

THE FIRST FINDINGS OF THE DIFFERENCES IN COMPLETE BLOOD COUNT BETWEEN DIPLOID AND TRIPLOID TENCH, *Tinca tinca* L.

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Abstract

Svobodová, Z., J. Kolářová, M. Flajšhans: *The First Findings of the Differences in Complete Blood Count Between Diploid and Triploid Tench, Tinca tinca L.* Acta vet. Brno 1998, 67: 243–248.

The aim of this work was to evaluate differences in complete blood count of diploid and triploid tench and concentration of total protein in blood plasma of these fish. Haematological examination of 78 specimens of three-year-old tench, consisting of 21 triploids and their 57 diploid siblings was performed in July 1996. Analysis of variance test confirmed a significantly lower erythrocyte count in triploids ($P < 0.01$), as well as a significantly lower haemoglobin content ($P < 0.05$), significantly higher medium corpuscular volume, higher medium corpuscular haemoglobin and lower medium corpuscular haemoglobin concentration (all at $P < 0.01$) compared to diploids. The haematocrit value of triploids was insignificantly lower compared to diploids. The differences of erythrocyte nuclear area, perimeter, average size, major axis and aspect ratio between diploids and triploids were found significant at $P < 0.01$ and the effect of ploidy level on these dimensions was considered highly significant ($P < 0.0001$). No effect of ploidy level was proven for erythrocyte nuclear minor axis. The leukocyte count of triploids was insignificantly lesser compared to diploids. Triploid tench were found of significantly lower concentration of total protein in blood plasma compared to diploids ($P < 0.01$). Triploid tench could be hypothesized to have a lower oxygenation capacity of blood, as well as a lower non-specific immunity level compared to diploids. Insignificantly higher erythrocyte count, haemoglobin content, haematocrit value, and significantly higher concentration of total protein in blood plasma ($P < 0.01$) was reported for diploid females compared to males.

Ploidy level, complete red blood count, erythrocyte nuclear dimensions, leukocyte, total protein

The haematological indices of diploid and triploid fish of various species were compared by several authors, mostly regarding the complete red blood count of salmonids (Benfey and Sutterlin 1984ab; Benfey et al. 1984; Small and Benfey 1987; Nakamura et al. 1989; Tambets et al. 1991; Biron and Benfey 1994; Thomas and Morisson 1995) or of other species mostly with that of cyprinids or silurids (Sezaki et al. 1977; Liu et al. 1978; Wolters et al. 1982; Beck and Biggers 1983; Barker et al. 1983; Sezaki et al. 1983; Krasznai et al. 1984; Ueno 1984; Suzuki et al. 1985; Wattendorf 1986; Sezaki et al. 1991; Boron 1994). Based on these data, the following values of complete red blood count indices of triploid fish can be generalized: triploids have erythrocytes of larger medium corpuscular volume caused above all by increment in size of the major erythrocyte axis. The differences in both erythrocyte cellular and nuclear dimensions between diploids and triploids are well described (e.g. Swarup 1959; Sezaki and Kobayasi 1978; Lemoine and Smith 1980; Wolters et al. 1982; Benfey et al. 1984; Flajšhans 1997 and others) and used for ploidy level discrimination in many fish species. Triploids have also a lower count of erythrocytes compared to diploids. The haematocrit value of triploids and diploids is similar. The

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amount of haemoglobin of triploids is usually lower than that of diploids. In contrary, the medium corpuscular haemoglobin of triploids is higher compared to diploids. The value of medium corpuscular haemoglobin concentration of erythrocytes of triploids and diploids is stated to be approximately at the same level.

The haematology of diploid tench, *Tinca tinca* L. was examined by several authors, mostly dealing with seasonal changes in some indices (Einzsporn-Orecka 1970; Svobodová et al. 1978; Collazos et al. 1993). Data on haematological indices of tench females and males were also reported (Svobodová et al. 1978; Habekovič 1991). From this point of view, tench was also used in studies on pollutant effects and other changes in the aquatic environment (Demaël et al. 1980; Kreutzmann 1984; Demaël et al. 1984; Al-Sabti 1986) and/or on the effects of three different anaesthetics on fish (Svobodová et al. 1988).

To the best of our knowledge, no references concern haematological comparison of diploid and triploid tench but the very first brief communication on image analysis of erythrocyte nuclei by Flajšhans (1996). The aim of this paper is to report on the results of haematological investigation of tench of both ploidy levels.

Materials and Methods

Triploid tench were produced at the authors' workplace, by cold shock as described by Flajšhans et al. (1993). Three-year-old triploids and their diploid siblings were sampled from pond performance tests, in total of 78 specimen, consisting of 21 triploids (138.6 ± 7.96 g body weight) and 57 diploids (173.3 ± 5.73 g bw) in July 1996.

Blood was sampled from the caudal vessel into a heparinized syringe. The following indices were determined according to Svobodová et al. (1986): erythrocyte count (Er), haematocrit value (PCV), haemoglobin content (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Erythrocyte nuclear dimensions (area, perimeter, average nuclear size, major axis, minor axis) were measured by microscope-coupled computer-assisted image analysis according to Flajšhans (1997). Aspect ratio was chosen as an additional index and computed as a ratio of minor axis: major axis.

Moreover, leukocyte count (Leuco) and concentration of total protein in blood plasma (TP) were determined according to Svobodová et al. (1986) with the latter index assessed refractometrically.

In order to test the effect of the ploidy level (diploidy vs. triploidy) and/or of the sex in diploids (female vs. male) on values of the respective haematological indices statistically, the analysis of variance (ANOVA) in Statgraphics 5.0 was used.

Results

Values of the respective haematological indices of diploid and triploid tench are given in Table 1. In triploids compared to diploids, there was evident a significantly lower erythrocyte count (ANOVA, $P < 0.01$), haemoglobin content (ANOVA, $P < 0.05$) and mean corpuscular haemoglobin concentration (ANOVA, $P < 0.01$), whereas the mean corpuscular volume and mean corpuscular haemoglobin were higher (ANOVA, $P < 0.01$). The haematocrit value of triploids was insignificantly lower (ANOVA, $P > 0.05$) compared to diploids. The differences of erythrocyte nuclear area, perimeter, average size, major axis and aspect ratio between diploids and triploids were found significant at $P < 0.01$ (ANOVA) and the effect of ploidy level on these dimensions was considered highly significant (ANOVA, $P < 0.0001$). No effect of ploidy level was proven for the dimension of erythrocyte nuclear minor axis (ANOVA, $P > 0.05$). Erythrocyte nuclei of triploid tench were found slightly more elliptic (Fig. 1). The leukocyte count of triploids was insignificantly lower (ANOVA, $P > 0.05$) compared to diploids. Triploids were found to have expressively lower concentration of total protein in blood plasma compared to diploids (ANOVA, $P < 0.01$).

Table 1
Body weight, indices of complete red blood count, erythrocyte nuclear dimensions, leukocyte count and total protein concentration in blood plasma of diploid and triploid tench (*Tinca tinca* L.) under study

Indices	Unit	Diploids		Triploids		Statistical significance
		n	mean \pm SD	n	mean \pm SD	
Body Weight	g	57	173.30 \pm 5.73	21	138.60 \pm 7.96	P < 0.01
Er	T.l ⁻¹	36	1.34 \pm 0.05	16	0.92 \pm 0.04	P < 0.01
PCV	l.l ⁻¹	52	0.34 \pm 0.01	20	0.32 \pm 0.02	
Hb	g.l ⁻¹	52	79.80 \pm 2.48	19	69.90 \pm 4.03	P < 0.05
MCV	fl	36	254.00 \pm 7.38	16	355.00 \pm 17.60	P < 0.01
MCHC	l.l ⁻¹	52	0.24 \pm 0.004	19	0.22 \pm 0.006	P < 0.01
MCH	mmol.l ⁻¹	36	60.70 \pm 1.74	16	75.30 \pm 4.65	P < 0.01
Nuclear Area	μ m ²	57	10.58 \pm 1.28	21	16.50 \pm 1.71	P < 0.01
Nuclear Perimeter	μ m	57	12.44 \pm 0.81	21	15.78 \pm 0.94	P < 0.01
Average Nuclear Size	μ m	57	4.00 \pm 0.24	21	5.05 \pm 0.23	P < 0.01
Nuclear Major Axis	μ m	57	4.67 \pm 0.38	21	6.06 \pm 0.49	P < 0.01
Nuclear Minor Axis	μ m	57	3.19 \pm 0.30	21	3.75 \pm 0.37	
Aspect Ratio	-	57	0.69 \pm 0.09	21	0.63 \pm 0.09	P < 0.01
Leuco	G.l ⁻¹	47	62.20 \pm 5.52	20	58.20 \pm 7.40	
TP	g.l ⁻¹	52	14.80 \pm 1.11	20	8.70 \pm 1.53	P < 0.01

Table 2
Body weight, indices of complete red blood count, leukocyte count and total protein concentration in blood plasma of diploid females and males of tench (*Tinca tinca* L.) under study

Indices	Unit	Females		Males		Statistical Significance
		n	mean \pm SD	n	mean \pm SD	
Body Weight	g	16	174.10 \pm 6.59	18	156.30 \pm 5.60	P < 0.05
Er	T.l ⁻¹	15	1.30 \pm 0.077	16	1.42 \pm 0.079	
PCV	l.l ⁻¹	16	0.33 \pm 0.022	17	0.38 \pm 0.019	
Hb	g.l ⁻¹	16	78.60 \pm 4.18	17	85.40 \pm 4.33	
MCV	fl	15	244.00 \pm 9.60	16	268.00 \pm 10.80	
MCHC	l.l ⁻¹	16	0.25 \pm 0.007	17	0.23 \pm 0.005	
MCH	mmol.l ⁻¹	15	61.90 \pm 3.41	16	60.60 \pm 1.91	
Leuco	G.l ⁻¹	15	54.80 \pm 9.00	16	73.80 \pm 12.60	
TP	g.l ⁻¹	16	18.70 \pm 2.39	17	11.70 \pm 1.01	P < 0.01

Table 2 shows the body weight and the haematological indices of diploid tench females and males. Body weight was higher for females compared to males (ANOVA, P < 0.05). There was found a higher erythrocyte count, haemoglobin content and haematocrit value of males compared to females. However, these differences were found insignificant (ANOVA, P > 0.05). Similarly, no significant differences between both sexes were found in other indices of complete red blood count (MCV, MCH, MCHC). Insignificantly higher leukocyte count was found in males compared to females (ANOVA, P > 0.05). In contrary, a significantly higher concentration of total protein in blood plasma of females was found compared to that of males (ANOVA, P < 0.01).

Discussion

To our knowledge, no data on the haematology of triploid tench are available. Comparing our results with those on triploid salmonids, other triploid cyprinids (common carp, *Cyprinus carpio* L. and herbivorous cyprinids) and triploid silurids, there is a good accordance with the data of Barker et al. (1983), Benfey and Sutterlin (1984a), Krasznai et al. (1984), Ueno (1984), Wattendorf (1986), Small and Benfey (1987), Flajšhans (1997) and others. Data on tench erythrocyte nuclear dimensions do not differ from those preliminarily published by Flajšhans (1996).

Based on significantly lower values for haemoglobin content and MCHC we could anticipate triploid tench to have a lower oxygenation capacity of blood compared to the diploid ones. Such a lower oxygenation capacity of blood that would appear in fish above all after exposure to stress, was reported e. g. for triploid rainbow trout (*Oncorhynchus mykiss*) by Virtanen et al. (1990), for triploid Atlantic salmon (*Salmo salar*) by Benfey and Sutterlin (1984b) and for triploid brook trout (*Salvelinus fontinalis*) by Stilwell and Benfey (1996).

Considering the lower leukocyte count and especially the markedly lower concentration of total protein in blood plasma of triploids, we suppose that triploids have a lower non-specific immunity level compared to diploids. Examination of the non-specific immunity of tench of both ploidy levels would be highly desirable for the future, since such data are missing in the literature. Moreover, they are not stated in studies of triploids of other fish species except for triploid rainbow trout by Yamamoto and Iida (1994), stating non-specific defence activities based on chemiluminescence determination of whole blood stimulated by zymosan.

The sex-related differences in values of complete red blood count of tench are in accordance with data of Einzsporn-Orecka (1970), Svobodová et al. (1978), Habekovič (1991), as well as with those for common carp (Svobodová 1973), northern pike, *Esox lucius* (Červinka and Pecha 1975; Hamáčková et al. 1975) and several other fish species. In all these cases, insignificantly or significantly higher erythrocyte count, haemoglobin content and haematocrit value were recorded for males as compared to females. Such increased levels in males are associated with a more intensive metabolism of males (Stroganov 1962). As found for common carp and tench (Svobodová 1973) this sex-related difference does not maintain the same level throughout the whole year: it is observed in certain periods only, and during some time of the year the difference in complete red blood count is insignificant or there is no difference at all. This is associated with the annual cycle of gonadal development.

There was recorded about $7 \text{ g} \cdot \text{l}^{-1}$ more total protein in tench females compared to males. A similar highly significant sex-related difference was recorded in wels, *Silurus glanis* L. ($6 \text{ g} \cdot \text{l}^{-1}$, Svobodová et al. 1981). Protein concentration in blood plasma is associated very closely to the sedimentation rate of erythrocytes (Karásek et al. 1960). Such a significantly higher erythrocyte sedimentation rate in tench females than in males was found by Habekovič (1991). This would refer to a higher protein concentration in blood plasma of females.

Odlišnosti krevního obrazu diploidních a triploidních línů, *Tinca tinca* L. - první nálezy

Cílem práce bylo posoudit rozdíly v červeném krevním obrazu diploidních a triploidních línů a rozdíly v počtu leukocytů a v obsahu celkových bílkovin v krevní plazmě u těchto ryb. Hematologické vyšetření bylo provedeno u 78 kusů tříletých línů obecných,

z toho u 21 triploidů a jejich 57 diploidních sourozenců v červnu 1996. Analýzou variance byl u triploidních linií potvrzen signifikantně nižší počet erytrocytů ($P < 0.01$), nižší obsah hemoglobinu ($P < 0.05$), vyšší střední objem erytrocytů, nižší střední barevná koncentrace a vyšší hodnota hemoglobinu v erytrocytu (vše na hladině $P < 0.01$) ve srovnání s diploidy. Hematokritová hodnota byla u triploidů nevýznamně nižší ve srovnání s diploidními linií ($P > 0.05$). Rozdíl ve velikosti plochy jádra erytrocytu, obvodu jádra, jeho průměrné velikosti, délce dlouhé osy a v poměru os jádra mezi diploidy a triploidy byly shledány signifikantními na úrovni $P < 0.01$ a vliv ploidie na tyto rozměry byl shledán vysoce významným ($P < 0.0001$). Nebyl prokázán vliv ploidie na délku krátké osy jádra erytrocytu. Počet leukocytů byl u triploidů nevýznamně nižší ve srovnání s diploidními linií ($P > 0.05$). U triploidních linií byl naměřen vysoce signifikantně nižší obsah celkových bílkovin v krevní plazmě ve srovnání s diploidními linií ($P < 0.01$). Na základě naměřených parametrů lze u triploidů předpokládat nižší oxygenační kapacitu krve a nižší úroveň nespecifické imunity ve srovnání s diploidy. Ve shodě s literárními údaji byl zjištěn nevýznamně vyšší počet erytrocytů, množství hemoglobinu, hematokritová hodnota, vyšší počet leukocytů ($P > 0.05$) a vysoce signifikantně vyšší koncentrace celkových bílkovin v krevní plazmě ($P < 0.01$) jikernaček ve srovnání s mlíčáky diploidních linií.

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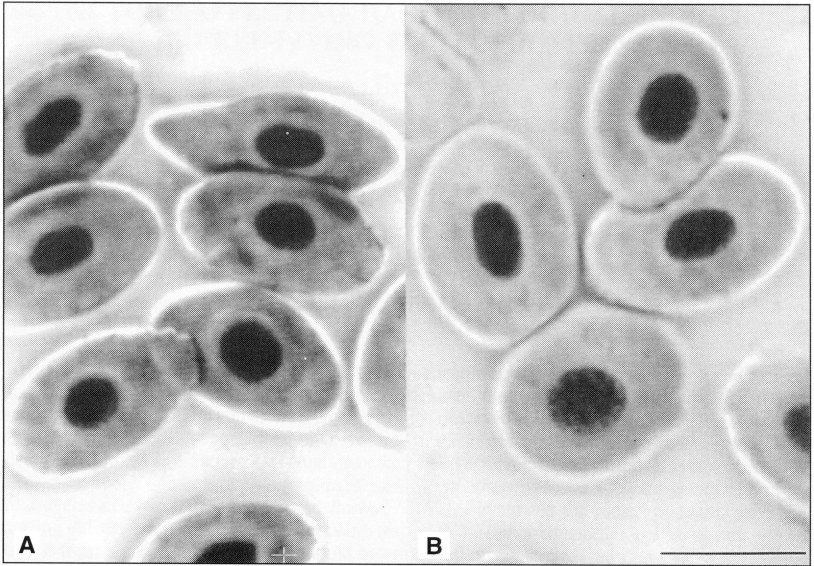


Fig. 1. Videoprintout of a computerized dark field microscopic image of erythrocytes of a diploid (A) and a triploid (B) tench, *Tinca tinca* L. Objective Olympus NCSPlan Dry 100 \times . Bar indicates 10 μ m.