Chemical composition of flesh of diploid and triploid population of tench (*Tinca tinca*, Linnaeus 1758)

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ABSTRACT: The objective of this study was to determine differences in relevant parameters of the basic chemical composition of flesh (descriptors: dry matter – DM, crude proteins – CP, net proteins – NP and net muscle proteins – NMP, fat – F, ash – A) of communal stock of diploid (2*n*) and triploid (3*n*) tench (*Tinca tinca* L.) of the same origin, in relation to sex (F: female vs. M: male) and age (T_3: 36 months, after overwintering vs. T_{3+}: 42 months, after one summer in the pond). Altogether 137 siblings of tench were studied. The basic chemical composition of flesh of 2*n* and 3*n* tench of T_3 age was found to be nearly the same. The only significant difference ($P < 0.05$) was found in DM content in diploids (5.7% higher DM content in females). Marked changes in the basic chemical composition were related to the increasing age of tench: after one summer in the pond (from T_3 to T_{3+}) the DM content increased significantly in all groups of tench (by 5.4% in 2*n* females, $P < 0.05$; by 6.5% in 2*n* males, 9.9% in 3*n* females and by 7.4% in 3*n* males, all at $P < 0.01$). The content of nitrogenous substances was found to increase significantly ($P < 0.01$) in flesh of 2*n* males: CP by 3.2%, NP by 4.2% and NMP by 3.3% and in flesh of 3*n* males: CP by 7.0%, NP by 6.6% and NMP by 7.3%. Muscles of 3n females contained 107.3% higher fat content ($P < 0.05$). The proportion of ash decreased in 2*n* and 3*n* by 2.5% and 1.7%, respectively ($P < 0.05$). The flesh quality of T_{3+} tench after one summer in the pond was higher if compared with that of T_3 tench after overwintering. Related to the ploidy level, more favourable chemical composition of flesh and more favourable flesh quality were found for triploids of T_{3+} age.

Keywords: tench; genome polyploidy; dry matter; crude proteins; net proteins; net muscle proteins; fat; ash

Flesh quality can be characterized by the results of laboratory analyses of the basic chemical composition of flesh. The main assessed descriptors are the content of dry matter, crude protein, lipids and ash. The quality of the proteinaceous component of flesh (crude protein) can be further assessed by means of analytical determination of other two qualitatively different descriptors: content of net protein and net muscle protein.

Marked changes in the basic chemical composition of flesh occur in mature fish in relation to the reproductive cycle (Lachowicz and Kołakowski,

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2001). These are mainly changes in the content of water and lipids in flesh, affecting its sensory (consistency, texture, colour, taste), technological and culinary properties.

No marked changes in the basic chemical composition of flesh are observed in reproductively sterile chromosomally-manipulated fish. Yearly stable colour of flesh of polyploid fish along with lower content of water and higher content of fat was reported by Lincoln (1987), who studied triploid populations of rainbow trout (*Oncorhynchus mykiss*).

The basic chemical composition of tench (*Tinca tinca*) flesh was described in the 1960s to the 1980s (e.g. Klejmenov, 1962; Jirásek and Laudát, 1984). Zięcik and Sławiński (1965) and Jevtić (1975) evaluated the chemical parameters of tench flesh also in relation to sex. Jevtić (1975) did not find any significant sex-related differences in any of the studied parameters. During the last decade, the topical studies of tench were focused mainly on the composition of fatty acids of lipids (Vácha and Tvrzická, 1995, 1998; Quirós and Alvariño, 1998; Steffens *et al*., 1998).

The basic chemical composition of fish flesh can also be affected by other endogenous (fish species, age, sex, health) or exogenous factors (living conditions, food availability, season), as shown in many papers (Love, 1975; Whittle *et al*., 1980; Hatae *et al*., 1995; Bandarra *et al*., 1997). In our experiment, we investigated the effect of some factors such as sex, ploidy level (diploidy, triploidy) and life cycle (age, season) on changes in the basic chemical composition of tench flesh. For the purposes of this study, diploidy is taken to mean a natural status of fish somatic cells containing two sets of chromosomes while triploidy is taken to mean an induced change in the genome constitution resulting in three sets of chromosomes in somatic cells of triploid fish.

The objective of this study was to determine differences in the basic chemical composition of flesh of diploid (2*n*) and triploid (3*n*) tench (*Tinca tinca* L.) of the same origin and reared in a communal stock, in relation to sex (F: female vs. M: male) and age (T_3: 36 months, after overwintering vs. T_{3+}: 42 months, after one summer in the pond) while 6 descriptors (dry matter – DM, crude proteins – CP, net proteins – NP and net muscle proteins – NMP, fat – F, ash – A) were determined analytically. Using these descriptors, this study should show a possibility to demonstrate and determine differences in the quality of flesh of diploid and triploid tench in order to assess the commercial production of fast-growing triploid tench for human consumption also from this aspect.

This study is a part of complex assessment of diploid and triploid tench and is associated with papers published by Buchtová and Vorlová (2002) and Buchtová *et al*. (2003a,b; 2004). Tench is an important supplemental species of pond aquaculture in the Czech Republic with annual production of about 200 t and triploidy represents an interesting possibility of production enhancement.

MATERIAL AND METHODS

Data concerning the description and origin of control (diploid) and experimental (triploid) populations of tench (Flajšhans and Linhart, 2000), their identification and ploidy level assessment (Vindelov and Christensen, 1990; Lecommandeur *et al*., 1994), rearing and sex determination (Flajšhans *et al*., 1993; Kvasnička and Flajšhans, 1993) were already published by the authors' team in this journal (Buchtová *et al*., 2003). Owing to this fact, only basic information is given on the origin of the studied fish populations.

Tench (*Tinca tinca*, L.) diploid and triploid populations originated from the hatchery of the Department of Fish Genetics and Breeding at Vodňany, Research Institute of Fish Culture and Hydrobiology of the University of South Bohemia. They were produced in 1998.

Altogether, 137 siblings of tench were studied. The experiment was carried out in two periods (T_3 old tench: 36 months, after overwintering; T_{3+} old tench: 42 months, after one summer in the pond).

We determined 6 descriptors of the basic chemical composition: content of dry matter in flesh (in g/kg) – DM, crude proteins – CP, net proteins – NP, net muscle proteins – NMP, fat – F and ash – A.

The DM content was determined gravimetrically following the reference method (Czech Standard No. 57 6021) for determination of moisture content in flesh by drying the sample with sand up to constant weight at +103 ± 2°C. The CP content was determined as an amount of organically bound nitrogen (conversion coefficient f_1 = 6.25) using a semiautomatic analyser Kjeltec System 1026 (Tecator, Sweden) by the method following the manufacturer's recommendation (AN 86/87). The NP content was determined by the same method

after precipitation with hot tannin solution. The NMP content was determined by subtracting the amount of collagen from NP content. The content of collagen was computed from the content of the amino acid hydroxyproline (conversion coefficient f_2 = 8). Hydroxyproline was determined quantitatively by photometric measurement of absorbance at 550 nm on Carl Zeiss Jena spectrophotometer (Germany). Fat content was determined quantitatively by extraction in diluents using a Soxtec semiautomatic system (Tecator, Sweden) by the method following the manufacturer's recommendation (AN 67/83). Ash content was determined gravimetrically by burning the weighed sample in a muffle oven (Elektro LM 212.11, Germany) at 550°C until disappearance of black carbon particles (Czech Standard No. 57 0185).

Basic statistics (mean ± S.D.) were calculated in Excel 97. The effect of ploidy and sex on the studied descriptors was evaluated separately for tench of T_3 and T_{3+} age by means of multifactorial ANOVA in STATGRAPHICS 5.0, whereas the effect of increasing age (T_3 – T_{3+}) on the studied descriptors was assessed separately for each group (F 2*n*, M 2*n*, F 3*n*, M 3*n*) at $^*P < 0.05$; $^{**}P < 0.01$ by means of one-way ANOVA using EXCEL 97.

RESULTS

Contents of the descriptors studied (DM, CP, NP, NMP, F, A) in flesh of tench in T_3 age (36 months, after overwintering) were not found significantly affected neither by ploidy level, nor by sex, except for the DM content of diploids (sex effect). Flesh of 2*n* females of T_3 tench contained 5.7% more DM ($P < 0.05$; Table 1).

The basic chemical composition of flesh of tench at T_{3+} age (42 months, after one summer in the pond) was significantly affected by ploidy level and by sex ($P < 0.05$) in 6 cases (Table 2). Higher values of DM content were registered in relation to ploidy level in T_{3+} triploids of both sexes and in the case of females the difference was found to be significant ($P < 0.05$). Significantly higher results of DM content ($P < 0.05$) were found for females of both ploidy levels (2*n* and 3*n*) (sex effect; Table 2).

The content of CP was not affected significantly by ploidy level or sex. Similarly, contents of the other two qualitatively different proteins (NP, NMP) were also found to be nearly the same in all tench groups under study, except for significant differences ($P < 0.05$) in contents of NP by 3.2% and NMP by 3.1% in males (ploidy level effect) and NMP by 3.5% in triploids (sex effect; Table 2).

No effects of ploidy level and sex on the contents of the other descriptors (F, A) were determined in flesh of tench of T_{3+} age (Table 2).

More marked changes in the basic chemical composition of tench flesh were related to the increasing age of fish, after one summer in the pond. The DM content (Figure 1) was found to increase significantly in all groups of tench at T_{3+} age (by 5.4% in 2*n* females, $P < 0.05$; by 6.5% in 2*n* males,

Table 1. Summary results of data on chemical parameters of flesh (fillet without skin) of diploid (2*n*) tench (F – female, M – male) of age T_3 (36 months, after overwintering) and their artificially induced triploid (3*n*) siblings (March 2001). The model of variance analysis: multiple range test. Groups with different alphabetic superscripts differ significantly at the given level of probability

Index	F 2*n* (*n* = 10) mean ± S.D.	M 2*n* (*n* = 9) mean ± S.D.	F 3*n* (*n* = 10) mean ± S.D.	M 3*n* (*n* = 10) mean ± S.D.	Statistical significance
Dry matter (g)	209.1 ± 1.24^a	197.8 ± 0.39^b	209.7 ± 0.59^a	203.2 ± 0.54^{a,b}	$P < 0.05$
Crude proteins (N × 6.25) (g/kg)	178.2 ± 0.42^a	175.9 ± 0.33^{a,b}	176.2 ± 0.39^{a,b}	173.8 ± 0.27^b	$P < 0.05$
Net proteins (N × 6.25 after precipitation with tannin) (g/kg)	164.8 ± 0.45	163.4 ± 0.27	167.3 ± 0.27	164.8 ± 0.31	*
Net muscle proteins (NP – hydroxyproline × 8) (g/kg)	160.0 ± 0.40	159.7 ± 0.25	162.4 ± 0.11	158.4 ± 0.30	*
Fat (g/kg)	16.2 ± 1.16	8.2 ± 0.40	11.0 ± 0.56	12.2 ± 0.36	*
Ash (g/kg)	11.6 ± 0.03	11.7 ± 0.06	11.4 ± 0.03	11.5 ± 0.02	*

*not significantly different at $P < 0.05$, values with superscripts a and b express significant differences ($P < 0.05$) between the groups compared

Table 2. Summary results of data on chemical parameters of flesh (fillet without skin) of diploid (2*n*) tench (F – female, M – male) of age T_{3+} (42 months, after one summer in the pond) and their artificially induced triploid (3*n*) siblings (October 2001). The model of variance analysis: multiple range test. Groups with different alphabetic superscripts differ significantly at the given level of probability

Index	F 2*n* (*n* = 20) mean ± S.D.	M 2*n* (*n* = 15) mean ± S.D.	F 3*n* (*n* = 19) mean ± S.D.	M 3*n* (*n* = 7) mean ± S.D.	Statistical significance
Dry matter (g/kg)	220.3 ± 1.11^{a}	210.6 ± 0.65^{b}	230.5 ± 0.93^{c}	218.2 ± 1.04^{a,b}	$P < 0.05$
Crude proteins (N × 6.25) (g/kg)	181.6 ± 0.49	181.5 ± 0.33	185.0 ± 0.41	186.0 ± 0.23	*
Net proteins (N × 6.25 after precipitation with tannin) (g/kg)	169.7 ± 0.39^{a}	170.3 ± 0.30^{a}	172.8 ± 0.45^{a,b}	175.7 ± 0.25^{b}	$P < 0.05$
Net muscle proteins (NP – hydroxyproline × 8) (g/kg)	163.4 ± 0.46^{a}	164.9 ± 0.31^{a}	164.3 ± 0.47^{a}	170.0 ± 0.25^{b}	$P < 0.05$
Fat (g/kg)	17.4 ± 1.36	12.5 ± 0.80	22.8 ± 1.44	17.9 ± 1.19	*
Ash (g/kg)	11.3 ± 0.03	11.4 ± 0.02	11.2 ± 0.04	11.3 ± 0.03	*

*not significantly different at $P < 0.05$

9.9% in 3*n* females and by 7.4% in 3*n* males, all at $P < 0.01$).

Considering the content of nitrogenous substances, positive qualitative and quantitative changes were noted. The content of all studied proteins significantly increased in flesh of males of both ploidy levels ($P < 0.01$): by 3.2%, 4.2% and 3.3% in diploids and by 7.0%, 6.6% and 7.3% in triploids, for CP, NP

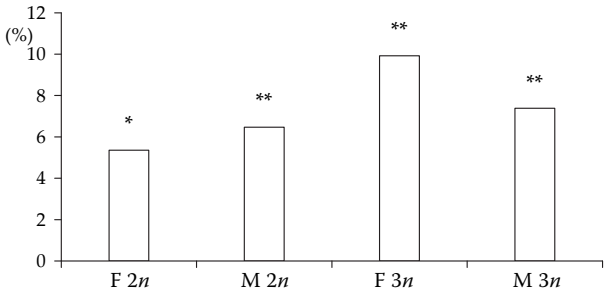

Figure 1. Comparison of the changes in % in the content of dry matter in fillets without skin of diploid (2*n*) and triploid (3*n*) tench (F – female, M – male) during the period from T_3 (36 months) to T_{3+} (42 months) of age. Asterisks *, ** express significant differences ($P < 0.05$, $P < 0.01$) between the groups compared. The model of variance analysis: one-way

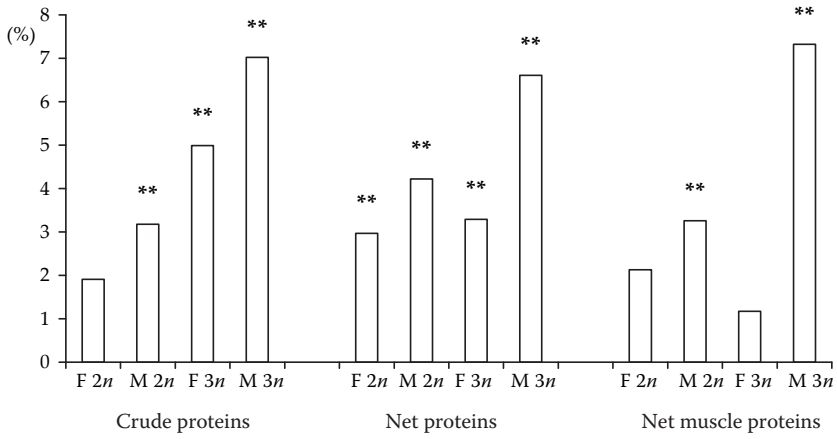

Figure 2. Comparison of the changes in % in the contents of crude, net and net muscle proteins in fillets without skin of diploid (2*n*) and triploid (3*n*) tench (F – female, M – male) during the period from T_3 (36 months) to T_{3+} (42 months) of age. Asterisks *, ** express significant differences ($P < 0.05$, $P < 0.01$) between the groups compared. The model of variance analysis: one-way

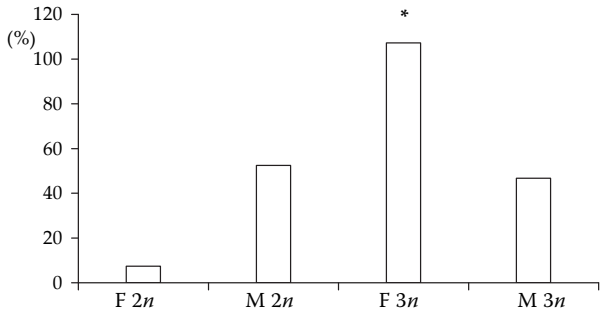

Figure 3. Comparison of the changes in % in fat content in fillets without skin of diploid (2*n*) and triploid (3*n*) tench (F – female, M – male) during the period from T_3 (36 months) to T_{3+} (42 months) of age. Asterisks *, ** express significant differences ($P < 0.05$, $P < 0.01$) between the groups compared. The model of variance analysis: one-way

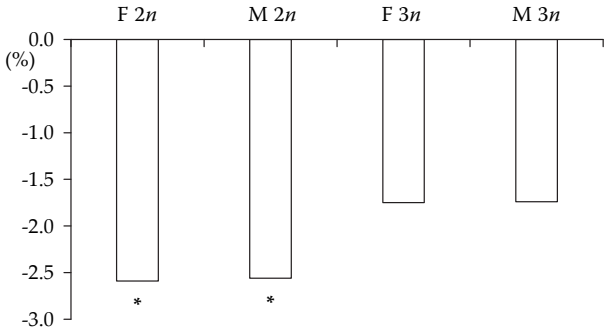

Figure 4. Comparison of the changes in % in ash content in fillets without skin of diploid (2*n*) and triploid (3*n*) tench (F – female, M – male) during the period from T_3 (36 months) to T_{3+} (42 months) of age. Asterisks *, ** express significant differences ($P < 0.05$, $P < 0.01$) between the groups compared. The model of variance analysis: one-way

and NMP, respectively. Triploid T_{3+} females were found to have significantly increased CP content by 5.0% ($P < 0.01$). The flesh of T_{3+} females of both ploidy levels contained a significantly higher proportion of NP (by 3.0% in diploids and by 3.3% in triploids; $P < 0.01$). However, the NMP values of females remained nearly unchanged with increasing age (Figure 2).

The flesh of 3*n* females at T_{3+} age was found to have 107.3% higher fat content ($P < 0.05$). No such differences were found in this descriptor between the other groups of tench under study, (Figure 3).

Related to increasing age, T_{3+} tench were found to have decreased A content in flesh: by 2.6% in diploids and by 1.7% in triploids. This decrease was found significant for diploids ($P < 0.05$; Figure 4).

DISCUSSION

The quality of tench flesh in our experiment was found to be affected strongly positively by the summer growing season, during which the fish organism was sufficiently saturated with necessary nutrients and energy. This fact results from a comparison of the values of the descriptors of basic chemical composition of tench flesh (DM, CP, NP, NMP, F, A) analysed in fish of two age categories: T_3 36 months, after overwintering, at the beginning of the experiment and T_{3+} 42 months, after one summer in the pond, at the end of the experiment. Apart from fish age, the flesh quality was also affected by ploidy level and by sex in particular cases.

The basic chemical composition of flesh was found to be nearly identical for all groups of tench at the beginning of the experiment. Results of laboratory analyses of the studied descriptors, e.g. high content of nitrogenous substances or low fat content (Tables 1, 2), refer to values published for this freshwater fish species (Zięcik and Sławiński, 1965; Jirásek and Laudát, 1984). Contrary to findings of Jevtić (1975), we found a sex-related significant difference in DM content for the diploid population at T_3 age ($P < 0.05$). This fact might probably be associated with the low final weight of immature (juvenile) 2*n* males of T_3, as revealed in the same groups of fish by Buchtová *et al.* (2003a,b). Higher content of water in the flesh of this group of tench is also connected with very low fat content (Table 1).

The basic chemical composition of flesh of all groups of tench was strongly positively affected by the summer growing period either qualitatively or quantitatively (Figures 1–4). Favourable living conditions during the summer period led to higher deposition of fat in flesh and, accordingly, to a decrease in water content. These changes were reflected most markedly in the heaviest reproductively sterile triploid females in which the fat content was found to increase by 107.3% ($P < 0.05$) and dry matter content by 9.9% ($P < 0.01$). Similarly, the other groups of T_{3+} tench under study were also found to have significantly increased content of dry matter in flesh but there were no accompanying significant changes in fat content (Figures 1 and 3). Positive effects of the studied factors (grow-

ing season, polyploidization) on fat content in flesh were also described in other fish species (trout by Lincoln, 1987; sardines by Bandarra *et al*., 1997).

Positive nitrogen balance in the fish organism caused the increase in nitrogenous substances (CP, NP, NMP) in all groups of tench during the period of study (Figure 2). Considering the males of both ploidy levels this highly significant increase ($P < 0.01$) in all three qualitatively different proteins (by 3.2%, 4.2% and 3.3% in diploids and by 7.0%, 6.6% and 7.3% in triploids for CP, NP and NMP, respectively; Figure 2) might be associated with the production of testosterone (anabolic effects), the secretion of which might be maintained also in reproductively sterile triploid males. A probable reason for significant differences in NP (by 3.2%) and NMP (by 3.1%) values of males in relation to the ploidy level ($P < 0.05$) and NMP (by 3.5%) values of triploids in relation to sex (Table 2) might be the increased utilization of nitrogenous substances in somatic tissues during the summer season, taking place in reproductively sterile triploid males at T_{3+} age with partially retarded testicular development. The acceleration of somatic growth of triploid tench due to preferential utilization of nutrients and energy by tissues as well as the retarded growth of gonads were reported by Flajšhans *et al*. (1993) and Flajšhans (1997). The progressive growth of active body mass during the summer growing period, described in identical groups of tench by Buchtová *et al*. (2003a,b), was associated with decreased ash content in flesh by 2.6% in diploids and 1.7% in triploids, which was found significant for diploids in relation to age ($P < 0.05$).

Resulting from the values of descriptors of the basic chemical composition, the quality of flesh of T_{3+} old tench after one summer in the pond was higher compared to that of T_3 old tench just after overwintering. Related to the ploidy level, the triploid population of tench showed more favourable composition and quality of flesh. These differences in the flesh composition were statistically significant in given cases (DM content of females, NP and NMP contents of males, $P < 0.05$; Table 2). As for the flesh of T_3 old tench, higher values of DM content were found for females of both ploidy levels also in tench of T_{3+} age ($P < 0.05$).

The study demonstrated that the quality of flesh of the triploid population of tench assessed by means of descriptors of the basic chemical composition (DM, CP, NP, NMP, F, A) is comparable to that of the diploids. After the summer growing period, the quality of flesh of triploid females in given cases (DM content) may be significantly higher ($P < 0.05$), as well as that of triploid males (NP and NMP contents).

We may conclude on the basis of this study as well as of other findings (Buchtová *et al*., 2003a,b, 2004) that using the chosen parameters, triploid tench in comparison with their diploid control did not show any changes in flesh quality or the changes in the composition and quality of flesh were positive. From this aspect, we may recommend the method of production of fast-growing triploid tench for human consumption to the fishery practice.

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