

Bernard *KŁYSZEJKO*, Grażyna *ŁYCZYWEK*

Fish physiology

**EFFECT OF A SUBLETHAL CONCENTRATION OF DELTAMETRIN
ON BIOCHEMICAL PARAMETERS OF THE BLOOD SERUM
OF CARP (*CYPRINUS CARPIO* L.)**

**WPLÝW SUBLETALNEJ KONCENTRACJI DELTAMETRYNY
NA WSKAŹNIKI BIOCHEMICZNE SUROWICY KRWI KARPIA
(*CYPRINUS CARPIO* L.)**

Department of Fish Physiology, Agricultural University of Szczecin, Poland

Effect of $0.14 \mu\text{m}/\text{dm}^3$ water concentrations of deltametrin on nine biochemical parameters of the blood serum of carp was studied. The experiment was conducted under laboratory conditions in aquaria in spring and in fall. The exposure times were 24 and 48 hours. In both experimental seasons the deltametrin solution caused fluctuations in the levels of: glucose, Cl^- , Na^+ , K^+ ions, total calcium, creatinine, urea nitrogen, and cholesterol. It did not affect the content of crude protein.

INTRODUCTION

Pyrethroid insecticides are commonly used in plant protection, because of their high toxicity for insects, short biodegradation period, and the lack of the tendency for accumulation in organism (Malinowski 1982a, 1982b; RóŹański 1985; Witkowski 1988). A common feature of these compounds is their mode of action consisting in blocking, in open position, ionic canals, which in turn hampers the conductivity in the nervous system (Clark and Brooks 1984; Lund 1984; Saldago et al. 1989). Pyrethroids have a negative temperature coefficient. They exhibit a moderate toxicity for homoiothermal animals and a higher toxicity for poikilothermal organisms (Malinowski 1982a, 1982b; RóŹański 1985).

In the case of fishes both low- and high concentrations of pyrethroids are harmful. High concentrations interfere directly with the process of neural transmission. Low concentrations cause changes in enzymatic and hormonal activity (Jungowska-Klin et al. 1992; Kozubek et al. 1992; Przybylska-Wojtyszyn et al. 1992) and anatomicopathological changes of the internal organs (Hłond 1982; Kumaraguru et al. 1982).

Deltametrin, assigned to the IV class of toxicity is an active substance of the insecticide known under the brand name of Decis 2.5 EC.

The lethal concentration of deltametrin for carp fry, determined under the criterion of $LC_{50}/96$ h is $3.5 \mu\text{m}/\text{dm}^3$ (Łakota et al. 1987; Łakota 1992).

Under long exposure times (34–60 days) deltametrin has effect on fishes in concentrations much lower (0.01 – $0.1 \mu\text{m}/\text{dm}^3$) causing increase of enzymes responsible for detoxification processes in the liver, kidney, and blood serum (Jungowska-Klin et al. 1992; Kozubek et al. 1992; Przybylska-Wojtyszyn et al. 1992) and by lowering the hematological parameters of the erythrocyte system (Wojtaszek et al. 1992).

The half-life period of deltametrin in aquatic environment is 4–8 days (Stefan 1970; Łakota et al. 1990; Szerow et al. 1996;) which means that under natural conditions this insecticide has relatively short effect on fishes when added to a body of water.

Changes in the blood of fishes in response to a toxic agent may be observed also in the biochemical parameters, which constitute a ground for evaluation of the condition state of cultured fishes.

The aim of the present work was to verify the assumption whether deltametrin in a sublethal concentration and in a short exposure time can influence changes in the values of the following parameters of the blood serum: glucose, Cl^- , Na^+ , K^+ ions, total calcium, crude protein, creatinine, urea nitrogen, and cholesterol.

MATERIAL AND METHODS

The present study was conducted in spring (Apr–May) and in fall (Oct–Nov) on a total of 90 mirror carp of the individual weight of 400–700 g. The carp originated in a cage-culture fish farm based on the discharge canal of the “Dolna Odra” Power plant near Szczecin.

The experiment was carried out in glass tanks of the capacity of 100 dm^3 in aerated tap water of the temperature of $20 \pm 1^\circ\text{C}$ (pH 7.4–8.2; oxygen 8.1–10.2 mg/dm^3). The adjustment period to the aquarium conditions was 3 days. In each season a total of 45 fish was studied. Each batch was randomly divided into 3 groups, 15 fish each (control and 2 experimental groups).

Deltametrin was used in the concentration of $0.14 \mu\text{m}/\text{dm}^3$ and the exposure time was 24 and 48 h. Deltametrin was added to the water in a single dose before the commencement of the experiment. The source of deltametrin was Decis 2.5 EC insecticide, manufactured by Hoechst Schering (Germany). After the intoxication the fish were clubbed, the body cavity was cut open and the blood was sampled from the arterial bulb of the heart. “Serum” syringes manufactured by Kabe-Laborteknik, adapted to direct centrifugation, were used. After retraction the blood was centrifuged for 15 minutes with the speed of 1200 revolu-

tions/min in a MPW 365 Centrifuge. The obtained serum was subjected to analysis after about three hours.

The content of sodium and potassium ions in the serum was determined with ionoselective electrode; total calcium—using a colorimetric method with methylene blue and monoethanolamine; chlorides—using Volhard method; crude protein—refractometrically; creatinine, glucose, urea nitrogen and cholesterol—using an enzymatic method with a diagnostic kits of “Human” company on an ABBOT biochemical analyzer.

At the time of the experiment behavioral reactions of the fish were observed.

The results were processed statistically using the procedure of multivariate analysis of variance (MANOVA) aided by the computer program Statgraphics v. 6.0. The confidence level was $P = 0.05$.

RESULTS AND DISCUSSION

The analyzed parameters are used in the full-scale rearing conditions for assessment of the metabolic performance, condition, and also nutritional values of feeds.

The material used in the present experiment originated in an intensive-rearing farm located in cooling water. Statistically significant seasonal differences in control groups were observed only for glucose and urea nitrogen, which were more abundant in the spring (Tab. 1). Because in the 3-day-long adjustment period to the conditions of the glass tanks (intended to minimize the results of the transport stress) and also in the course of the experiment the fish were not fed—it is possible that the lack of seasonal differences in the majority of the studied parameters of the blood serum reflected relatively stable rearing conditions in cooling water (temperature, feeding regime, kind of feed).

The content of glucose in the blood of cultured fishes depends chiefly on the kind of ingested carbohydrate feed (Chavin et al. 1970). Hyperglycemia is associated with stressful situations (Wedemeyer 1972; Fletcher 1975) and bacterial infections (Herbell et al. 1979), while hypoglycemia may accompany intensive physical effort (Blac et al. 1966; Miles et al. 1974; Driedzic and Kiceniuk 1976). The present study revealed, that in the case of glucose deltametrin solution caused statistically significant decrease of the level of this compound in the blood serum only in spring, following 48-h exposure of the fish (Tab. 1).

The content of Cl^- , Na^+ , K^+ ions and the level of the total calcium in the blood indicates a direction and range of the ionic-osmotic regulation (Richards and Fromm 1970; Jonson 1973; Eddy 1976; Girard 1976). The present study demonstrated that deltametrin in sublethal concentration can change osmotic regulation processes. After 24 hours of the exposure in both experimental seasons the following changes were observed: statistically significant decrease in the content of chlorides and Na^+ ions, increase of total calcium, and (only in the spring) statistically significant increase of K^+ ions. After 48 hours of the expo-

sure in both experimental seasons, levels of chlorides, total calcium, and K^+ ions regained the values of control groups. In the case of Na^+ ions the changes were reversed in the spring only, while in the fall after 48 hours of exposure a further statistically significant decrease was observed (Tab. 1).

Crude protein level in the blood serum is linked to the kind of feed, experimental season (Stefan 1970; Szerow et al. 1996), and also to the health status of the fish (Wedemeyer and Mc Leay 1981). The present study did not demonstrate any effect of deltametrin solution nor study season on the level of crude protein in the blood serum of the carp originating from a cooling-water culture.

Amount of urea nitrogen in the blood serum is an indicator of protein metabolism. Creatinine content may be regarded as a measure of glomerular filtration rate (Murray et al. 1995). In the case of urea nitrogen there were seasonal differences between control groups. Deltametrin solution after 24 hours of exposure caused an increase of this parameter. This change was statistically significant only in the fall, whereas after 48 hours the changes were reversed. In the case of creatinine, the solution of deltametrin caused statistically significant decrease of its level in fall after 48 hours of exposure.

Cholesterol content in the blood is linked to lipid metabolism and depends on the calorific value of the feed. As a consequence of action of negative environmental factors (stress and pollution) the same fish species exhibited hypercholesterolemia (Biliński and Lau 1969; Mazeaud 1969; Perrier et al. 1972) and also decrease of cholesterol level (Donaldson and Mc Bride 1974; Mc Leay and Brown 1974; Wedemeyer 1976).

The present study demonstrated that the exposure of the fish to deltametrin solution after 24 hours caused statistically significant decrease of the level of cholesterol, more pronounced in the fall. After 48 hours of the exposure in both seasons an increase of the cholesterol level was observed. In the spring this level regained the value of control group.

Observation of the behavioral reactions revealed that throughout the entire experiment, the fish immersed in $0.14 \mu\text{g}/\text{dm}^3$ solution of deltametrin behaved identically as those in control groups. There were no signs of stress symptoms (anxiety, excessive mucus secretion, changes in the respiratory rhythm etc.) known as a response of fishes to various hazards (Węgrzynowicz et al. 1984; Kłyszajko 1992, 1996, 1998).

The acquired results demonstrated that changes of selected blood serum parameters (increase as well as decrease) can be observed in carp following their 24-hour exposure to sublethal concentrations of deltametrin. This is much earlier detection than observations in chronic experiments of enzymatic activity changes (Jungowska-Kiln et al. 1992; Kozubek et al. 1992; Przybylska-Wojtyszyn et al. 1992).

Table 1

Effect of deltamethrin in the concentration of $0.14 \mu\text{g}/\text{dm}^3$ on biochemical parameters of carp blood ($\bar{x} \pm \text{SD}$)

| Blood serum parameters | Spring | | | Autumn | | |
|---|--------------------|--------------------|--------------------|--------------------|---------------------|--------------------|
| | Control | Exposure time | | Control | Exposure time | |
| | | 24 h | 48 h | | 24 h | 48 h |
| Glucose [mg/dm^3] | 163.45 \pm 1.31* | 185.57 \pm 1.93 | 132.70 \pm 1.22* | 150.07 \pm 1.32* | 177.18 \pm 1.98 | 163.55 \pm 1.28 |
| Chlorides [mmol/dm^3] | 122.53 \pm 2.08 | 112.80 \pm 2.74* | 122.47 \pm 2.06 | 124.18 \pm 2.20 | 113.72 \pm 2.65* | 121.70 \pm 1.99 |
| Na ⁺ [mmol/dm^3] | 146.52 \pm 2.25 | 138.46 \pm 2.56* | 146.67 \pm 2.25 | 145.62 \pm 2.40 | 135.14 \pm 1.98* | 121.69 \pm 2.65* |
| K ⁺ [mmol/dm^3] | 3.19 \pm 0.85 | 3.78 \pm 1.05* | 2.80 \pm 0.85 | 2.95 \pm 1.15 | 3.38 \pm 0.74 | 2.70 \pm 0.84 |
| Total Ca ⁺ [mg/dm^3] | 12.26 \pm 0.19 | 13.72 \pm 0.19* | 11.81 \pm 0.15 | 12.31 \pm 0.19 | 13.22 \pm 0.20 | 12.03 \pm 0.18 |
| Crude protein [g/dm^3] | 4.00 \pm 0.60 | 4.03 \pm 0.66 | 4.13 \pm 0.53 | 4.19 \pm 0.60 | 4.04 \pm 0.66 | 4.15 \pm 0.48 |
| Creatinine [mg/dm^3] | 0.30 \pm 0.07 | 0.37 \pm 0.09 | 0.32 \pm 0.07 | 0.32 \pm 0.08 | 0.34 \pm 0.10 | 0.16 \pm 0.07* |
| Urea nitrogen [mmol/dm^3] | 6.01 \pm 1.26* | 7.06 \pm 1.63 | 5.55 \pm 0.76 | 2.19 \pm 1.46* | 6.56 \pm 1.31* | 3.70 \pm 0.67 |
| Cholesterol [mg/dm^3] | 280.71 \pm 28.66 | 201.26 \pm 22.9* | 283.40 \pm 19.52 | 284.60 \pm 28.76 | 151.33 \pm 29.77* | 237.93 \pm 16.37 |

*statistically significant differences

Despite continuing the exposure for another 24 hours, the majority of changes observed in the blood serum were reversed to the levels of control groups, which would indicate that the changes constituted a non-specific stress response. Such response, considering the lack of visible behavioural reactions may be useful in determination of excitability threshold of carp to sublethal concentrations of deltamethrin.

The present results could also be an indication that a short contact with deltamethrin in the concentration of $0.14 \mu\text{g}/\text{dm}^3$ does not constitute a significant hazard for carp. Such conclusion would require, however, studies conducted on wider material, with particular emphasis on the post-intoxication period.

CONCLUSIONS

1. No seasonal differences in the content of glucose, chlorides, total calcium, Na^+ and K^+ ions, crude protein, creatinine, cholesterol were detected in the blood serum of carp delivered for the experiment from the cooling water culture. Seasonal changes were observed only in the levels of urea nitrogen.
2. In the additional 24-h period of exposure (up to 48 hours) to $0.14 \mu\text{g}/\text{dm}^3$ of deltamethrin in the spring and fall, statistically significant fluctuations of : glucose, Cl^- , Na^+ , K^+ ions, total calcium, urea nitrogen, creatinine, and cholesterol were observed in the blood serum of carp. No statistically significant changes were observed in the levels of crude protein.

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Bernard KŁYSZEJKO, Grażyna ŁYCZYWEK

WPLYW SUBLETALNEJ KONCENTRACJI DELTAMETRYNY NA WSKAŹNIKI
BIOCHEMICZNE SUROWICY KRWI KARPIA (*CYPRINUS CARPIO* L.)

STRESZCZENIE

Karpie o masie jednostkowej 400–700 g pochodzące z hodowli w wodzie pochłodniczej przez okres 24 i 48 godzin poddawano działaniu deltametryny w koncentracji $0,14 \mu\text{g}/\text{dm}^3$ – subletalnej dla tego gatunku. Źródło deltametryny stanowił insektycyd pyretroidowy o nazwie handlowej Decis 2.5 EC podawany bezpośrednio do wody akwarium doświadczalnego.

Po intoksykacji pobierano krew z serca. W uzyskanej surowicy oznaczano: glukozę, chlorki, jony Na^+ i K^+ , wapń całkowity, białko całkowite, azot mocznikowy, kreatyninę i cholesterol.

Doświadczenia wykonano w sezonie wiosennym i powtórzono w sezonie jesiennym. Stwierdzono, że w obu sezonach deltametryna w koncentracji subletalnej spowodowała zmiany w większości badanych wskaźników surowicy krwi – nasilone po 24 godzinach ekspozycji i przemijające po 8 godzinach przebywania ryb w roztworze toksykanta. Nie stwierdzono wpływu zastosowanej koncentracji deltametryny na behawioralne reakcje ryb.

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Author's address:

Bernard Kłyszajko PhD DSc
Grażyna Łyczywek MSc eng
Department of Fish Physiology
Agricultural University of Szczecin
Kazimierza Królewicza 4, 71-550 Szczecin, Poland