

BIOCHEMICAL PROFILE OF BLOOD PLASMA OF TENCH (*Tinca tinca* L.) DURING PRE- AND POSTSPAWNING PERIOD

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Abstract

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The aim of this study was to assess the biochemical profile of tench blood plasma during pre- and postspawning period under the conditions of hormonally-induced artificial reproduction. A total of 59 females and 27 males were examined during the postspawning period of 1999 and 52 females and 25 males were examined during the prespawning period of 2000, as well as 48 females after reproduction. Biochemical indices determined in blood plasma were as follows: cortisol, glucose, total protein (TP), triacylglycerols (Tcg), cholesterol (Chol), transaminases (ALT and AST), creatine kinase (CK), alkaline phosphatase (ALP) and electrolytes (Na). In females in the pre-spawning period, higher values of TP ($P < 0.05$) and Tcg ($P < 0.01$) were found compared to males. Immediately after reproduction, males had higher TP ($P < 0.01$) and Chol ($P < 0.01$) than females. No significant sex-related differences were found in other indices under study. Higher values of glucose ($P < 0.01$), Tcg ($P < 0.01$), Chol ($P < 0.05$), AST ($P < 0.01$) and ALP ($P < 0.01$) were found for females after reproduction in June compared to values found in April, i.e. two months prior to reproduction. Differing water temperature (10.3 °C in April; 22 °C in June) associated with metabolic rate also played an important role. Induction of ovulation by GnRH synthetic analogue and carp pituitary was not successful in all females. However, between the spawned and unspawned female fish, differences were found in glucose concentration ($P < 0.01$) but non-significant differences were recorded for other biochemical indices. The blood plasma biochemical profile enabled to assess the state of internal milieu of broodstock during the reproduction period.

Stress, conditions, lipid analytes, enzymes, artificial reproduction, sex differences

Great attention has been recently paid to biochemical characterization of fish blood as to an index of the state of internal milieu (Lusková 1997b; Böttcher 1998; Edsall 1999). For assessment of biochemical indices of fish blood, analysis of blood plasma is preferred to that of blood serum (Hrubec and Smith 1999). Data on the values of biochemical indices either in blood serum, or blood plasma of tench are very sporadic.

Most papers dealt with the concentration of glucose and/or of proteins in blood plasma. Values of glucose concentration in blood plasma of tench were used as indicators of temperature stress (Demaël and Garin 1978), as well as an indicator of stress caused by drop of water pH (Demaël et al. 1984). De-Pedro et al. (1998) evaluated the daily rhythm and effect of diurnal changes in tench also by means of changes in concentration of plasma glucose and cortisol. Significant day/night changes in plasma cortisol concentrations were detected with a peak at the beginning of the dark phase. Glucose concentration showed the highest values at 4 h after feeding. Seasonal variability of various haematological indices of tench were studied by Collazos et al. (1993). Apart from haematological examination, they also studied the concentrations of plasma proteins

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(albumin, α -, β 1- β 2- and γ -globulin). The highest protein concentrations were found during the summer period while the lowest ones were found in winter. In summer, there were high concentrations of globulins while the highest concentrations of albumin were found at the onset of reproduction (spring). Total protein concentration in 78 specimens of three-year-old tench, consisting of 21 triploids and 57 diploids was reported by Svobodová et al. (1998). Triploids were found to have significantly lower concentration of total protein in blood plasma compared to diploids.

In this study, the selected biochemical indices were used in order to assess the changes in internal milieu of the organism of brood tench during pre- and postspawning period of artificial reproduction. Both the natural and artificial reproduction of fish belong to factors seriously affecting internal milieu of the organism (Marquez 1976; Hlavová 1989; Lusková and Lusk 1995a, 1995b; Hochachka and Mommsen 1995; Lusková 1997a; Svoboda et al. 2000).

The goal of this study was to assess the values of selected biochemical indices of blood plasma of tench during pre- and postspawning period of artificial reproduction.

Materials and Methods

A group of 4-to-5-year-old brood tench, both females and males were kept in monoculture in a pilot-scale pond. Individual females were marked cryogenically by numeric signs. Males were not marked. In April 1999, the fish were harvested, separated according to sex and each sex was placed into a separate pond. There was sufficient natural food in the ponds, apart from that the fish were given supplemental cereal feeding. In June 1999, both ponds were harvested and fish stocked into 4 flow-through troughs supplied with pond water. Five days later, ovulation was induced in the first group of females by a synthetic superactive analogue of gonadotropin releasing hormone (GnRH) at a dose of 20 $\mu\text{g}\cdot\text{kg}^{-1}$ (Kouřil et al. 1986). Females of the second group were injected with suspension of carp pituitary at a dose of 2 $\text{mg}\cdot\text{kg}^{-1}$ (Kouřil 1987). Females of the third group (control) were not treated hormonally but handled identically to the injected females. Males were placed in the 4th trough. Spermiation was induced by means of carp pituitary similarly to the 2nd group of females. All injections were single im. doses. Artificial reproduction was performed on June 20, 1999 and blood was sampled immediately afterwards. In part of the hormonally-stimulated females, as well as in the control females, no ovulation occurred (the fish did not spawn). At the same time, blood sampling was performed in these fish. Blood was sampled from 59 females and 27 males. Water temperature was 21 °C. Both the spawned and not-spawned females were stocked together with males into a pond on the next day for further culture.

In spring 2000, the entire procedure was repeated on the same brood fish, now 5- to 6-year-old. Blood was sampled two times, in April (the 15th) after pond harvest (from 52 females and 25 males at water temperature 10.3 °C) and in June (the 17th) 24 h after artificial reproduction. Blood was also sampled from a control group of fish and from hormonally-stimulated but not spawned females at the same time. In June 2000, blood was sampled from 48 females at water temperature of 22 °C. Either in 1999, or in 2000, all fish under study were without clinical and pathoanatomical changes. Details about induced ovulation by hormonal preparations, along with the course of artificial reproduction in tench females is reported by Kouřil et al. (2001).

In order to assess the status of internal milieu of the brood tench, the following biochemical indices of blood plasma were chosen

- stress indices (cortisol, glucose)
- indices of condition (total protein, TP)
- lipid analytes (triacylglycerols, Tgc, cholesterol, Chol)
- enzymes – tests of permeability and integrity of cell membranes (alanine aminotransferase – ALT, aspartate aminotransferase – AST); monitoring of the status of skeletal musculature and its disorders (creatine kinase – CK); supplement of information on the status of parenchymatous tissues, liver - kidney (alkaline phosphatase – ALP)
- electrolytes (sodium, Na)

These values were found and compared in:

- females and males of tench during the pre- and postspawning period (sex differences)
- females of tench during the pre- and postspawning period
- spawned and not-spawned females of tench after induction of ovulation by means of GnRH synthetic analogue and/or carp pituitary.

Blood (approximately 1 ml per fish) was sampled from v. caudalis. Prior to sampling, the fish were anaesthetised in 2-phenoxyethanol and weighed. Heparin sodium salt (50 i.u. per 1 ml blood) was used as anticoagulant (Svobodová et al. 1991). Concentration of cortisol in blood plasma was determined by means of

radioimmunoanalytical method using a commercial kit of Immunotech. Concentration of glucose, total protein (TP), triacylglycerols (Tcg), cholesterol (Chol), transaminases (ALT, AST), creatine kinase (CK), alkaline phosphatase (ALP) and sodium were determined by means of COBAS MIRA automatic analyser (F. Hoffmann, La-Roche and Co., Switzerland). Activity of plasma enzymes (ALT, AST, CK, ALP) was determined at 37 °C. Due to high values of CK activity, blood plasma was diluted 5-10 times with saline.

The results were statistically analysed by means of one-way ANOVA.

Results

Comparison of biochemical profile of blood plasma of tench females and males

Sex differences in the values of biochemical indices of brood tench in spring 2 months prior to reproduction are given in Table 1. Body weight of females was higher than that of males

Table 1
Comparison of biochemical profile of blood plasma of tench females and males during the prespawning period (15th April 2000)

| Indices | Unit | Females | | Males | | Statistical significance |
|-------------|----------------------|---------|----------------|-------|----------------|--------------------------|
| | | n | Mean ± SD | n | Mean ± SD | |
| Body weight | g | 52 | 775.4 ± 138.19 | 25 | 567.8 ± 104.57 | $P < 0.01$ |
| Glucose | mmol.l ⁻¹ | 52 | 5.93 ± 1.579 | 25 | 6.05 ± 1.142 | - |
| TP | g.l ⁻¹ | 52 | 37.5 ± 4.85 | 25 | 35.1 ± 3.09 | $P < 0.05$ |
| Tcg | mmol.l ⁻¹ | 52 | 1.40 ± 0.428 | 25 | 1.08 ± 0.230 | $P < 0.01$ |
| Chol | mmol.l ⁻¹ | 52 | 3.58 ± 0.978 | 25 | 3.45 ± 0.830 | - |
| ALT | µkat.l ⁻¹ | 50 | 0.17 ± 0.117 | 25 | 0.14 ± 0.101 | - |
| AST | µkat.l ⁻¹ | 52 | 4.72 ± 1.848 | 25 | 5.86 ± 3.807 | - |
| ALP | µkat.l ⁻¹ | 37 | 0.21 ± 0.196 | 18 | 0.32 ± 0.218 | - |

($P < 0.01$). Similarly, the indices of condition (TP) and lipid analytes (Tcg) were higher in females compared to males ($P < 0.05$ and $P < 0.01$, respectively). Values of other indices under study, i.e. glucose, cholesterol, transaminases and alkaline phosphatase were comparable for both sexes.

Values of biochemical indices of females and males of brood tench immediately after reproduction are given in Table 2. Body weight of females was higher than that of males

Table 2
Comparison of biochemical profile of blood plasma of tench females and males during the postspawning period (20th June 1999)

| Indices | Unit | Females | | Males | | Statistical significance |
|-------------|----------------------|---------|---------------|-------|---------------|--------------------------|
| | | n | Mean ± SD | n | Mean ± SD | |
| Body weight | g | 20 | 809.7 ± 126.1 | 27 | 567.5 ± 96.28 | $P < 0.01$ |
| Glucose | mmol.l ⁻¹ | 20 | 9.53 ± 3.049 | 27 | 9.77 ± 1.791 | - |
| TP | g.l ⁻¹ | 20 | 35.1 ± 5.64 | 26 | 42.4 ± 7.94 | $P < 0.01$ |
| Tcg | mmol.l ⁻¹ | 20 | 1.42 ± 0.251 | 27 | 1.33 ± 0.467 | - |
| Chol | mmol.l ⁻¹ | 20 | 3.60 ± 0.891 | 27 | 4.82 ± 1.058 | $P < 0.01$ |
| ALT | µkat.l ⁻¹ | 20 | 0.22 ± 0.113 | 27 | 0.29 ± 0.252 | - |
| AST | µkat.l ⁻¹ | 20 | 4.39 ± 1.773 | 27 | 5.13 ± 2.351 | - |
| CK | µkat.l ⁻¹ | 20 | 94.2 ± 51.85 | 27 | 125.9 ± 72.13 | - |
| ALP | µkat.l ⁻¹ | 19 | 0.77 ± 0.406 | 21 | 0.55 ± 0.291 | - |

($P < 0.01$). Higher values of total protein (TP) ($P < 0.01$) and cholesterol (Chol) ($P < 0.01$) were recorded in blood plasma of males compared to females. No significant differences between females and males were found for other indices under study (glucose, Tcg, ALT, AST, CK, ALP and Na).

Comparison of biochemical profile of blood plasma of tench females during pre- and postspawning period

is given in Table 3. Values compared were those found in April (i.e. 2 months prior to reproduction) and in June 2000. Two groups of females were analysed in June, the first one was the control group and the second one was analysed 24 h after reproduction. No ovulation was induced in the control group. At the time of blood sampling, these fish were in fact in state before natural spawning. In the second group of females, ovulation was induced by means of GnRH analogue and carp pituitary.

Differences between the compared groups were found for body weight ($P < 0.05$) and for the value of triacylglycerols ($P < 0.01$). These values were the lowest in females 2 months before reproduction, followed by rising values for the control group before natural spawning and group of females 24 h after reproduction. The lowest concentration of glucose and cholesterol, as well as the lowest activity of both AST and ALP were recorded for females in April, i.e. 2 months before reproduction. Higher values of these indices ($P < 0.05$ Chol; $P < 0.01$ glucose, AST, ALP) were found in June in both the control group and the group of females 24 h after reproduction. Concentration of total protein (TP) and ALT were comparable in all groups of females. A higher cortisol level was found for the control group compared to the group of females 24 h after reproduction but this difference was not significant. Similarly, higher activity of creatine kinase was found for the group of females 24 h after reproduction compared to the control group but this difference was not significant as well.

Comparison of biochemical profile of blood plasma of spawned and not spawned females of tench

Biochemical indices of blood plasma of spawned and not spawned tench females are compared in Tables 4 and 5. Values of all indices under study were similar for both spawned

Table 3
Comparison of biochemical profile of blood plasma of tench females during the pre- and postspawning period (15th April and 17th June 2000)

| Indices | Unit | 15 th April 2000 | | 17 th June 2000 control | | 17 th June 2000 spawned | |
|-------------|---------------------------|-----------------------------|---------------------------------|------------------------------------|---------------------------------|------------------------------------|-------------------------------|
| | | n | Mean \pm SD | n | Mean \pm SD | n | Mean \pm SD |
| Body weight | g | 52 | 775.4 \pm 138.19 ^a | 20 | 878.5 \pm 159.31 ^b | 14 | 1020 \pm 182.1 ^c |
| Cortisol | mmol.l ⁻¹ | | - | 20 | 515.8 \pm 210.6 | 14 | 440.6 \pm 212.43 |
| Glucose | mmol.l ⁻¹ | 52 | 5.93 \pm 1.579 ^a | 20 | 9.52 \pm 3.687 ^b | 14 | 8.24 \pm 1.842 ^b |
| TP | g.l ⁻¹ | 52 | 37.5 \pm 4.85 | 20 | 36.4 \pm 4.64 | 14 | 38.1 \pm 5.09 |
| Tcg | mmol.l ⁻¹ | 52 | 1.40 \pm 0.428 ^a | 20 | 1.83 \pm 0.346 ^b | 14 | 2.33 \pm 0.616 ^c |
| Chol | mmol.l ⁻¹ | 52 | 3.58 \pm 0.978 ^a | 20 | 4.34 \pm 0.916 ^b | 14 | 4.54 \pm 1.242 ^b |
| ALT | μ kat.l ⁻¹ | 50 | 0.17 \pm 0.117 | 20 | 0.15 \pm 0.064 | 14 | 0.16 \pm 0.082 |
| AST | μ kat.l ⁻¹ | 52 | 4.72 \pm 1.848 ^a | 20 | 7.01 \pm 2.161 ^b | 14 | 7.11 \pm 1.561 ^b |
| CK | μ kat.l ⁻¹ | 52 | - | 20 | 160.8 \pm 88.82 | 14 | 203.4 \pm 167.37 |
| ALP | μ kat.l ⁻¹ | 37 | 0.21 \pm 0.196 ^a | 17 | 0.78 \pm 0.377 ^b | 12 | 0.78 \pm 0.547 ^b |

Values with superscript ^a and ^b and ^c express significant difference ($P < 0.05$, $P < 0.01$, respectively) among of groups compared.

and not spawned females either immediately after spawning (Table 4), or 24 h later (Table 5). Different values were recorded in glucose concentration only. Higher values of glucose in blood plasma of spawned fish were recorded in 1999, i.e. in females immediately after reproduction ($P < 0.01$). In contrary, in 2000 when the study was focused on analyses of fish 24 h after reproduction, higher values of glucose in blood plasma were recorded for the not spawned fish ($P < 0.01$).

Table 4
Comparison of biochemical profile of blood plasma of tench females spawned and not spawned (20th June 1999)

| Indices | Unit | Spawned | | Not spawned | | Statistical significance |
|-------------|---------------------------|---------|-------------------|-------------|-------------------|--------------------------|
| | | n | Mean \pm SD | n | Mean \pm SD | |
| Body weight | g | 20 | 809.7 \pm 126.1 | 15 | 777.5 \pm 135.6 | - |
| Glucose | mmol.l ⁻¹ | 20 | 9.53 \pm 3.049 | 15 | 6.29 \pm 2.149 | $P < 0.01$ |
| TP | g.l ⁻¹ | 20 | 35.1 \pm 5.64 | 15 | 37.8 \pm 5.61 | - |
| Teg | mmol.l ⁻¹ | 20 | 1.42 \pm 0.251 | 15 | 1.26 \pm 0.498 | - |
| Chol | mmol.l ⁻¹ | 20 | 3.60 \pm 0.891 | 15 | 2.93 \pm 1.105 | - |
| ALT | μ kat.l ⁻¹ | 20 | 0.22 \pm 0.113 | 15 | 0.18 \pm 0.097 | - |
| AST | μ kat.l ⁻¹ | 20 | 4.39 \pm 1.77 | 15 | 4.84 \pm 1.97 | - |
| CK | μ kat.l ⁻¹ | 20 | 94.2 \pm 51.85 | 15 | 110.0 \pm 70.10 | - |
| ALP | μ kat.l ⁻¹ | 19 | 0.77 \pm 0.41 | 15 | 0.79 \pm 0.39 | - |

Table 5
Comparison of biochemical profile of blood plasma of tench females spawned and not spawned (17th June 2000)

| Indices | Unit | Spawned | | Not spawned | | Statistical significance |
|-------------|---------------------------|---------|-------------------|-------------|-------------------|--------------------------|
| | | n | Mean \pm SD | n | Mean \pm SD | |
| Body weight | g | 14 | 1020 \pm 182.1 | 14 | 1044 \pm 146.1 | - |
| Cortisol | mmol.l ⁻¹ | 14 | 440.6 \pm 212.4 | 14 | 554.9 \pm 202.7 | - |
| Glucose | mmol.l ⁻¹ | 14 | 8.24 \pm 1.842 | 14 | 11.60 \pm 3.564 | $P < 0.01$ |
| TP | g.l ⁻¹ | 14 | 38.1 \pm 5.09 | 14 | 34.5 \pm 4.79 | - |
| Teg | mmol.l ⁻¹ | 14 | 2.33 \pm 0.616 | 14 | 2.09 \pm 0.327 | - |
| Chol | mmol.l ⁻¹ | 14 | 4.54 \pm 1.24 | 14 | 4.22 \pm 0.82 | - |
| ALT | μ kat.l ⁻¹ | 14 | 0.16 \pm 0.082 | 14 | 0.18 \pm 0.061 | - |
| AST | μ kat.l ⁻¹ | 14 | 7.11 \pm 1.561 | 14 | 6.67 \pm 1.558 | - |
| CK | μ kat.l ⁻¹ | 14 | 203.4 \pm 167.4 | 14 | 211.1 \pm 111.8 | - |
| ALP | μ kat.l ⁻¹ | 12 | 0.78 \pm 0.547 | 13 | 0.64 \pm 0.253 | - |

Discussion

Fish reproduction is one of the factors seriously affecting the internal milieu of the organism. Therefore, great attention is paid to the study of haematological and biochemical indices during the reproduction period. Changes in haematological indices of tench during the reproduction period are reported by Einszporn-Orecka (1970) and Svobodová et al. (1978). A drop in values of haematological indices (erythrocyte count Er, haemoglobin content Hb, haematocrit value PCV) was described by Einszporn-Orecka (1970) during the reproduction period of brood tench from lakes; (an expressive

decrease of values was found for females). Similar character of changes in haematological values (Er, Hb, PCV) in tench females and males after artificial reproduction was reported by Svobodová et al. (1978).

Repeatedly, no differences in biochemical indices were recorded between two groups of females treated with GnRH analogue, or carp pituitary (Kouřil et al. 2001). Therefore, the results presented in this study are assessed regardless to the way of induction of ovulation.

For the assessment of fish stress, the determination of glucose concentration in blood plasma was used, as well as cortisol concentration in some cases. No sex-related difference in glucose concentration was found for the brood tench neither 2 months prior to the reproduction, nor immediately after it. Literature data for various fish species also do not state important differences in glucose concentration in blood plasma of females and males (Edsall 1999; Bhatnagar and Saksena 1989). In contrary, in tench females there were found significantly higher values of glucose concentration in blood plasma in the period of reproduction compared to those found 2 months before reproduction ($P < 0.01$). This finding confirms the presumption of Robertson et al. (1961), Plisecka and Kuzminova (1971), Svobodová (1977) and Zuim et al. (1988) which state that gonad development is one of the reasons inducing higher concentration of glucose in fish blood plasma.

After induction of ovulation by hormones some females spawned while some other females did not spawn. These two groups differed in glucose concentration in the 1999 study, as well as in 2000. Increase ($P < 0.01$) in glucose concentration was found in females immediately after the reproduction in 1999. It was a persisting response to physiological and handling stress due to artificial reproduction. Similar values are reported by Jeney and Jeney (1992) and Svobodová et al. (1997) for blood plasma of females of common carp and wels immediately after an artificial reproduction. On the other hand in 2000, where the analysis was performed 24 h after the reproduction, higher values of glucose were recorded for not spawned fish ($P < 0.01$). This may indicate a certain stress of the organism of not spawned females probably due to no release of mature oocytes or, appropriately to other factors. Such stress also revealed a slight non-significant increase in cortisol level of the not spawned females compared to those 24 h after reproduction. There are also many papers stating cortisol in blood plasma of teleosts as an indicator of primary stress, as particularly reviewed by Mommsen et al. (1999).

Body weight of tench females surpassed that of males ($P < 0.01$), both in 1999 and 2000. This is a natural sex difference in this fish species (Krupauer 1968).

Concentration of total protein in blood plasma was used as a basic index of condition and health status of brood tench, in accordance with data reported for other fish species (Mulcahy 1971; Svobodová and Párová 1977; Hille 1982; Pravda 1986; Jirásek et al. 1993; Řehulka 1996, 1998). A significant sex difference in total protein concentration in blood plasma of brood tench was found either 2 months before reproduction, or immediately after it. In the pre-spawning period, there was a higher TP concentration in females compared to males ($P < 0.05$) while results of analyses immediately after reproduction were reciprocal ($P < 0.01$). Svobodová et al. (1981) in accordance with this finding reported a significantly higher TP concentration in wels females compared to males in springtime ca. 3 months before reproduction. Immediately before the reproduction, values of TP concentration in blood plasma of wels females and males were comparable (Svobodová et al. 1998). Similarly, Pravda et al. (1989) did not find any important sex-related differences in TP concentration of brood pike in reproduction time. In brood common carp at the time of the first blood sampling (April 7, 1999) the concentration of TP in blood plasma did not differ for females and males. Nevertheless, during the next periods compared (May 27, May 29 and June 14) males were found to have lower TP compared to females ($P < 0.01$) (Svoboda et al. 2000). Lusková

(1997b, 1998) who observed the TP content in blood plasma throughout the year, stated sex-related differences in grayling only whereas in brown trout, nase and chub no sex-related differences in TP content in blood plasma were found.

As evident from the literature sources reported hereby as well as from our results, a question on the effect of sex on TP concentration in blood plasma cannot be answered unequivocally. Often this effect is overlapped with other factors such as annual season associated with metabolism intensity (Lusková 1997b, 1998; Edsall 1999), nutritional status of the fish (Pravda et al. 1989), fish health status (Mulcahy 1971; Řehulka 1996, 1998) etc. There is a relatively good accordance among authors stating the lowest TP concentrations in various fish species during the reproduction time, e.g. Miller et al. (1983) in rainbow trout, Kovacheva and Tchekov (1993) in common carp and herbivorous fish, Lenhardt (1992) in pike, Svobodová et al. (1997) in wels. With the tench females under study, this fact cannot be confirmed. As shown in Table 3, values of TP concentration in blood plasma of tench females 2 months before reproduction and in the reproduction time were comparable. Theoretically, even in this case a finding of these TP concentrations in blood plasma of females could be considered a finding of minimum concentrations after overwintering (April), as well as in time of reproduction (June). Low TP concentrations in blood plasma of cyprinids after overwintering are reported by Pravda (1986) and Spurný and Mareš (1989).

Results of examination of blood plasma of brood tench 2 months before reproduction showed a sex-related difference ($P < 0.01$) in concentration of triacylglycerols. Higher concentrations of triacylglycerols were detected in females in which synthesis of vitellogenin took part during the prespawning period (phase of vitellogenesis type I, vitellogenesis type II). The process of vitellogenesis is highly energy-demanding and fats are preferred as a source of energy (Bon et al. 1997). At the time of reproduction, concentrations of triacylglycerols in blood plasma were comparable for both sex.

Differences ($P < 0.01$) in concentration of plasma triacylglycerols were found for tench females before and after reproduction. Higher triacylglycerole concentrations in blood plasma of females at the time of reproduction (June) compared to those in the pre-spawning period (April) can be associated with different water temperature (10.3 °C in April versus 22 °C in June) and thus with different metabolic rate and differing need for sources of energy. Higher need for energy can be also expected for females after reproduction compared to those before reproduction which have not been stimulated hormonally for ovulation. This ensues from significantly different concentrations of plasma triacylglycerols ($P < 0.01$) found in control fish (before reproduction without hormonal stimulation of ovulation) and in fish 24 h after reproduction (Table 3). Higher concentration of plasma triacylglycerols were also found in tench females both immediately after and 24 h after spawning, compared to values found in not spawning females which had been hormonally stimulated for ovulation. However, these differences were not significant.

Concentration of plasma cholesterol in tench females and males in the pre-spawning period (2 months before reproduction) was nearly the same. Sex-related differences in concentration of plasma cholesterol were found in tench immediately after reproduction, with values for females being higher. We suggest that cholesterol is incorporated into membranes and endogenous structures of eggs and thus its concentration in blood plasma of females decreases at the time of reproduction. Diwan and Krishnan (1986) stated a fluctuation of serum cholesterol in males and females of *Etroplus suratensis* as related to maturity. Cholesterol concentration in blood plasma of females was the lowest when the gonadosomatic index (GSI) was the highest and vice versa. We therefore suggest that there is a comparable incorporation of cholesterol into the developing eggs in birds (Vorlová et al. 2001) similar to that found by us in fish.

Similarly to the concentration of triacylglycerols, cholesterol concentration in blood

plasma of tench females was also significantly higher in the reproduction period (June) than during the pre-spawning period (April). This can be also associated with different water temperature (10.3 °C in April versus 22 °C in June) and thus with different metabolic rate.

Enzyme activity in blood plasma was determined to assess the internal milieu of tench during reproduction. No sex-related differences were found in brood tench in the activity of transaminases (ALT, AST), creatine kinase (CK) and alkaline phosphatase (ALP) neither in the prespawning period, nor immediately afterwards. Higher values ($P < 0.01$) of AST and ALP were found in females in June (control group without hormonal induction of ovulation and group of females 24 h after spawning) compared to values found in females in April. Similarly to lipid analytes, this difference in enzyme activity can be related to water temperature (10.3 °C in April versus 22 °C in June) followed by different metabolic rate. Moreover, Johnston et al. (1987) reported that *Salmo salar* had markedly elevated plasma ALP in female spawners during vitellogenesis and that it appears to be an important enzyme system providing a source of phosphate for the hepatic synthesis of vitellogenin. Activity of plasma AST, ALT, CK a ALP was compared in tench females either immediately after spawning (1999), or 24 h thereafter (2000) to the enzyme activity of not-spawned females which had been hormonally stimulated for ovulation. No significant difference was found.

Similarly to brood tench, no important changes in enzyme activity were found in brood common carp within the course of artificial reproduction (Svoboda et al. 2000). In contrary, mainly in the course of natural reproduction of salmonids Lusková (1997a, 1997b) found a marked effect of the reproductive process (spawning) on the activity of enzymes studied. In ALT and AST, there were 3-6fold increased activities, in creatine kinase the enhancement was even 100fold. The source of these enzymes in blood plasma remains unknown. Lusková (1997ab) anticipates that gonads are one of the most important sources of enzymes in blood circulation during the reproductive period.

Mean values of Na concentration in blood plasma range in 144 and 145 mmol.l⁻¹ in all cases. Comparable Na concentration values, very stable either before, or after the reproductive period were also detected in brood common carp (Svoboda et al. 2000).

Biochemický profil krevní plazmy lína obecného (*Tinca tinca* L.) v před a v povýtěrovém období

Cílem práce bylo posoudit biochemický profil krevní plazmy u lína v před a povýtěrovém období při hormonálně indukované umělé reprodukci. V roce 1999 bylo vyšetřeno 59 jikernaček a 27 mlíčáků v povýtěrovém období, v roce 2000 52 jikernaček a 25 mlíčáků v předvýtěrovém období a 48 kusů jikernaček v povýtěrovém období. Stanoveny byly následující biochemické ukazatele krevní plazmy: kortizol, glukóza, celkové bílkoviny (TP), triacylglyceroly (Tcg), cholesterol (Chol), transaminázy (ALT a AST), kreatinkináza (CK), alkalická fosfatáza (ALP) a elektrolyty (Na). V předvýtěrovém období byly u jikernaček zjištěny vyšší hodnoty TP ($P < 0,05$) a Tcg ($P < 0,01$) ve srovnání s mlíčáky. Bezprostředně po výtěru byly u mlíčáků naměřeny vyšší hodnoty TP ($P < 0,01$) a Chol ($P < 0,01$) ve srovnání s jikernačkami. V hodnotách ostatních ukazatelů nebyly zjištěny signifikantní sexuální difference. Vyšší hodnoty glukózy ($P < 0,01$), Tcg ($P < 0,01$), Chol ($P < 0,05$), AST ($P < 0,01$) a ALP ($P < 0,01$) byly zjištěny u jikernaček v měsíci červnu po výtěru ve srovnání s hodnotami zjištěnými v dubnu, tj. dva měsíce před výtěrem. Významnou roli sehrála i rozdílná teplota vody (duben 10,3 °C; červen 22 °C) a s tím související intenzita metabolismu. Po indukci ovulace hormonálními přípravky (syntetický analog GnRH a kapří hypofýza) došlo k výtěru u části jikernaček, část jikernaček se nevytřela. Mezi těmito dvěma skupinami

byly zjištěny rozdíly ($P < 0,01$) v koncentraci glukózy, rozdíly v hodnotách dalších biochemických ukazatelů nebyly signifikantní. Zvolený biochemický profil krevní plazmy umožňuje v základních rysech hodnotit stav vnitřního prostředí generačních ryb v období reprodukce.

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References

- BHATNAGAR, S., SAKSENA, D. N. 1989: Observations on certain haematological and biochemical parameters of blood in an air-breathing teleost, *Clarias batrachus* (Linn.). J. Anim. Morphol. Physiol. **36**: 163-168
- BON, E., CORRAZE, G., KAUSHIK, S., LE-MENN, F. 1997: Effects of accelerated photoperiod regimes on the reproductive cycle of the female rainbow trout: 1. Seasonal variations of plasma lipids correlated with vitellogenesis. Comp. Biochem. Physiol. **118A**: 183-190
- BÖTTCHER, K. 1998: Untersuchungen zu klinisch-chemischen Parametern im Blutplasma von Karpfen (*Cyprinus carpio*). Dissertation, Tierärztliche Hochschule, Hannover, 158 p.
- COLLAZOS, M. E., BARRIGA, C., DE-SANDE, F., ORTEGA, E. 1993: Seasonal variations and influence of gender on several haematological parameters in the cyprinid fish *Tinca tinca*. Actas del IV. Congreso Nacional de Agricultura, Spain, pp. 173-178
- DEMAEL, A., GARIN, D. 1978: Effects of thermal stress on some metabolic parameters of tench living in different oxygenated waters. Cah. Lab. Hydrobiol. **7**: 15-26
- DEMAEL, A., GUSTIN, P., LEPOT, D. 1984: Influence of a small decrease in water pH on some enzymatic activities of liver and on certain blood components in tench (*Tinca tinca* L.). Ichthyophysiol. Acta **8**: 75-91
- DE-PEDRO, N., DELGADO, M. J., PINILLOS, M. L., ALONSO-GÓMEZ, A. L., ALONSO-BEDATE, M. 1998: Daily rhythms in NAT activity, cortisol, glucose, glycogen and catecholamines in tench (*Tinca tinca* L.). Pol. Arch. Hydrobiol. **45**: 321-329
- DIWAN, A. D., KRISHNAN, L. 1986: Levels of cholesterol in blood serum and gonads in relation to maturation in *Etroplus suratensis* (Bloch). Indian J. Fish. **33**: 241-245
- EDSALL, C. C. 1999: A blood chemistry profile for lake trout. J. Aq. Animal Health **11**: 81-86
- EINSZPORN-ORECKA, T. 1970: Quantitative changes in the circulating blood of tench (*Tinca tinca* L.) in the annual cycle. Pol. Arch. Hydrobiol. **17**: 435-444
- HILLE, S. 1982: A literature review of the blood chemistry of rainbow trout, *Salmo gairdneri*. J. Fish Biol. **20**: 535-569
- HLAVOVÁ, V. 1989: Enzyme activities in the blood plasma of grayling, *Thymallus thymallus* (Linn.), in the breeding season. J. Fish Biol. **31**: 779-789
- HOCHACHKA, P. W., MOMMSEN, T. P. 1995: Biochemistry and molecular biology of fishes. Metabolic biochemistry. Elsevier Amsterdam, 515 pp.
- HRUBEC, T. C., SMITH, S. A. 1999: Differences between plasma and serum samples for the evaluation of blood chemistry values in rainbow trout, channel catfish, hybrid tilapias, hybrid striped bass. J. Aq. Anim. Health **11**: 116-122
- JENEY, Z., JENEY, G. 1992: Primary and secondary stress responses of common carp (*Cyprinus carpio* L.) caused by artificial propagation. Proc. Conf. Fish Reproduction, RIFCH Vodňany, pp. 27-30
- JIRÁSEK, J., PALÁČKOVÁ, J., MAREŠ, J. 1993: The effect of a different quality of feed on selected indicators of the internal environment of the rainbow trout. Proc. 3rd Ichthyohaematol. Conf., RIFCH Vodňany, pp. 40-45
- JOHNSTON, C. E., GRAY, R. W., McLENNAN, A., PETERSON, A. 1987: Effects of photoperiod, temperature, and diet on the reconditioning response, blood chemistry, and gonad maturation of Atlantic salmon kelts (*Salmo salar*) held in freshwater. Can. J. Fish. Aq. Sci. **44**: 702-711
- KOŮŘIL, J. 1987: Induced ovulation of the female tench (*Tinca tinca* L.): Effect of the numbers and levels of pituitary injections on the results of stripping. Práce VÚRH Vodňany **16**: 53-61
- KOŮŘIL, J., BARTH, T., HAMÁČKOVÁ, J., FLEGEL, M. 1986: Induced ovulation in tench (*Tinca tinca* L.) by various LH-RH synthetic analogues: Effect of site of administration and temperature. Aquaculture **54**: 37-44
- KOŮŘIL, J., SVOBODA, M., BARTH, T., HAMÁČKOVÁ, J., KALÁB, P., LEPIČOVÁ, A., RENDON, P.M., SAVINA, L., SEDOVÁ, M., SVOBODOVÁ, Z., VYKUSOVÁ, B. 2001: The effect of repeated application of hormonal treatment by different preparations in artificial propagation of tench (*Tinca tinca* L.) females on gained indices of reproduction, survival and blood biochemistry profile. Czech J. Anim. Sci. in press.
- KOVACHEVA, N. P., TCHEKOV, A. G. 1993: Physiological method for a control of the brood fish maturity before and after spawning. Proc. 3rd Ichthyohaematol. Conf., RIFCH Vodňany, pp. 64-67
- KRUPAUER, V. 1968: Vliv pohlaví na váhu lina obecného. Buletín VÚR Vodňany, **4**: 8-13.
- LENHARDT, M. 1992: Seasonal changes of some blood chemistry parameters and in relative liver and gonad weights of pike (*Esox lucius*, L.) from the River Danube. J. Fish Biol. **40**: 709-718

- LUSKOVÁ, V. 1997a: Influence of spawning on enzyme activity in the blood plasma of fish. *Pol. Arch. Hydrobiol.* **44**: 57–66
- LUSKOVÁ, V., 1997b: Annual cycles and normal values of hematological parameters in fishes. *Acta Sc. Nat. Brno* **31**, 70 pp.
- LUSKOVÁ, V. 1998: Factors affecting haematological indices in free-living fish populations. *Acta vet. Brno* **67**: 249-255
- LUSKOVÁ, V., LUSK, S. 1995a: Enzyme activities in the blood plasma of brown trout, *Salmo trutta* m. *fario* during spawning. *Folia Zool.* **44**: 81–89
- LUSKOVÁ, V., LUSK, S. 1995b: Enzyme activities in the blood plasma of nase, *Chondrostoma nasus*, during spawning. *Folia Zool.* **44**: 131–136
- MARQUEZ, E. D. 1976: A comparison of glutamic-oxalacetate transaminase, lactate dehydrogenase, alpha-hydroxybutyrate dehydrogenase and creatine phosphokinase activities in non-spawning, pre-spawning and spawning pink salmon. *Comp. Biochem. Physiol.* **54B**: 121–123
- MILLER, W. R., HENDRICKS, A. C., CAIRNS, J. Jr. 1983: Normal ranges for diagnostically important hematological and blood chemistry characteristics of rainbow trout (*Salmo gairdneri*). *Can. J. Fish. Aq. Sci.* **40**: 420-425
- MOMMSEN, T. P., VIJAYAN, M.M., MOON, T. W. 1999: Cortisol in teleosts: dynamics, mechanisms of action and metabolic regulation. *Rev. Fish Biol. Fish.* **9**: 211-268
- MULCAHY, M.F. 1971: Serum protein changes associated with ulcerative dermal necrosis (UDN) in the trout *Salmo trutta* L. *J. Fish Biol.* **3**: 199-201
- PLISECKAJA, E. M., KUZMINA, V. V. 1971: Uroveň glikemii v organach kruglorotych Cyclostomata i ryb Pisces. *Vop. Ichtiol.* **11**: 1077-1087
- PRAVDA, D. 1986: Shortened hematological conditional test (SHCT) and its application in prognosis of breeding effect in carp fry. *Proc. 1st Ichthyohaematol. Conf., Litomyšl*, pp. 38-44
- PRAVDA, D., PALÁČKOVÁ, J., PECHA, O. 1989: Sexual differences in some basic haematological parameters of breeding pikes during the spawning period. *Proc. 2nd Ichthyohaematol. Conf., Litomyšl*, 7 pp.
- ROBERTSON, O. H., KRUPP, N. A., FAVOUR, C. B., HANE, S., THOMAS, S. F. 1961: Physiological changes occurring in the blood of the pacific salmon (*Oncorhynchus tshawytscha*) accompanying sexual maturation and spawning. *Endocrinol.* **68**: 325-337
- ŘEHULKA, J. 1996: Blood parameters in common carp with spontaneous spring viremia (SVC). *Aquaculture International* **4**: 175-182
- ŘEHULKA, J. 1998: Blood indices of the rainbow trout, *Onconhyrchus mykiss* (Walbaum) in aeromonas-induced ulcerous dermatitis. *Acta vet. Brno* **67**: 317-322
- SPURNÝ, P., MAREŠ, J. 1989: Dynamics of changes in selected haematological indices of two weight categories of common carp fry in the course of overwintering. *Proc. 2nd Ichthyohaematol. Conf., Litomyšl*, 9 pp.
- SVOBODA, M., LUSKOVÁ, V., KOUŘIL, J. 2000: The effect of sex on selected blood plasma indices of adult common carp (*Cyprinus carpio* L.) during hormonally induced artificial reproduction. *Proc. 4th Czech Ichthyol. Conf., USB RIFCH Vodňany*, pp. 249–252
- SVOBODOVÁ, Z. 1977: Influence of sex on the glucosemia and glycogen content in hepatopankreas and musculature of the carp (*Cyprinus carpio* L.). *Acta vet. Brno* **46**: 253-258
- SVOBODOVÁ, Z., PÁROVÁ, J. 1977: The use of some physiological parameters of fish for the evaluation of feeding tests. *Buletin VÚRH Vodňany* **13**: 12-19
- SVOBODOVÁ, Z., KOLÁŘOVÁ, J., FLAJŠHANS, M. 1998: The first findings of the differences in complete blood count between diploid and triploid tench, *Tinca tinca* L. *Acta vet. Brno* **67**: 243–248
- SVOBODOVÁ, Z., KOUŘIL, J., HAMÁČKOVÁ, J. 1978: The values of some haematological indices in parent tench (*Tinca tinca* L.). *Živočišná výroba* **23**: 825-833
- SVOBODOVÁ, Z., KOUŘIL, J., HAMÁČKOVÁ, J. 1981: The effect of sex on some haematological and biochemical indices in fish. *Proc. Conf. Reproduction, genetics and hybridization of fish, RIFCH Vodňany*, pp. 11-18
- SVOBODOVÁ, Z., PRAVDA, D., PALÁČKOVÁ, J. 1991: Unified methods of haematological examination of fish. *Research Institute of Fish Culture and Hydrobiology, Vodňany, Edition Methods, No. 22*, 31 pp.
- SVOBODOVÁ, Z., KOLÁŘOVÁ, J., KOUŘIL, J., HAMÁČKOVÁ, J., VYKUSOVÁ, B., KALÁB, P. 1997: Haematological investigations in *Silurus glanis* L. females during pre- and postspawning period. *Pol. Arch. Hydrobiol.* **44**: 67-81
- SVOBODOVÁ, Z., KOLÁŘOVÁ, J., MODRÁ, H., VAJCOVÁ, V., HAMÁČKOVÁ, J., KOUŘIL, J., KOZÁK, P. 1998: Values of haematological indices of wels (*Silurus glanis* L.) in relationship to the level of nutrition during the prespawning period. *Acta vet. Brno* **67**: 235-242
- VORLOVÁ, L., SIEGLOVÁ, E., KARPÍŠKOVÁ, R., KOPŘIVA, V. 2001: Cholesterol content in shelled eggs during the hen's laying period. *Acta vet. Brno*, in press
- ZUIM, S. M. F., ROSA, A. A. M., CASTAGNOLLI, N. 1988: Sex and sexual cycle influences over metabolic parameters in pacu *Piaractus mesopotamicus* (Holmberg, 1887). *Proc. Aquacult. Int. Congr., Vancouver*, p. 74