments. The highest concentration of free amino acids was stated in the cellular compartment of skeletal muscles serving also as the main protein reservoir in the body (Filho et al. 1997). The concentration of amino acids in serum is the result of their intestine absorption as well as metabolic processes in the liver and skeletal muscles (Canepa et al. 2002). The amino acid concentration in serum is maintained at a relatively constant level in the post-absorptive state in healthy animals and humans. However, significant changes of amino acid concentration in serum may be related to different growth and development stages, lactation, catabolic conditions and disease (Flynn et al. 2000, 2009; Field et al. 2002; Manso Filho et al. 2009; Wu 2009). Sex-related differences in amino acid concentration in different tissue compartments are also possible and may be due to differences of growth rate and metabolic processes intensity, especially resulting from differentiated endocrine system functions of males and females, determined by biological actions of the androgens and estrogens (Arciero et al. 1993; Tatara et al. 2012).

Free amino acids in serum and skeletal muscles in males and females. In this study, significant sex-related differences of free amino acid concentration in serum were found in case of Pro, Ala and Tyr, while the other analyzed amino acids reached similar concentrations in males and females. The

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concentration of all these amino acids was higher in males than in females and coexists with their higher body size and weight. In this study, the body weight of males was by 25% higher than in females. Although Pro is classified as non-essential amino acid, it is also considered as functional amino acid with particular importance for growth and homeostasis maintenance processes (Hou et al. 2015). Proline and its hydroxylated form (hydroxyproline (Hyp)) are two main amino acids building collagen type I structure in bone tissue of the skeleton. In a previous study on silver foxes, higher skeletal size and bone mass was observed in males in comparison to females (Chmielowiec et al. 2014). Proline together with Lys undergo hydroxylation process to form Hyp and hydroxylysine providing structural stabilization and better mechanical endurance of the collagen fibres (Viguet-Carrin et al. 2006). Behind structural functions, Ala and Tyr play important roles in metabolic processes including gluconeogenesis, transamination, phosphorylation, nitrosation and sulfation. All these processes are crucial for energy transformation and systemic development on cellular and tissue level (Wu 2009). In skeletal muscle compartment, the concentration of Tau and Gly was significantly higher in males than in females, while Gln has shown similar tendency to be increased in males by over 31%. Glycine is an indispensable amino acid for spatial configuration of bone collagen structure and contributes to one third of the triple collagen alpha-helix (Viguet-Carrin et al. 2006). As shown in the previous and the current studies, Tau is an ubiquitous amino acid present at high concentrations in skeletal and cardiac muscles. It is considered as a relatively inert compound that modulates basic cellular processes such as osmotic pressure, oxidative stress, cation homeostasis, enzyme activity, receptor function and cell signaling and development (Schaffer et al. 2010). Dietary Tau deficiency in canides and rodents leads to developmental cardiomyopathies and disturbances in muscle contraction (Pion et al. 1987; Moise et al. 1991; Kittleson et al. 1997). Glutamine as the most abundant free amino acid in skeletal muscles plays important roles in gene expression, protein turnover, anti-oxidative defence, nutrient metabolism, immunity and acid-base balance (Xi et al. 2011). The proper course of all these processes is important for optimal systemic growth and development, including skeletal muscles. Negative protein balance in skeletal muscles due to hypercatabolic conditions such as infections, sepsis, injury, burns and cancer is associated with the depletion of intracellular and extracellular Gln concentration (Fraga Fuentes et al. 1996; Xi et al. 2011). In fast growing turkeys, a 38% higher concentration of Gln in skeletal muscle in males was associated with their higher body weight when compared to females (Tatara et al. 2012). Experimental Gln infusion to rat skeletal muscles has increased protein synthesis and has inhibited protein breakdown (MacLennan et al. 1987, 1988). Moreover, it was shown that increased extracellular Gln concentration improves protein synthesis and decreases protein degradation in dose-dependent manner in chicken skeletal muscles (Wu and Thompson 1990). In the case of Adi, its muscle concentration was significantly lower in males than in females; however, this amino acid does not contribute to physiological groups of structural and functional amino acids. Its role in living organism is related to synthesis of Lys and acetyl-CoA (Bellance et al. 2012).

In this study, the classification of the evaluated amino acids to the BCAAs and AAAs groups revealed also significantly higher concentration of AAAs in serum in male foxes. The observed sex-related differences reached 46%. In the skeletal muscle compartment, neither the BCAAs group nor the AAAs group showed differentiated concentrations in male and female foxes. These results are opposite to the observations in humans between 23 and 92 years of life where the concentration of BCAAs was significantly higher in men than in women. However, similarly to the current study and higher concentration of AAAs in serum of males, the previous studies in humans have shown significantly higher serum concentration of Tyr, Phe and Trp in men (Pitkanen et al. 2003). In the other study in humans suffering from insuline resistance (mean age of 52 years), blood concentrations of Asp, β-alanine, Cit, Glu, Pro, Leu, Ile, Val, Met, Orn, Phe, Tyr, Trp and sarcosine were significantly higher in men than in women. The only amino acid showing higher concentration in women was Gly (Seibert et al. 2015). In female chickens, plasma concentration of Ser was significantly higher than in males, while the concentration of asparagine (Asn) was higher in males (Garcia et al. 1986). Investigations of sex-related differences in skeletal muscle compartment

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in 20-week-old turkeys have shown a significantly higher concentration of Gln, Trp, Ser and Asn when compared to the group of females in which the concentrations of Cya, Tau and Val were found to be higher. As opposite to the current study on trapezius muscle model, the previous investigations were executed on breast muscle samples (Tatara et al. 2012).

Differences of amino acid concentration between serum and muscle compartments. Based on the results obtained in this study, significant differences of the most of the analyzed amino acids between serum and muscle compartments were observed. In females, the concentrations of Cya, Tau, Asp, Glu, Gln, Ala, GABA and Adi were significantly higher over 10 times (12–27 times) in skeletal muscles than in serum. In males, such comparison has shown similar scale of the differences between both these compartments for Cya, Asp and Ala. Taurine concentration in trapezius muscle was over 94 times higher than in serum. The comparison of the concentrations of Gln, Glu, Pro and Hyp for both these compartments in males was not possible due to technical difficulties. In the case of Thr, Ser, Gly, Cit, Val, Leu, Eta, Lys and His, their concentrations in males and females were significantly higher up to 9 times (1.6–8.8 times) in the skeletal muscles compartment. Similar results were found in males for the concentrations of Ctn, Ile, Tyr, Phe and Arg, as well as for GABA in females. The concentrations of Asn, Cys, Met, Orn and alpha-amino-butyrate (Aab) were not differentiated between serum and muscle compartments both in males and females. Similar findings were seen for the concentrations of Ctn, Ile, Tyr, Phe, Arg and Adi in males, as well as for Pro in females. Tryptophan was the only amino acid with its lower concentration in skeletal muscle than in serum compartment and the difference was statistically confirmed for the group of male foxes. The whole group of BCAAs has shown significantly higher concentration in skeletal muscle compartment by approximately two times when compared to serum. Similar observations were obtained in female foxes when comparing AAAs concentrations in both the analyzed compartments. The results obtained in the current study correspond to amino acid determinations in growing children, where amino acid concentration gradient between plasma and skeletal muscle compartments was evaluated. Skeletal muscle compartment was characterized by higher concentrations of 20 analyzed amino acids in comparison to blood plasma. It is worth to underline that the previous study presented a muscle-plasma amino acid gradient for 3 different age groups of children (< 1 year, 1–4 years, and 5–15 years of age) and for adults. Total amino acid content in muscles was 7.4–8.7 times higher than in plasma (Hammarqvist et al. 2010). Free amino acids determinations in plasma and skeletal muscles in healthy patients and patients suffering from liver cirrhosis have also shown the amino acid gradient between plasma and skeletal muscle compartments. Isoleucine concentration was found to be 1.4–3.1 times higher in human muscles than in plasma, while in the foxes this muscle : serum ratio reached 1.2 in males and 2.1 in females. Leucine concentration was found to be 1.8–3.0 times higher in human muscles compartment, while in the foxes the differences of the ratio reached 2.0 in males and 2.5 in females. The muscle : plasma ratio for the whole group of BCAAs in humans reached the values between 1.7 and 2.2 (Montanari et al. 1988). Experimental studies in 7–8 months old female Beagles (*Canide* family) have revealed a significantly higher concentration of most of the analyzed free amino acids in skeletal muscles (*musculus semitendinous*) in comparison to plasma compartment. The most significant differences of free amino acid concentrations between both the analyzed tissue compartments were stated for Glu (132.2 times), Asp (88.2 times), cysteine (13.3 times), Gln (11.3 times) and Ala (8.1 times). For Gly, Ser, Asn, Thr, Lys, Arg, His, Ile, Leu, Tyr, Met, Phe and Pro the values of the muscle : serum ratio reached 5.9, 5.3, 5.1, 3.3, 3.1, 2.9, 2.7, 2.1, 2.1, 2.0, 1.7, 1.7 and 1.5, respectively. The concentration of Val was very comparable in both the muscle and plasma compartments. Similarly to the current study showing significantly lower Trp content in skeletal muscles (1.4 times in females and 4.5 times in males), analogical observations were obtained in Beagles where muscle content of Trp was 8.6 times lower than in the plasma compartment. Total amino acid content in semitendinous muscle was 7.3 times higher than in plasma. Moreover, based on the results obtained in the previous studies on humans and dogs, it was concluded that experimental studies on Beagles may be interpolated for humans in many aspects of the amino acid metabolism (Stinnett et al. 1982). The other studies on amino acids in humans suffering from hip disorders

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and the analyses of plasma and skeletal muscle compartments have confirmed higher muscle content of Val, Leu, Ile, Phe, Tyr, Met, Thr, Lys, Gln, Ala, Gly, Arg, His, Orn, Tau, Asp, Ser, Glu, Pro and Aab. The highest differences of the muscle : plasma ratio values were stated for Asp (389), Tau (254) and Glu (128). Contrary to the current study showing lack of significant differences between muscle and plasma Orn concentrations in male and female foxes, the results obtained in the previous studies on humans show the value of 6.4 for the muscle : plasma ratio of Orn (Askanazi et al. 1980).

CONCLUSION

This study has shown significantly higher serum concentrations of Pro, Ala, Tyr and AAAs in males than in females. Higher concentrations of the amino acids in males coexist with sex-related differences of body size and final body weight. In skeletal muscle compartment, the concentration of Tau and Gly was significantly higher in males than in females, while Gln has shown similar tendency to be increased in males by over 31%. Neither BCAAs nor AAAs in skeletal muscles were sex-differentiated in males and females. Muscle concentrations of Cya, Tau, Asp, Glu, Gln, Ala, GABA and Adi were significantly higher over 10 times than in serum in females. Similar scale of the differences between both tissue compartments was observed for Cya, Asp and Ala in males. Taurine concentration in trapezius muscle in males was over 94 times higher than in serum. Muscle concentrations of Thr, Ser, Gly, Cit, Val, Leu, Eta, Lys and His in males and females were significantly higher up to 9 times when compared to serum. Similar findings were found in males for the concentrations of Ctn, Ile, Tyr, Phe and Arg, as well as for GABA in females. The concentrations of Asn, cystine, Met, Orn and Aab were not differentiated between serum and muscle compartments in both groups. Similar findings were seen for the concentrations of Ctn, Ile, Tyr, Phe, Arg and Adi in males, and for Pro in females. Tryptophan was the only amino acid with its lower concentration in skeletal muscle than in serum. The concentration of BCAAs in skeletal muscles was approximately two times higher than in serum in both sexes. Similar results were obtained for AAAs in females. The elaborated experimental model may serve for further studies focused on amino acid metabolism regulation in *Canide* and other monogastric mammals, especially with the use of environmental, dietary, pharmacological and toxicological factors affecting whole body growth and development and protein homeostasis maintenance. Precise formulations and administration of the diet to farm foxes, and a relatively large population of the experimental animals maintained at the same environmental conditions on a farm are considered as much more advantageous than experimentations on dogs. Due to obligatory slaughter procedures of farm foxes, their use for experimental procedures may be an alternative to reduce the number of dogs undergoing experimental euthanasia, and may replace some experiments on dogs.

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