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Fish physiology

**EFFECT OF RELANIMAL (DIAZEPAM) ON FREE FATTY ACID (FFA)
LEVEL IN CARP (*CYPRINUS CARPIO* L.) PLASMA
UNDER THERMAL STRESS**

**WPŁYW RELANIMALU (DIAZEPAM) NA POZIOM WOLNYCH
KWAŚÓW TŁUSZCZOWYCH (FFA) W OSOCZU KARPIA
(*CYPRINUS CARPIO* L.) W STRESIE TERMICZNYM**

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The aim of this work was to determine the effect of an abrupt growth in temperature (from 24 to 35°C) on the FFA level in carp plasma, for the fish kept in water without and with Relanibal at concentration of 0.5 ml/l.

It was noted, for the fish kept in pure water, the FFA level in fish plasma to decrease, respectively, by 20 and 70% after 15 and 30 min. exposure to increased temperature. With the Relanibal added the FFA content, in carp plasma, decreased by 5.4 and 10.8% respectively, after 15 and 30 min. exposure

INTRODUCTION

Thermal stress has been recognized as a causative agent of some hormonal (Farkas 1967; Mazeaud and Mazeaud 1981), enzymatic (Kayama et al. 1986), hematological (Mazeaud et al. 1977; Głębocka 1978) changes in fish as well as changes in lipids metabolism (Farkas 1967; Leibson et al. 1968; Brodziak 1969; Plysetskaja 1980).

Like in farm animal cultures (Fitko 1974), stress effects in fish can be soothed by pharmacological agents (Głębocka 1978; Cybulska 1981; Węgrzynowicz et al. 1981; Kłyszajko 1986).

The aim of this work was to estimate changes in free fatty acids (FFA) level in carp plasma after an abrupt growth in temperature (from 24 to 35°C) and to sooth the effect of those changes with the Relanibal (an active substance - diazepam: $C_{16}H_{13}ClN_2O$).

MATERIAL AND METHODS

Surveys were carried out on 50 carp individuals, 300 - 500 g each, collected from the Freshwater Fish Culture in Nowe Czarnowo, between 4th and 18th May. Experiments were conducted in aquarium - 200 l in volume, in aerated tap water of pH 7.2 to 7.5 and oxygen content between 8.0 - 9.5 mg/l. Fast transfer of fish from temperature 24°C to 35°C was the stress agent applied. As to sooth stress effect 0.5 ml of Relanimal (product of Polfa) per 1 l of water was added.

Fish as a whole, were divided, at random, into 3 groups:

1. Control group, kept at 24°C (10 individuals),
2. Group under stress (20 individuals),
3. Group under stress + Relanimal (20 individuals).

Blood samples, collected from the tail artery, after 15 and 30 min exposure of fish to increased temperature, were centrifuged immediately and plasma samples were stored in ice, in vacuum flask. The FFA level in plasma was estimated by the colorimetric method (Duncome 1964; cited by Kokot 1969), 3 hours after the blood samples were collected.

RESULTS AND DISCUSSION

Results of the work gathered in Table 1 and on Fig. 1 show, that rapid increase in water temperature, from 24° to 35°C, resulted in marked decrease in FFA content, in carp plasma. For fish exposed to thermal stress for 15 min. the FFA level in fish plasma decreased by 20% and after 30 min. exposure lowered by 70%, when compared to the one for an unstressed fish (0.130 mM/l - control group). Such result confirmed those of previous surveys on thermal stress to effect carp and other fish species (Mazeaud 1969; Fletcher 1984).

According to the Mazeaud et al. (1977) classification, changes in the FFA content in fish plasma belong to the secondary stress symptoms; nevertheless, when based on such changes, estimation of the stress intensity is difficult. The FFA level in fish plasma depends on fish species and sometimes even on the variety within the species. It is also affected by season, diet and condition of the fish (Farkas 1967; Leibson et al. 1968; Mazeaud 1969; Plyetskaja 1980).

When exposed to various stress agents (exercise, changes in water temperature, oxygen deficiency, etc.) the FFA level in fish plasma is, usually, decreasing. According to several authors such decrease can be, possibly, caused (in between) by accelerated glycogenolysis, noted for the fish under stress, resulting in hyperglykemia, which slows down the FFA synthesis from the lipid tissue (Hattingh 1967; Plyetskaja 1980).

Results of this works proved, the decrease in the FFA content of carp plasma to be much lower for the fish exposed to thermal stress with Relanimal present, than for fish stressed with no Relanimal added (Tab. 1, Fig. 1).

Table 1

The level of serum FFA in carp

Groups	Control group	Thermal stress		Thermal stress + Relanimal (0.5 ml/l of the water)	
Temperature of water (°C)	24 ± 1.5	35 ± 1.5		35 ± 1.5	
Exposition time (min)	≥ 3 days acclimation	10	30	10	30
Number of fish	10	10	10	10	10
FFA (mM/l)					
$\bar{x}$	0.130	0.104	0.039	0.123	0.116
±SE	±0.003	±0.002	±0.003	±0.004	±0.004
%	100	80	30	94	89.2

Diazepam - an active substance of Relanimal belongs to benzodiazepins, commonly applied in medicine as psychotropic drugs and used as stress symptoms soothing agent for farm animals and fish (Fitko 1974; Węgrzynowicz et al. 1981).

The benzodiazepins mode of action is to active physiological protective mechanisms within the central nervous system, by making an inhibitory transfer within the gabergic synapses easier (Valzelli 1973; Haefely 1984; Costa 1984). It was noted for benzodiazepins to have the strongest effect on the limbic system and hypothalamus (Schallek et al. 1971). Benzodiazepins reduce the catecholamines and dopamine formation (Corrodi et al. 1971) and decrease catecholamines level in fish when under stress (Cybulska 1981).

Fig. 1. Effect of thermal stress and Relanimal on FFA serum level in carp plasma. I - the water without of Relanimal; II - the Relanimal - concentration 0.5 ml/l in the water

Presumably, a limited increase in catecholamines level (less intensive glycogenolysis and hiperglicemia - in result) in fish exposed to thermal stress, with Relaninal added, could be one of possible causes of lower changes range of the FFA level in carp plasma, noted in this work.

CONCLUSIONS

1. Fast increase in water temperature from 24 to 35°C result in decrease of the free fatty acid (FFA) level in carp plasma: by 20% after 15 min. and by 70% after 30 min. exposure.
2. Relaninal, added to water at the concentration of 0.5 ml/l lowers down the drop of the FFA content in plasma of carp under thermal stress by 5.4% after 15 min. and by 10.8% after 30 min. exposure when compared to the standard level for unstressed fish.

REFERENCES

- Brodziak A., 1969: The homeostasis of glucose and free fatty acids. *Acta Med. Pol.*, **10** : 325-326.
- Corrodi H., K. Fuxe, P. Lindbrink, L. Olson, 1971: Minor tranquillizers stress and central catecholamine neurons. *Brain Research*, **29** : 1-16.
- Costa E., 1984: Neurologia lęku. [Fear neurobiology]. *Wszechświat*, **85**, 7-8 : 155-159. (In Polish).
- Cybulska N., 1981: Zmiany poziomu amin katecholowych we krwi karpi pod wpływem Relaninalu, w hodowlach wielkostadnych, w wodach zrzutowych. [Changes in catecholamines level in carp blood due to Relaninal, in farm cultures in discharged waters]. Praca nie publikowana, maszynopis - Zakład Fizjologii Ryb AR Szczecin. (In Polish).
- Farkas T.L., 1967: The effects of catecholamines and adreno-corticotropoics hormones on blood and adipose tissue FFA levels in the fish, *Cyprinus carpio*. *Progr. Biochem. Pharm.*, **3** : 314-319.
- Fitko R., 1974: Antystresowe właściwości preparatu Relaninal u świń. [Anti-stress effects of Relaninal in pigs]. *Nowości Wet.*, **2** : 165-175. (In Polish).
- Fletcher D.J., 1984: Plasma glucose and plasma fatty acids level of *Limanda limanda* (L.) in relation to season, stress, glucose loads and nutritional state. *J. Fish Biol.*, **25**, 6 : 629-648.
- Głębocka G., 1978: Zmiany hematologiczne u karpia *Cyprinus carpio* L. w szoku temicznym oraz próby ich łagodzenia trankwilizatorami. [Hematological changes in carp *Cyprinus carpio* L. under thermal stress and soothing effect of tranquilizers]. Praca nie publikowana, maszynopis - Zakład Fizjologii Ryb AR Szczecin. (In Polish).
- Haefely W.E., 1984: System gabergiczny i benzodwiazepiny. [Gabergic system and benzdiazepins]. *Wszechświat*, **85**, 6 : 125-131. (In Polish).
- Hattingh J., 1976: Blood sugar as an indicator of stress in the freshwater fish *Labeo capensis* Smith. *J. Fish Biol.*, **10** : 191-195.
- Kayama M., M. Hirata, T. Hisai, 1986: Effect of water temperature on the desaturation of fatty acid in carp. *Bull. Jap. Soc. Sci. Fish.*, **52**, 5 : 853-857.
- Kłyszajko B., 1986: Wpływ diazepamu jako antystresora na przebieg zmian hematologicznych u karpia (*Cyprinus carpio* L.). [Effect of diazepam - an anti-stress agent on the course of hematological changes in carp (*Cyprinus carpio* L.)]. AR Szczecin, *Rozprawy*, **109** : 59 pp. (In Polish).
- Kokot F., 1969: Metody badań laboratoryjnych stosowanych w klinice. [Methods of the laboratory surveys applied in clinic]. PZWL, Warszawa : 257-258. (In Polish).
- Leibson L.G., E.M. Plysetskaja, T. Mazina, 1968: Concentration of non-esterified fatty acids in blood plasma of cyclostomes and fishes and it's changes under the influence of adrenalin and insulin. *Zhum. Evol. Biochim. Fiziol.*, **4**, 2 : 121-128. (In Russian).
- Mazeaud F., 1969: Acides gras libres plasmatiques et glycémie de la carpe. *C. r. Séanc. Soc. Biol.*, **159** : 2159-2161.
- Mazeaud M.M., F. Mazeaud, E.M. Donaldson, 1981: Primary and secondary effects of stress in fish: some new data with a general review. *Trans. Am. Fish. Soc.*, **106** : 201-212.

- Mazeaud M.M., F. Mazeaud, 1981: Adrenergic responses to stress in fish. In: Stress and Fish [A.D. Pickering ed.], Acad. Press, London-New York : 49-75.
- Plysetskaja E., 1980: Fatty acid levels in blood of cyclostomes and fish. *Env. Biol. Fish.*, 5, 3 : 273-290.
- Schallek W., J. Thomas, 1971: Effects of benzodiazepines on spontaneous electrical activity of subcritical areas in brain of cat. *Arch. Int. Pharmacodyn.*, 192 : 321-337.
- Valzelli L., 1973: Activity of benzodiazepines on aggressive behavior in rats and mice. In: The Benzodiazepines [S. Garattini, E. Mussini, L.O. Randall ed.], Paven Press, New York : 405-417.
- Węgrzynowicz R., B. Kłyszajko, G. Głębocka, R. Zbanyszek, 1981: Wpływ preparatu Relanibal na przyrosty masy ciała pstrąga tęczowego i karpia w hodowlach wielokostadnych w sadzach. [Effect of Relanibal on increase in body weight of the rainbow trout and carp in fish farming in live boxes]. *Gospodarka Rybna*, 10 : 12-13. (In Polish).

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WPŁYW RELANIMALU (DIAZEPAM) NA POZIOM WOLNYCH KWASÓW
TŁUSZCZOWYCH (FFA) W OSOCZU KARPIA (*CYPRINUS CARPIO* L.)
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STRESZCZENIE

W osoczu karpia o masie ciała 300 - 500 g, po szybkiej (skokowej) zmianie temperatury wody z 24 do 35°C, badano metodą kolorymetryczną poziom wolnych kwasów tłuszczowych (FFA).

Stwierdzono, że po upływie 15 minut od zmiany temperatury wody, poziom FFA obniżył się o 20%, a po 30 minutach obniżenie osiągnęło wartość 70%.

Dodatek do wody Relanimalu (substancja czynna - diazepam) w ilości 0,5 ml/l wpłynął łagodząco na obniżenie poziomu FFA. W obecności Relanimalu, w osoczu u ryb poddanych działaniu stresu termicznego, poziom FFA obniżył się po upływie 15 minut o 5,4% i po 30 minutach o 10,8%.

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