Free amino acid content in muscle tissue of bighead carp and wels catfish

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Summary

The present research was aimed at a comparative analysis of free amino acid (FAA) levels in the muscle tissue of herbivorous and predatory fish. The FAA concentration in the muscle tissue samples from bighead carp and wels catfish was determined by ion-exchange chromatography. The bighead carp muscle tissue, as compared to that of wels catfish, showed significantly higher concentrations of arginine, histidine, methionine, phenylalanine, alanine, asparagine, serine, glycine, and taurine, but significantly lower levels of isoleucine, leucine, threonine, valine, glutamine, cystathionine, β -alanine, ethanolamine, as well as aspartic, glutamic, cysteic, α - and γ -aminobutyric acids. The muscle tissues of bighead carp and wels catfish did not differ significantly in the levels of cystine, lysine, tryptophan, tyrosine, α -aminoadipic acid, citrulline, ornithine and 1-methyl-histidine. Proline was detected only in the wels catfish muscle. The results obtained have shown differences in free amino acid concentration in muscle tissue of examined fish. A differentiated natural diet induces changes in free amino acid content in fish tissues. Knowledge of levels of free amino acids which are precursors for biogenic amines facilitates setting the safety criteria for fish and fishery products from species other than those mentioned in the Commission Regulation No 2073/2005.

Keywords: freshwater fish, free amino acids, bighead carp, wels catfish

Studies on free amino acid (FAA) composition in various organisms have shown that free amino acid composition reflects changes in biological evolution (23). Free amino acid content in various fish species is also differentiated (7, 8, 15, 20). Free amino acid concentration also depends on growth stage, starving, spawning migration, seasonal variation, water temperature and hardness, the storage and processing conditions of fresh fish as raw material, as well as diet. Bighead carp and wels catfish are important food fish (www.fao.org/fishery/statistics). Bighead carp is considered as herbivorous fish and feeds phytoplankton, zooplankton and detritus (10). Wels catfish is predatory fish and feeds a lot of fish species in natural ponds, while under fish farming conditions feeds weed and cultivated fish species (26).

As the available literature lacks relevant data comparing the concentrations of free amino acids in bighead carp and wels catfish, the present research aimed at a comparative analysis of free amino acid levels in

the muscle tissue of these fish species subject to a different natural diet. Insight into free amino acid content in fish muscle tissues provides information on levels of precursors for biogenic amines.

Material and methods

Sample collection. The studies were conducted from October 2013 through January 2014. The research material consisted of the muscle tissue of bighead carp (*Aristichthys nobilis*) and wels catfish (*Silurus glanis*). Fish of both species were obtained from two fish farms located in eastern Poland. Bighead carp and wels catfish were cultured together with carp in a farm pond. The fish fed on natural food exclusively. The free amino acid content was determined in 12 fish of each species. After fish harvesting and killing, the body weight and length of each fish were measured. The mean body weight and length were 2.64 ± 0.30 kg and 50.38 ± 1.58 cm for bighead carp, and 2.68 ± 0.38 kg and 74 ± 3.74 cm for wels catfish. Within an hour, the fish were transported at 0°C - 4°C to the laboratory, where they

were gutted and filleted. A laboratory sample consisted of a pair of filets from each fish, minced twice and then homogenized. The muscle samples were stored at -36°C until amino acid analysis.

Ion-exchange analysis of amino acid concentration in muscle tissues. To evaluate free amino acid concentration in the muscle tissue samples collected from each fish, 1.0 g samples (analytical sample) were homogenized in 10 ml of 6.0% sulphosalicylic acid buffered to pH 2.9. The homogenized samples were centrifuged for 15 minutes at 12 000 rpm. The supernatants obtained served to determine free amino acids by ion-exchange chromatography (INGOS AAA-400 apparatus for automatic analysis of amino acids, Ingos Corp., Prague, Czech Republic). Amino acids were separated using an analytic column OSTION LG FA 3 mm $\times$ 200 mm and five lithium cytrate buffers (pH 2.9, 3.1, 3.35, 4.05, and 4.9). Then they were derivatized with ninhydrin and identified by means of a photocell combined with a computer on the basis of retention time compared with the standards provided by Ingos Corp. The evaluation of amino acids was performed by the original software MIKRO version 1.8.0 (Ingos Corp., Prague, Czech Republic).

The amino acids under study were divided into two groups depending on their content in fish muscle tissue, i.e. equal to and greater than 1 $\mu\text{mol/g}$ of tissue or lower than 1 $\mu\text{mol/g}$ of tissue.

Statistical analysis. All values are presented as means $\pm$ SD. The statistical analysis was done using Statistica software (version 6.0) and non-paired Student's *t*-test for non-dependent variables. The differences between mean values were considered as statistically significant at $P < 0.05$.

Results and discussion

The content of free amino acids in the muscle tissue of the examined fish species is presented in Table 1. The bighead carp muscle tissue, as compared to that of wels catfish, showed significantly higher concentrations of arginine, histidine, methionine, phenylalanine, alanine, asparagine, serine, glycine, and taurine ($P < 0.05$), but significantly lower levels of isoleucine, leucine, threonine, valine, glutamine, cystationine, β -alanine, ethanolamine, as well as aspartic, glutamic, cysteic, α - and γ -aminobutyric acids ($P < 0.05$). The muscle tissues of bighead carp and wels catfish did not differ significantly in the levels of cystine, lysine, tryptophan, tyrosine, α -aminoadipic acid, citrulline, ornithine and 1-methyl-histidine ($P > 0.05$). Proline was detected only in the wels catfish muscle at a concentration of $0.906 \pm 0.43 \mu\text{mol/g}$. The amino acids found in the muscle tissue of both fish species at a level of no less than 1 $\mu\text{mol/g}$ were glycine, taurine, alanine, and ethanolamine. Additionally, histidine was found in bighead carp, and aspartic acid in wels. The concentrations of the other amino acids were lower than 1 $\mu\text{mol/g}$.

The present research has shown that the muscle tissue of bighead carp and wels catfish was the richest in glycine, followed by taurine, alanine, and ethanolamine. Amino acids present in the lowest amounts in the muscle tissue of both species were tryptophan,

Tab. 1. Free amino acid concentrations ($\mu\text{mol/g}$ tissue; mean, SD) in fish muscles

Amino acid	Bighead carp (n = 12)	Wels catfish (n = 12)
Arginine	0.106 ± 0.03	$0.063 \pm 0.02^*$
Cystine	0.002 ± 0.00	0.002 ± 0.00
Histidine	5.355 ± 0.95	$0.193 \pm 0.06^*$
Isoleucine	0.101 ± 0.03	$0.187 \pm 0.04^*$
Leucine	0.179 ± 0.05	$0.363 \pm 0.14^*$
Lysine	0.413 ± 0.07	0.399 ± 0.11
Methionine	0.079 ± 0.02	$0.062 \pm 0.02^*$
Phenylalanine	0.085 ± 0.02	$0.061 \pm 0.01^*$
Threonine	0.337 ± 0.05	$0.792 \pm 0.18^*$
Tryptophan	0.001 ± 0.00	0.001 ± 0.00
Tyrosine	0.076 ± 0.02	0.066 ± 0.02
Valine	0.213 ± 0.03	$0.283 \pm 0.10^*$
Alanine	3.858 ± 0.30	$2.974 \pm 1.17^*$
Asparagine	0.009 ± 0.01	$0.003 \pm 0.00^*$
Aspartic acid	0.496 ± 0.06	$1.215 \pm 0.42^*$
Serine	0.891 ± 0.11	$0.355 \pm 0.11^*$
Glutamine	0.001 ± 0.00	$0.020 \pm 0.03^*$
Glutamic acid	0.221 ± 0.06	$0.460 \pm 0.25^*$
Glycine	15.335 ± 1.97	$7.309 \pm 2.26^*$
Taurine	8.687 ± 2.20	$5.986 \pm 1.71^*$
Cysteic acid	0.068 ± 0.00	$0.092 \pm 0.02^*$
α -Aminoadipic acid	0.012 ± 0.01	0.019 ± 0.03
Citrulline	0.003 ± 0.01	0.002 ± 0.00
α -Aminobutyric acid	0.015 ± 0.01	$0.037 \pm 0.03^*$
Cystationine	0.002 ± 0.00	$0.013 \pm 0.01^*$
β -Alanine	0.017 ± 0.01	$0.292 \pm 0.14^*$
γ -Aminobutyric acid	0.013 ± 0.01	$0.171 \pm 0.05^*$
Ethanolamine	1.213 ± 0.07	$1.877 \pm 0.5^*$
Ornithine	0.209 ± 0.03	0.279 ± 0.12
1-Methyl-histidine	0.010 ± 0.01	0.016 ± 0.02

Explanations: * significant at $P \leq 0.05$

cystine, citrulline, and asparagine. Glutamine and cystationine were also scarce in bighead carp. In wels, the concentrations of free amino acids, such as methionine (by 21%), alanine (23%), phenylalanine (28%), taurine (31%), arginine (41%), glycine (52%), serine (60%), asparagine (67%) and histidine (96%) were lower than in bighead carp. Further, concentrations of free amino acids were higher in wels catfish than in bighead carp by 25%-95% for valine (25%), cysteic acid (26%), ethanolamine (35%), isoleucine (46%), leucine (51%), glutamic acid (52%), threonine (57%), aspartic acid (59%), α -aminobutyric acid (59%), cystationine (85%), γ -aminobutyric acid (92%), β -alanine (94%), and glutamine (95%). There were no significant differences between wels catfish and bighead carp in the levels of cystine, lysine, tryptophan, tyrosine, α -aminoadipic acid, citrulline, ornithine, and 1-methyl-histidine.

The free amino acid concentrations found in the present study in tissues of freshwater fish are markedly different from those observed in marine fish. White muscles of Atlantic cod (*Gadus morhua*) (15) contained the highest amount of taurine (13.856 $\mu\text{mol/g}$ fresh weight), followed by arginine (3.514 $\mu\text{mol/g}$), alanine (3.458 $\mu\text{mol/g}$), and glycine (2.444 $\mu\text{mol/g}$). The concentrations of taurine and arginine were higher, respectively, by 37% and 97% those established in the tissues of bighead carp and, respectively, by 57%, and 98% those found in wels catfish. The alanine content in Atlantic cod was close to those in bighead carp and wels catfish, whereas the glycine level was lower, respectively, by 84% and 67%. The concentrations of aspartic acid (0.085 $\mu\text{mol/g}$) and histidine (0.155 $\mu\text{mol/g}$) in Atlantic cod were also lower. The aspartic acid content in wels catfish muscle tissue and the histidine content in bighead carp muscle tissue were higher, respectively, by 93% and 97% as in Atlantic cod. As in wels catfish and bighead carp, tryptophan was the scarcest amino acid in Atlantic cod tissue, but its content in cod (0.047 $\mu\text{mol/g}$) was 47 times as high as in bighead carp and wels catfish. The concentrations of the other amino acids (except ethanolamine) detected in the muscle tissue of wels catfish and bighead carp were lower than 1 $\mu\text{mol/g}$. Compared with the white muscle of Atlantic cod, bighead carp muscle tissue contained less of cystine, isoleucine, leucine, phenylalanine, threonine, tyrosine, valine, asparagine, glutamine, and glutamic acid, similar amounts of lysine and methionine, and more of aspartic acid and serine. Wels catfish muscle tissue, on the other hand, contained less of cystine, methionine, phenylalanine, tyrosine, asparagine, and glutamine, more of proline, and similar quantities of histidine, isoleucine, leucine, lysine, threonine, valine, serine, and glutamic acid as the white muscle of cod. The concentrations of the abovementioned free amino acids in the white muscle of Atlantic cod were also lower than 1 $\mu\text{mol/g}$. The levels of the other free amino acids in bighead carp and wels catfish muscle tissue, that is cysteine acid, α -aminoadipic acid, citrulline, α -aminobutyric acid, cystathionine, β -alanine, γ -aminobutyric acid, ethanolamine, ornithine and 1-methyl-histidine, cannot be compared with those in Atlantic cod, as these values for cod are not available in the literature.

Among the free amino acids determined in the white muscle tissue of Atlantic salmon (*Salmo salar* L.) (8), the most abundant were taurine (9.97 $\mu\text{mol/g}$ of fresh tissue), glycine (8.72 $\mu\text{mol/g}$), alanine (2.65 $\mu\text{mol/g}$), and glutamine (1.34 $\mu\text{mol/g}$). The taurine content in the white muscle of Atlantic salmon was 13% higher than in bighead carp and 40% higher than in wels catfish. Atlantic salmon contained 43% less glycine and 31% less alanine than bighead carp, but, compared with wels catfish, salmon had 16% higher concentration of glycine and a similar concentration of alanine. Notable differences were observed in the glutamine content. In

the white muscle tissue of Atlantic salmon, the glutamine level was 1340 times as high as in bighead carp muscle tissue and 67 times as high as in wels catfish. Tyrosine levels in the muscle tissue of these three species were comparable. Compared to Atlantic salmon, bighead carp had higher aspartic acid, serine and ornithine contents (by 62%, 57%, and 43%, respectively), but lower asparagine and glutamic acid contents (by 96% and 52%, respectively). In wels catfish, the serine and glutamic acid levels were similar to those found in Atlantic salmon, but ornithine, proline, and aspartic acid concentrations were higher (by 57%, 59%, and 84%, respectively). Besides, the asparagine content was lower by 98.8%.

The data presented here confirm species-dependent differences in free amino acid concentrations in fish muscle tissue. These differences were observed not only between the freshwater and marine fish species, but also between the two freshwater species, that is bighead carp and wels catfish. In freshwater fish (bighead carp and wels catfish), the predominant amino acid was glycine, whereas in marine fish (Atlantic cod and Atlantic salmon) it was taurine. This is consistent with the results of research on 4 freshwater natural populations of mountain trout (*Salmo trutta macrostigma*), in which glycine was reported as the prevalent free amino acid in all the samples (12). In Atlantic cod, as bighead carp and wels catfish, the scarcest amino acid was tryptophan. The available literature provides no data on the concentration of this amino acid in Atlantic salmon muscle tissue. Moreover, it was not determined in fingerling rainbow trout either (25). Lyndon et al. (15) point out the omission of tryptophan content unexplained in the literature. This omission is probably due to the low levels of tryptophan in tissues. For example, in white muscle rainbow trout (*Oncorhynchus mykiss* Walbaum) tryptophan concentration was also low (0.11 $\mu\text{mol/g}$ of fresh tissue) (7), although it was over 100 times as high as those found in the present research in the bighead carp and wels catfish tissue.

However, free amino acid levels in muscle tissue depend not only on a fish species, but also on the type of muscle. The red and white muscle tissue of mahi-mahi (*Coryphaena hippurus*) and yellowfin tuna (*Thunnus albacares*) had significantly different histidine contents. In both species, higher levels of this amino acid were found in the white muscles. Similarly, lysine concentration in mahi-mahi was significantly higher in white muscle (4). The red and white muscles of the cyprinid fish *Carassius auratus* L. (goldfish) differed significantly in their levels of free histidine, taurine, glycine, valine, isoleucine, glutamic acid, and aspartic acid. In the red muscles taurine, histidine, and other free amino acids accounted, respectively, for 50%, 25% and 25% of the free amino acid pool, whereas in the white muscles their share amounted to 40%, 30% and 30% of the free amino acid pool, respectively (5).

Other factors are known to affect free amino acid concentration as well. Free amino acid content in dry-cured grass carp (*Ctenopharyngodon idellus*) produced traditionally in China was shown to rise gradually during processing and storage. Twelve free amino acids were identified in the fresh fish (aspartic acid, serine, glutamic acid, glycine, alanine, histidine, threonine, valine, methionine, isoleucine, leucine and lysine) with a total concentration of 2.7 g/kg dry weight. During processing, the fish contained 15 free amino acids (additionally cysteine, tyrosine, and phenylalanine) with total concentration 10.1 g/kg dry weight, and the final product (after storage) had 17 free amino acids (additionally proline and arginine) with total concentration 35.4 g/kg dry weight (27).

The levels of some free amino acids also changed during starvation. Studies conducted on milkfish (*Chanos chanos*) during the 60-day starvation period revealed changes in histidine, taurine, and glycine contents, as well as interdependence among these amino acids. On day 0, their percentage shares in the total free amino acids were 42%, 31%, and 9%, respectively, that is 82% altogether. On day 60 histidine made up only 27% of the total free amino acids, while taurine and glycine accounted for 40% and 13%, respectively, that is 81% in total. It follows that these free amino acids may have had a compensation effect on the free amino acids pool in milkfish muscle during the food deprivation period. Other free amino acids were reported only in small quantities in the starving fish, so they made a relatively minor contribution to the change in the total free amino acids (21). In the skipjack tuna (*Katsuwonus pelamis*) white muscle, histidine declined to 30% and 4% of control levels after 5 and 12 days of starvation, respectively (2). The free histidine content in sockeye salmon (*Oncorhynchus nerka*) muscle tended to decline during the spawning migration. The initial histidine concentration in white muscle was nearly 4 $\mu\text{mol/g}$, but fell to less than one tenth of this initial value after a 400 km spawning migration and remained at that level for the rest of migration period (17).

The histidine concentration in the white muscle of milkfish (*Chanos chanos*) increased along with body weight and age (20). Histidine content ranged from 15.8 to 92.8 $\mu\text{mol/g}$ in dark-fleshed fish, from 4.06 to 20.3 $\mu\text{mol/g}$ in intermediate fish, and from 0.0734 to 0.931 $\mu\text{mol/g}$ in white-fleshed fish (1). Concerning the above levels, a histidine content in the tissues of the fish analyzed in the present study corresponds to intermediate fish in the case of bighead carp and to white-fleshed fish in the case of wels catfish. A study on milkfish (*Chanos chanos*) white muscle also revealed some interdependencies among the contents of free histidine, taurine, and glycine observed during an 8-month growth period. The total amount of these free amino acids in the initial test fish accounted for about 36% of the total free amino acid pool. This ratio rose to as high as 75% in 1-month reared fish, and after a 4.5-month fish culture these three free amino acids

peaked at 85% to remain relatively constant thereafter. A rise in histidine concentration triggered a decrease in taurine and/or glycine (20).

A characteristic fish flavor is related to with a free amino acid level in muscle tissue. Glycine, glutamic acid and alanine contents were reported to vary between the red and white muscle of bigeye tuna (*Thunnus obesus*). A higher concentration of these amino acids with stronger flavour were determined in the red muscle tissue (19). Glutamic acid, followed by methionine, glycine, aspartic acid, and lysine is regarded as the most important for the flavor of mountain trout (*Salmo trutta macrostigma*) (12). Glutamate, histidine, and glycine substantially contribute to the taste, texture and quality of seafood (14).

In Atlantic salmon (*Salmo salar* L.), high temperature caused lower muscle free amino acid levels. An average content of total free amino acids in white muscle tissue was lower at 19°C than at 13°C throughout a 24 h sampling period, but the difference became statistically significant only at 8 h, 12 h, 16 h, and 20 h (24). Buentello and Gatlin (6) emphasize the effect of water hardness on the free amino acid concentration in the muscles of juvenile channel catfish (*Ictalurus punctatus*). Fish were maintained under two different regimes of water hardness, i.e. 17.9 mg/L (soft water) and 407 mg/L (hard water) as CaCO_3 . The fish adapted to hard water by accumulating taurine, alanine, tyrosine, valine, and phenylalanine in the muscle free amino acid pool. In these fish, compared with the fish held in soft water, the taurine level was by 50% higher, while the levels of phenylalanine, valine, tyrosine, and alanine were by 12%-26% higher.

Differences in the free amino acid content in tissues can be attributed to diet nutrient composition. Yamamoto et al. (25) observed that tissue free amino acid levels in fingerling rainbow trout (*Oncorhynchus mykiss*) were affected by dietary fat levels. The levels of asparagine, glycine, methionine, tyrosine, and phenylalanine were significantly higher in the dorsal white muscle of rainbow trout fed low-protein diets with a balanced amino acid diet+high fat diet, as compared to rainbow trout receiving low-protein diets with a balanced amino acid diet+low fat diet. There was some variation in the free pool concentrations of individual amino acids in the white muscle of Atlantic salmon (*Salmo salar* L.) fed maize gluten dietary protein. The free amino acid concentrations of histidine, leucine, alanine, aspartic acid, and proline increased significantly along with a growing maize gluten content in the diet. However, threonine, hydroxyproline, serine and lysine displayed a contrary trend. No significant differences were reported in the free amino acid concentrations of all other amino acids (16).

Hwang et al. (13) found seasonal variations in free amino acids in the muscle of puffer *Takifugu rubripes*. Seasonal variation in total free amino acids was more pronounced in puffer cultured in Ilan Prefecture than

it was in puffer from Taipei Prefecture. In general, the highest contents of total free amino acids in the samples from Ilan Prefecture were observed in January, and the lowest in March. The level of total free amino acids in specimens from Taipei Prefecture was the lowest in November. The concentration of free amino acids in garfish (*Belone belone belone*) fillets was higher in spring (9799 ppm) than in autumn (8648 ppm), and the difference was statistically significant for alanine, glycine, isoleucine, methionine, phenylalanine, proline, serine and valine (11).

The above results demonstrate that free amino acid concentration in fish muscle tissue depends on several factors. Apart from the fish species, it is significantly affected by the type of muscle, the kind of diet and its supplementation, growth stage, starving, spawning migration, seasonal variation, water temperature and hardness, as well as the storage and processing conditions of fresh fish as raw material. Moreover, the free amino acid content in fish muscle tissue can serve as a freshness indicator, since the free amino acid concentration changes during spoilage. According to Antoine et al. (4), free amino acids in fish reflect microbial spoilage, are precursors of biogenic amines, and indicators of fish decomposition. Precursors for main biogenic amines involved in food poisoning incidents are histidine (precursor of histamine), tyrosine (of tyramine), tryptophan (of tryptamine), lysine (of cadaverine), ornithine (of putrescine) and arginine (precursor of spermine and spermidine) (22). Even though a number of biogenic amines have been found in fish, those significant for fish safety and quality determination are only histamine, cadaverine, and putrescine (3). Histamine is commonly recognized as a quality index for fish species rich in histidine, whereas putrescine and cadaverine are regarded as the most objective indicators of quality in histidine-poor fish (18) and whereby quality index for bighead carp muscle tissue could be histamine content, whereas cadaverine and putrescine concentration for wels catfish muscle tissue. Knowledge of free amino acid levels in fish muscle tissue is critical for food safety criteria for fishery products. Fishery products from fish species associated with elevated histidine level, especially from the family *Scombridae*, *Clupeidae*, *Engraulidae*, *Coryfenidae*, *Pomatomidae* and *Scombrosidae* have defined maximum histamine limits (9).

The results obtained in this study have revealed significant differences in muscle tissue free amino acid content in bighead carp and wels catfish. The observed species-related differences concerning concentration of free amino acid in muscle tissue result from a differentiated diet in natural conditions. Knowledge of levels of free amino acids which are precursors for biogenic amines facilitates setting the safety criteria for fish and fishery products from fish species other than those mentioned in the Commission Regulation No 2073/2005 (9).

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