Amino Acid Composition of Muscle Proteins of Diploid and Triploid Tench (*Tinca tinca,* Linnaeus 1758)

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Received February 10, 2005
Accepted June 6, 2005

Abstract

Buchtová H., M. Smutná, L. Vorlová, Z. Svobodová, M. Flajšhans: Amino Acid Composition of Muscle Proteins of Diploid and Triploid Tench (*Tinca tinca*, Linnaeus 1758). Acta Vet. Brno 2005, 74: 329-337.

The aim of the study was to determine the differences in the composition of amino acids (in % of total determined amino acids) between diploid (2n) and triploid (3n) tench of identical genetic specifications and raised under the same conditions, in relation to sex (F-female vs. M-male) and age (T_3-36 months vs. T_{3+}- 42 months). A total of 38 mixed samples of muscle tissue from 137 tench divided by sex, ploidy and age were analyzed. The effect of ploidy and sex on the composition of the spectrum of amino acids of muscle proteins was greater in the T_3 tench population. The following differences in values were found: female fish (ploidy effect): Thr ($p < 0.05$), Arg ($p < 0.05$), Pro ($p < 0.01$) Gly ($p < 0.01$); male fish (ploidy effect): Ile ($p < 0.01$); diploids (sex effect): Arg ($p < 0.05$); triploids (sex effect): Pro ($p < 0.01$). In the T_{3+} tench population, highly significant ($p < 0.01$) differences in Gly contents in female fish (ploidy effect) and triploids (sex effect) were found. Lower levels of the following amino acids were found in muscle tissue of T_{3+} tench compared with the T_3 (younger) tench population: Val, Phe, Lys, His, Arg (in F 2n and M 3n), Pro (in F 3n) and Tyr. Levels of Thr, Ile, Leu, Arg (in M 2n and F 3n), Asp, Glu, Gly and Ala, on the other hand, were higher in T_{3+} tench. Results of the present study suggest that the qualitative and quantitative compositions of some amino acids bound in tench muscle proteins may be statistically significantly influenced by such biotic factors like sex, ploidy and age.

Fish, tench, genome manipulations, polyploidy, essential and non-essential amino acid

A large number of research teams study the quantitative and qualitative composition of amino acids of bodily tissues and fluids of fish. They mainly focus on investigating the effects that diets (different contents of proteins and lipids) may have on the composition of free amino acids (FAA). In different species of fish, considerable variations in the composition of amino acids, particularly in studies of free essential amino acids (FEAA) in blood plasma, are demonstrated. A high degree of correlation between diet composition and FEAA levels in blood plasma in the common carp has been reported by, e.g., Plakas et al. (1980), Dabrowski (1982), Schwarz et al. (1984), Zeitler et al. (1984) and Ogata (1986), in the rainbow trout by Kaushik and Luquet (1977 a,b), Walton and Wilson (1986), Yokoyama and Nakazoe (1991) and Yokoyama et al. (1994), in the salmon by Ogata and Murai (1994) and in the sturgeon by Kaushik et al. (1994).

Schwarz and Kirchgessner (1988) found that dietary levels of crude protein (CP) exceeding 41% cause a significant increase in certain free non-essential amino acids (FNEAA), while high-energy diets (over 18.2 MJ) will cause their reduction.

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FAA levels in bodily tissues of fish (liver, muscle tissues, brain) are less affected by dietary composition (Yamamoto et al. 2000).

The quantitative and qualitative composition of amino acids taking part in the building of muscle proteins is very similar among fish of the same as well as different species (Wilson and Cowey 1985; Wilson 1989; Mohanty and Kaushik 1991; Yamamoto et al. 2000). Fu et al. (2000), who studied effects of diets with different protein contents (20 %, 30 %, 40 %) on the composition of AA in transgenic populations of the common carp, found significant ($p < 0.05$) differences in some AA (Lys, Leu, Arg, Ala) between the control (natural) and the transgenic group of carp fed with diets with the same protein content. The differences found may be related to changes in specific genetic information in transgenic fish on the basis of which muscle proteins are synthesized.

Quantitative changes in the DNA content in cells also occurs in genetically modified polyploid fast-growing fish, which, in theory, might influence the composition of amino acids in bodily tissues of the fish. This issue, however, has never been investigated experimentally. Neither has there been any study of possible effects of sex (and age) on changes in AA composition.

The aim of the present study was to monitor the differences in the muscle protein composition between diploid (2n) and triploid (3n) tench of identical genetic specifications and reared under the same conditions in relation to sex and age of the fish based on an analysis of the quantitative and qualitative composition of the amino acid (AA) spectrum, total content of essential amino acids (EAAsum) and other amino acids (NEAAsum).

The study is a part of a comprehensive assessment of diploid and triploid tench reported also in other published papers by Buchtová and Vorlová (2002), Buchtová et al. (2003ab) and Buchtová et al. (2004).

Materials and Methods

A genetically identical tench population was established in 1998 by artificial spawning in the hatchery of the Department of Fish Genetics and Breeding of the Research Institute of Fish Culture and Hydrobiology at the South Bohemian University in České Budějovice. A part of fertilized eggs of this diploid (2n) tench population was subject to a cold shock to induce triploidy (3n) by a fusion with the secondary polar body according to Flajšhans and Linhart (2000).

The tench were reared under identical conditions in experimental ponds of the Research Institute in VodÀany. The 2n tench fry were freeze-branded using a matrix, while the 3n population was left unbranded.

At the beginning of each experiment (harvest date: 27.3.2001 – T_3, 10.10.2001 - T_{3+}) the fish were kept in tanks with original pond water under stress-eliminating conditions (O_2 above 80% saturation, constant water temperature).

Sex of the tench was determined on the basis of expressed sexual dimorphism (120 tench), pathological and anatomical examination of gonads (10 tench) and histological preparations according to Flajšhans et al. (1993) and Kvasnička and Flajšhans (1993) (7 tench). Diploid tench were identified by their freeze-branding on the skin.

Ploidy level in 3n tench was checked in blood sampled by puncture from the caudal vein (vena caudalis) by flow cytometry as a relative DNA content in cell nuclei according to Vindelov and Christensen (1990).

A total of 38 mixed samples of skinned fillets ("muscle tissue") obtained by filleting trimmed carcases (carcases without the head, fins and internal organs) of 137 siblings of 2n and 3n tench (*Tinca tinca*, L.) T_3 and T_{3+}. Each mixed sample was composed of tissues from 2 to 4 different fish.

To prepare samples for amino analysis, 0.5 g from each mixed sample with 0.0001 g accuracy (PRECISA 240 A, France) were used (homogenization: Moulinex ILLICO Y92, Ireland). The samples were prepared by acid hydrolysis (HCl = 6 mol.l-1) for 24 hours at 110 °C. The amino acid assay was performed on the AAA T 339 automatic amino acid analyzer (Mikrotechna Praha, CR). For their separation, sodium – citrate elution buffers in a chromatographic column with catex (OSTION LG ANB, CR) were used. After colour reaction with the ninhydrin, separated amino acids were detected in a flow photometer. AMIK software 3.0. (CR) was used to calculate retention times and areas of individual amino acid peaks, and to process data. Reagents necessary for the preparation of samples, buffers and AAA operation were supplied by the amino analyzer manufacturer. Solution of standard amino acid mixtures also supplied by the AAA manufacturer were used as external amino acid standards.

The Met content was deducted from the total sum of every mixed sample analyzed and was not included in experimental results because of degradation which takes place during acid hydrolysis of sulphuric amino acids. In the total muscle protein ("MP") obtained after the deduction of Met content, the proportion (in %) of each of the remaining 15 amino acids was calculated.

The parameters studied included the quantitative and qualitative composition of amino acids (acid hydrolysis) of the muscle protein and the sum of essential (EAA_{sum}) and other amino acids ($NEAA_{sum}$) and their differences between diploid (2n) and triploid (3n) tench of an identical genetic specification and rearing conditions in relation to sex (F: female vs. M: male) and fish age (T_3: 36 months, after overwintering vs. T_{3+}: 42 months, after one summer in the pond).

Basic statistical values (means, S.D.) of the indexes studied were processed in Excel 97. Evaluation of statistical significance of results was performed according to ploidy and sex of the tench by a multiple range variance analysis (ANOVA, Statgraphics 5.0) separately for T_3 and T_{3+} age categories. Evaluation of statistical significance of age of the fish (T_3 - T_{3+}) on indexes studied was performed at the * $p < 0.05$; ** $p < 0.01$ level of significance by a one-way variance analysis (ANOVA, Excel 97) separately for each group of the tench (F 2n, M 2n, F 3n, M 3n).

Results

The values (means ± S.D.) obtained in the study of amino acids (EAA, NEAA) and their sums (EAA_{sum}, $NEAA_{sum}$) for T_3 and T_{3+} tench according to ploidy (F 2n vs. F 3n, M 2n vs. M 3n) and sex (F 2n vs. M 2n, F 3n vs. M 3n) are summarized in Table 1 and Table 2. They also give the statistical significance (ANOVA, Statgraphics 5.0) of the effect of ploidy and sex on individual indexes and tench groups (F 2n, M 2n, F 3n, M 3n).

Table 1. Summary of data on amino acids (in % of total amino acids investigated), essential amino acid sum (EAAsum) and non-essential amino acids sum (NEAAsum) of T3 (36 months old) diploid (2n) tench (F-female, M-male) and their artificially induced triploid (3n) siblings (March 2001). The model of variance analysis: multiple range test.

Index %	F 2n Mean ± SD	n 5	M 2n Mean ± SD	n 5	F 3n Mean ± SD	n 5	M 3n Mean ± SD	n 5	Statistical significance
Thr	4.6 ± 0.22^{a}		4.8 ± 0.37^{ab}		5.0 ± 0.13^{b}		4.9 ± 0.19^{ab}		$p < 0.05$
Val	5.8 ± 0.33		6.0 ± 0.31		6.1 ± 0.42		5.4 ± 2.16		-*
Ile	5.2 ± 0.24^{ab}		5.0 ± 0.17^{a}		5.4 ± 0.08^{ab}		5.6 ± 0.25^{b}		$p < 0.01$
Leu	9.5 ± 0.44		9.8 ± 0.39		9.7 ± 0.13		9.4 ± 0.19		-*
Phe	4.5 ± 0.54		3.7 ± 1.42		3.4 ± 0.43		4.6 ± 0.72		-*
Lys	11.4 ± 0.38		12.1 ± 0.46		11.1 ± 0.20		11.2 ± 0.93		-*
His	3.2 ± 0.09		3.2 ± 0.13		2.6 ± 0.07		3.0 ± 1.00		-*
Arg	6.6 ± 1.76^{a}		3.4 ± 0.07^{bc}		3.4 ± 0.07^{c}		5.0 ± 2.26^{ac}		$p < 0.05$
EAA_{sum}	50.7 ± 1.35a		48.1 ± 0.93^{b}		46.7 ± 0.63^{b}		49.0 ± 2.27^{ab}		$p < 0.05$
Asp	11.8 ± 0.72		12.4 ± 0.37		12.1 ± 0.19		12.2 ± 0.60		-*
Ser	4.5 ± 0.13		4.6 ± 0.17		4.7 ± 0.47		4.6 ± 0.39		-*
Glu	16.4 ± 0.54		17.1 ± 0.43		17.0 ± 0.07		17.1 ± 0.62		-*
Pro	0.8 ± 0.78^{a}		1.9 ± 0.60^{ab}		2.3 ± 0.12^{b}		1.0 ± 0.21a		$p < 0.01$
Gly	5.7 ± 0.08^{a}		5.9 ± 0.27^{ab}		6.3 ± 0.29^{b}		6.2 ± 0.35^{ab}		$p < 0.01$
Ala	6.8 ± 0.22		7.0 ± 0.21		7.1 ± 0.09		7.0 ± 0.34		-*
Tyr	3.3 ± 0.07		3.0 ± 0.91		3.5 ± 0.55		3.1 ± 0.67		-*
$NEAA_{sum}$	49.3 ± 1.19^{a}		52.0 ± 0.92^{b}		53.2 ± 0.58^{b}		51.1 ± 2.18^{ab}		$p < 0.05$

-* Not significantly different at $p < 0.05$
Values with superscript "a" and "b" and "c" express significant difference ($p < 0.05$, $p < 0.01$ respectively) among groups compared.

Significant ($p < 0.05$) differences in EAA_{sum} and $NEAA_{sum}$ values were found in T_3 female fish (effect of ploidy) and T_3 diploids (effect of sex) (Table 1). A statistically significant effect of ploidy or sex on the content of specific AA in T_3 tench was found in 7 specific cases. The following differences in values were found: female fish (effect of ploidy): Thr ($p < 0.05$), Arg ($p < 0.05$), Pro ($p < 0.01$) Gly ($p < 0.01$); male fish (effect of ploidy): Ile ($p < 0.01$); diploids (effect of sex): Arg ($p < 0.05$); triploids (effect of sex): Pro ($p < 0.01$) (Table 1).

Effects of ploidy and sex on the content of EAA_{sum} and $NEAA_{sum}$ were not statistically significant in T_{3+} tench (Table 2). Contents of specific AA with the exception of Gly in T_{3+} tench were practically identical. Highly significant ($p < 0.01$) differences in Gly content in T_{3+} female fish (ploidy effect) and T_{3+} triploids (sex effect) were found. The highest levels of this NEAA were found in 3n female fish (Table 2).

Table 2. Summary data on amino acids (in % of total amino acids investigated), essential amino acid sum (EAA_{sum}) and non-essential amino acids sum ($NEAA_{sum}$) of T_{3+} (42 months old) diploid (2n) tench (F-female, M-male) and the-ir artificially induced triploid (3n) siblings (October 2001). The model of variance analysis: multiple range test.

Index %	F 2n Mean ± SD	n 5	M 2n Mean ± SD	n 5	F 3n Mean ± SD	n 5	M 3n Mean ± SD	n 3	Statistical significance
Thr	6.7 ± 1.58		7.2 ± 1.42		7.4 ± 1.49		7.7 ± 1.42		-*
Val	3.6 ± 1.53		3.0 ± 0.78		2.5 ± 0.30		2.8 ± 0.10		-*
Ile	6.3 ± 0.39		6.1 ± 0.25		5.9 ± 0.87		6.3 ± 0.17		-*
Leu	10.3 ± 0.76		10.5 ± 0.54		10.3 ± 0.99		10.2 ± 0.69		-*
Phe	1.0 ± 0.05		1.3 ± 0.42		1.1 ± 0.08		1.0 ± 0.06		-*
Lys	10.2 ± 0.51		10.8 ± 1.14		10.6 ± 1.24		10.0 ± 0.53		-*
His	1.9 ± 0.15		1.8 ± 0.05		1.9 ± 0.05		2.1 ± 0.21		-*
Arg	4.0 ± 0.19		3.8 ± 0.16		4.0 ± 0.13		4.0 ± 0.06		-*
EAA_{sum}	44.1 ± 1.01		44.5 ± 0.95		43.8 ± 0.68		44.0 ± 1.39		-*
Asp	3.6 ± 0.85		13.8 ± 0.34		14.0 ± 0.35		13.9 ± 0.25		-*
Ser	4.1 ± 0.92		4.2 ± 0.82		4.5 ± 0.91		4.3 ± 1.18		-*
Glu	20.2 ± 0.94		19.9 ± 0.29		19.9 ± 0.69		20.2 ± 0.47		-*
Pro	0.9 ± 1.14		1.1 ± 1.02		0.2 ± 0.39		0.5 ± 0.68		-*
Gly	7.0 ± 0.27^{a}		6.9 ± 0.13^{a}		7.6 ± 0.24^{b}		7.0 ± 0.10^{a}		$p < 0.01$
Ala	8.4 ± 0.52		7.8 ± 0.17		8.6 ± 0.80		8.0 ± 0.12		-*
Tyr	1.8 ± 0.21		1.9 ± 0.25		1.5 ± 0.30		2.0 ± 0.10		-*
$NEAA_{sum}$	55.9 ± 0.98		55.5 ± 1.01		56.3 ± 0.74		56.0 ± 1.39		-*

-* Not significantly different at $p < 0.05$

A comparison between EAA_{sum}, NEAAsum and specific AA (%) in tench from different age groups is given in Figs 1 and 2. The values of indexes studied in T_3 tench made up 100 %.

A highly significant decrease ($p < 0.01$) of EAA_{sum} and increase of $NEAA_{sum}$ with increasing age (T_3 - T_{3+}) of tench populations were found (Fig. 1). In T_{3+} tench populations, EAA_{sum} reached only 87 % (F 2n), 93 % (M 2n), 94 % (F 3n) and 90 % (M 3n) of T_3 values, while $NEAA_{sum}$ values were 13 % (F 2n), 7 % (M 2n), 6 % (F 3n) and 10 % (M 3n) higher compared with T_3 values (Fig. 1).

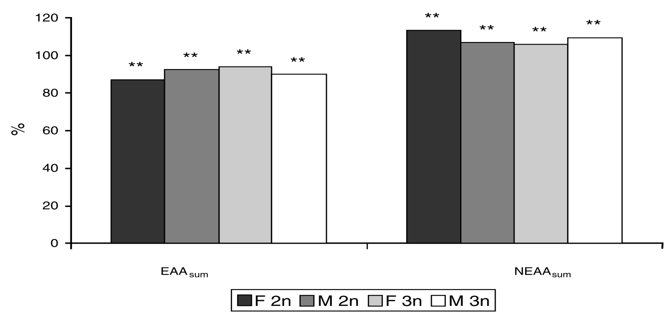

Fig. 1. Comparison of changes (in %) of the contents of essential amino acid sum (EAA_{sum}) and non-essential amino acids sum ($NEAA_{sum}$) of diploid (2n) and triploid (3n) tench (F-female, M-male) between the age of 36 months (T_3 tench) and 42 months (T_{3+} tench). Asterisk * and ** express significant difference of $p < 0.05$ and $p < 0.01$, respectively, between groups compared. The model of variance analysis: one-way.

Lower levels of some EAA, specifically of Val, Phe, Lys, His, Arg (in F 2n and M 3n) were found in T_{3+} tench groups compared with the T_3 population. The difference was highly significant ($p < 0.01$) in Phe levels, which in T_{3+} tench made only 22% (F 2n), 35% (M 2n), 32% (F 3n) and 22% (M 3n) of Phe values in T_3 tench (Fig. 2).

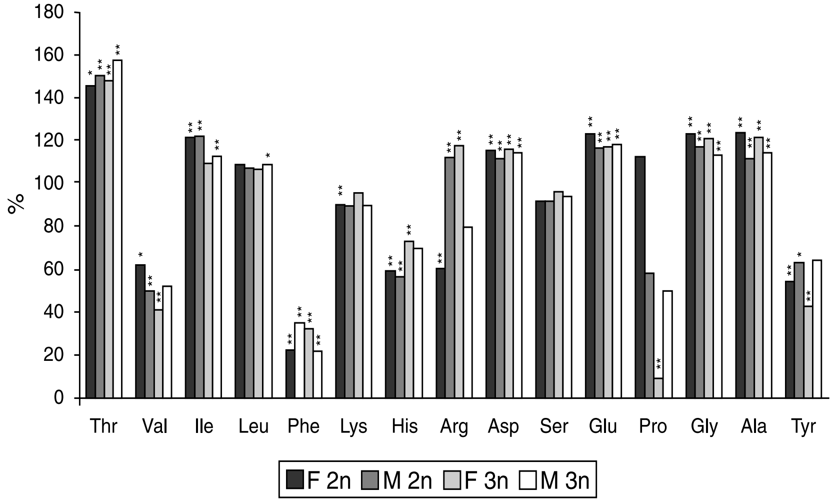

Fig. 2 . Comparison of changes (in %) of the contents of amino acids composition of diploid (2n) and triploid (3n) tench (F-female, M-male) from the age of 36 months (T_3 tench) to 42 months (T_{3+} tench). Asterisks * and ** express significant difference of $p < 0.05$ and $p < 0.01$, respectively, between groups compared. The model of variance analysis: one-way.

The levels of other EAA, i.e. Thr, Ile, Leu, Arg (in M 2n and F 3n), were higher in T_{3+} tench. The greatest increase was recorded in Thr levels. They were higher by 46% ($p < 0.05$), 50% ($p < 0.01$), 48% ($p < 0.01$) and 57% ($p < 0.01$) in F 2n, M 2n, F 3n, M 3n groups of T_{3+} tench, respectively, than Thr levels in T_3 tench (Fig. 2).

Statistically significant age-related differences (T_3 - T_{3+}) were also found in some NEAA. There was a highly significant increase ($p < 0.01$) of Asp, Glu, Gly, Ala in T_{3+} tench (Fig. 2).

The most marked decrease in NEAA was found in Pro contents in muscle tissues of triploid T_{3+} female tench. Age-related differences in Pro levels in F 3n were highly significant ($p < 0.01$): T_{3+} Pro levels were only 9 % of Pro levels in T_3 population. In the remaining groups of tench, no statistically highly significant age-related differences in Pro levels were found, although an increase of 13% in Pro levels in diploid T_{3+} female tench and a drop to 58% and 50% in diploid and triploid T_3 male tench, respectively, were recorded.

The content of another NEAA, specifically of Tyr, showed a statistically highly significant decrease to 55% ($p < 0.01$ F 2n), 63% ($p < 0.05$, M 2n), 43% ($p < 0.01$, F 3n) and 65% ($p < 0.05$, M 3n) of Tyr values in T_3 tench (Fig. 2).

Ser contents were practically identical in both age categories studied (Fig. 2).

Discussion

The statistical evaluation of amino analysis of muscle protein of 2n and 3n tench from T_3 and T_{3+} age groups showed a higher frequency of ploidy and sex effects on the composition of amino acids in T_3 tench populations, where several cases (Thr, Ile, Pro, Gly, Arg) of a significant effect of one of the factors were identified. Effects of ploidy and sex in T_{3+} tench populations were statistically highly significant only in non-essential Gly.

The differences in the levels of individual amino acids (Thr, Ile, Pro, Gly, Arg) in T_3 tench population may be connected with significant differences in some biometric and weight parameters and relevant indexes that were investigated in identical tench groups in another study by the same authors (Buchtová et al. 2003ab). Because the experimental design of the study consisted of only acid hydrolysis of samples and corrections of individual AA contents and their respective sums to the Met content (and not to other sulphuric amino acids, e.g. cystein and taurin and the aromatic amino acid tryptofan), it was not possible to make a genuine comparison between the results of quantitative and qualitative composition of AA in tench muscle tissues obtained here with results of amino analytical studies published by other authors. In spite of its experimental design, however, the present study confirmed that amino acids with the highest values in muscle proteins of the tench included Glu, Asp, Lys, Leu and Ala, which is in agreement with data by Hochachka and Mommsen (1995).

The reason for the significantly lower ($p < 0.05$) content of the essential amino acid Thr in the muscle protein of F 2n T_3 tench (ploidy effect) can lie in its insufficient dietary uptake, because the lowest Fulton's coefficient of physical condition was recorded in this tench group (Buchtová et al. 2003b). However, because no significant effects of ploidy (or sex) on this parameter in T_3 populations of the tench studied were demonstrated, we may hypothesize that the low content of Thr in muscle proteins of F 2n T_3 tench reflects the priority utilization of Thr by the developing ovaries in this group of fish, whose weight was highly significantly ($p < 0.001$) affected by both ploidy and sex (Buchtová et al. 2003b). In the final stage of metabolism, threonine produces acetyl-CoA and pyruvate. Acetyl-CoA can be used by the ovaries to synthesize cholesterol, steroid hormones and other compounds, and to produce energy (ATP). Pyruvate, the other metabolite, plays a key role in glycolysis (ATP) and in the synthesis of non-essential amino acids (transamination) necessary for the growth and development of the ovaries. Comments on the results obtained are based on the authors' own knowledge and experience.

The significantly higher ($p < 0.01$) content of the essential Ile in muscle proteins of 3n T_3 tench testifies to its sufficient dietary uptake (the highest Fulton's coefficient of condition). Its positive stimulatory effect for the proteosynthesis of somatic tissues was demonstrated in this tench group compared with the M 2n population (ploidy effect) by highly significant ($p < 0.001$) differences in biometric parameters and total weight (Buchtová et al. 2003b). As a branched amino acid, isoleucin is used by fish in skeletal muscles and the liver (glucoplastic and ketoplastic amino acid) and it may have a positive effect on the total weight and biometric parameters in tench (Hochachka and Mommsen 1995).

The highly significantly higher ($p < 0.01$) content of non-essential amino acids Pro and Gly in muscle tissue of 3n T_3 tench (ploidy effect) is probably due to the higher content of stromal proteins (reticulation) in muscle tissues, whose main component in fish is collagen. A conspicuous characteristic of collagen is the presence of glycine at every third position in the treble helix section of the chain. This primary structure is necessary because glycine is the only amino acid inside the central nucleus of the triple helix. The repeated structure represented by the sequence (Gly-X-Y)n is absolutely necessary for the existence of the triple helix, where X and Y may be any other amino acids. But approximately 100 amino acids in the X position represent proline and approximately 100 amino acids in the Y position hydroxyproline, which is produced by the posttranslation hydroxylation of proline (Murray et al. 2000). Because of their topographic location between the myomeres and around myofibrils, stromal proteins provide for the cohesion of muscle tissue, whose ratio was significantly the highest in F 3n T_3 tench (Buchtová et al. 2003b). A significant effect ($p < 0.01$) of ploidy on the content of Gly was also demonstrated in T_{3+} female tench (Table 2). Muscle tissue of F 3n T_{3+} tench contained very little proline

(0.2 ± 0.39 % MP), which is probably due to the histological structure of infertile ovaries in this fish (Flajšhans 1997; Sedláček 1999).

Significant differences ($p < 0.01$) in the content of Pro in the 3n population of T_3 tench and of Gly in 3n population of T_{3+} tench (effect of sex) may be related to significant differences ($p < 0.001$) found in biometric parameter and total weight. Another reason might also be a different histological structure of the male and female gonads, and significant differences ($p < 0.001$) in the size of reproductive organs in 3n T_{3+} tench population (Buchtová et al. 2003b).

The significantly lower ($p < 0.05$) level of Arg (ploidy effect) in muscle tissue of F 3n T_3 tench compared with F 2n tench may be due to its insufficient endogenous synthesis during overwintering, which is not sufficient to saturate proteins of rapidly grown somatic tissues of 3n female fish with this amino acid. Significantly lower ($p < 0.05$) Arg content in the muscle protein of M 2n T_3 tench (effect of sex) may be related to the lowest growth capabilities of this tench group. An absence of the ureosynthetic cycle (arginine producer) probably contributes to these effects during the vegetation period (Smutná et al. 2002).

In older tench populations (T_3 - T_{3+}), there was a highly significant ($p < 0.01$) decrease in EAA_{sum}. EAA_{sum} in T_{3+} tench populations was only 87% (F 2n), 93% (M 2n), 94% (F 3n) and 90% (M 3n) of the total in T_3 populations (Fig. 1). The responsibility for this – from the nutritional point of view – undesirable trend probably rests with the highly significant decrease ($p < 0.01$) in the Phe content: T_{3+} tench recorded only 22% (F 2n), 35% (M 2n), 32% (F 3n) and 22% (M 3n) of Phe values found in T_3 tench (Fig. 2).

We may hypothesize that this finding is related to endocrinous, metabolic and osmoregulatory changes in the fish organism that take place as a consequence of their stress in the period following overwintering. Higher Phe levels in muscle tissue of T_3 tench may correlate with increased excretion of catecholamines (adrenalin, noradrenalin) by the chromaiffinous tissue of the head part of the kidneys and their higher blood plasma concentrations. During the vegetation period, stress responses of fish are minimized by better living conditions, and requirements for endogenous synthesis of Phe are reduced, which might have theoretically been manifested by the lower content in the muscle tissue of T_{3+} tench.

The most important role in the highly significant ($p < 0.01$) increase in NEAAsum of T_{3+} populations by 13% (F 2n), 7% (M 2n), 6% (F 3n) and 10% (M 3n) compared with $NEAA_{sum}$ T_3 populations was played by dicarboxyl amino acids Asp and Glu and neutral aliphatic amino acids Gly and Ala (Fig. 2).

Rising Arg levels in muscle protein of 2n male and 3n female tench were probably due to their growth capability related to the increasing age of the fish, enhanced by natural foods received during the vegetation period (Buchtová et al. 2003b).

Amino acid levels in muscle tissue most affected by tench age were those of Ser (Fig. 2). The differences found are probably related to differences in the histological and anatomical structure of gonads between 2n and 3n tench. According to Flajšhans (1996) and Sedláček (1999), who made a histological study of tench gonads in relation to ploidy and sex, the differences between 2n and 3n tench gonads are not only in the different stages of oogenesis and spermatogenesis, but also in the presence of connective (and adipose) tissues. According to these authors, the two components did not usually constitute more than 5% of the ovaries of 2n female tench but the ovaries of infertile 3n female tench consisted of up to 20% of connective tissue (up to 40% adipose tissue). While in 2n male tench, connective tissue made about 10% of their testes, in infertile 3n male tench it exceeded 10%.

The priority utilisation of endogenously synthesized Pro by the developing gonads of 2n and 3n tench was probably decisive for the trend found in the present study between Pro level in muscle protein and the tench age.

Results of the present study demonstrated that, under the conditions of the experiment, the qualitative and quantitative compositions of some amino acids bound in muscle proteins of fish may be statistically significantly influenced by such biotic factors like sex, ploidy and age. There are, however, no studies on the effect of those factors on amino acid composition of tench proteins. It would therefore be very useful to extend the experimental design of the experiment in the future to cover the entire range of amino acids in order that the findings can be compared and the effect of the factors investigated reassessed.

Analýza složení aminokyselin diploidní a triploidní populace lína obecného (*Tinca tinca* Linnaeus 1758)

Cílem práce bylo stanovení rozdílů aminokyselinového složení mezi diploidními (2n) a triploidními (3n) líny identické genetické specifikace a chovných podmínek v závislosti na pohlaví (F - jikernačky vs. M-mlíčáci) a věku ryb (T_3-36 měsíců vs. T_{3+}- 42 měsíců). Celkem bylo analyzováno 38 směsných vzorků svaloviny ze 137 kusů lína rozdělených v závislosti na pohlaví, ploidii a věku ryb. Vliv ploidie a pohlaví na složení spektra aminokyselin svalových proteinů se uplatnil výrazněji u populace línů T_3. Zjištěny byly následující rozdíly hodnot: jikernačky (vliv ploidie): Thr ($p < 0.05$), Arg ($p < 0.05$), Pro ($p < 0.01$) Gly ($p < 0.01$); mlíčáci (vliv ploidie): Ile ($p < 0.01$); diploidi (vliv pohlaví): Arg ($p < 0.05$); triploidi (vliv pohlaví): Pro ($p < 0.01$). U populace línů T_{3+} byly zjištěny vysoce signifikantní ($p < 0.01$) rozdíly v obsahu Gly u jikernaček (vliv ploidie) a triploidů (vliv pohlaví). V závislosti na zvyšujícím se věku (T_3 - T_{3+}) línů byl zjištěn ve svalovině u línů T_{3+} nižší obsah následujících aminokyselin: Val, Phe, Lys, His, Arg (u F 2n a M 3n), Pro (u F 3n) a Tyr. Obsahy následujících aminokyselin: Thr, Ile, Leu, Arg (u M 2n a F 3n), Asp, Glu, Gly a Ala byly u línů T_{3+} vyšší. Práce ukazuje, že kvalitativní a kvantitativní složení některých aminokyselin vázaných ve svalových proteinech línů může být statisticky významně ovlivňováno některými biotickými faktory jako jsou pohlaví, ploidie nebo věk.

Acknowledgements

This experimental study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Research Plans MSM6215712402) and carried out in cooperation with University of South Bohemia âeské Budûjovice, Research Institute of Fish Culture and Hydrobiology (USB RIFCH) at VodÀany. The authors_ thanks are due to all collaborators from USB RIFCH and students of the University of South Bohemia âeské Budûjovice for their help during spring and autumn processing the fish and for technical assistance.

The authors would also like to thank Doc. Ing. Eva Straková, Ph.D. and other workers of the Department of Nutrition, Dietetics, Zoohygiene and Plant Products, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, for qualitative and quantitative analyses of amino acids on the amino acid automatic analyser T339.

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