

RESISTANCE IN CARP (CYPRINUS CARPIO L.) AFFECTED BY A NATURAL BACTERIAL INFECTION

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Sick carp, affected by acute or chronic forms of erythrodermatitis (CE) were studied in order to obtain pathophysiological data which would permit evaluation of their clinical status and prognosis at two, ages (23 or 28 months). The number of carp and dynamics of changes in nonspecific humoral and cell mediated resistance and in the levels of selected serum proteins were determined. The ingestion index of neutrophilic granulocytes and percent of neutrophilic granulocytes capable of ingestion was significantly higher in sick carp as compared to healthy carp, at each age and for both forms of the disease. The metabolic activity of neutrophilic granulocytes, expressed as the percentage of NBT+ cells nitroterozolium blue reduction test was the same in carp with acute CE and in the control fish. On the other hand in carp suffering from the chronic form of the disease a significant decrease in the number of cells was observed NBT index and the amount of formazan in fishes with acute CE demonstrated a decrease when compared to thee corresponding values in control carp. Carp with chronic CE showed increases in the indices. The level of lysozyme (LZM) was higher in 23 and 28 month - old carp with acute CE than in healthy fishes. In carp affected by chronic CE no significant differences in LZM levels were found when compared to the healthy carp. Myeloperoxidase activity in the sick carp, particularly those with acute CE, was significantly higher than in the control carp. On the other hand serum globulin and total protein levels in the sick carp were lower than those in the control carp. The two indices showed a particularly extensive decrease in carp affected by the acute form of the disease. When all the parameters estimated in fishes with acute CE were taken into account, no differences associated with age (23 or 28 months) were noted which could be related to tendencies and directions of the observed changes.

Key words: neutrophilic granulocytes, lysozyme, myeloperoxidase, serum proteins, sick carps.

INTRODUCTION

The growing interest in mechanisms of resistance in fishes and cultured fishes in particular results first of all from the numerous infectious (bacterial, viral) diseases and invasive diseases. The infections and invasions lead to disturbed development or death of the fish and, thus, to material losses. Adequate measures of protection of animals against diseases, particularly against bacterial and viral diseases, represents an important research goal, also from the practical and economic point of view. The most rational solution within the preventive measures and prophylaxis of fish disease should take advantage of the natural protective mechanisms of an organism or immune system (Lamers, 1985; Stosik, and Deptula, 1990, 1995; cit Frost, 1991; Van Muiswinkel, 1992; Kodoma et al., 1993; Myszkowski, 1993). Possibly complete information on the role and importance of individual elements of resistance mechanisms in of various ages is of practical application in increasingly intense culture of the animals which are exposed to a growing scale of hazards. The present study is aimed at finding out the direction of change in quantitative indices and alterations in activity of selected resistance indices of cell mediated and humoral types and in protein levels in carp of different ages affected by acute or chronic bacterial infection, i. e. by erythrodermatitis.

MATERIAL AND METHODS

The studies were performed on 87 sick carp and 60 healthy carp (Table 1), divided in 6 groups:

Table 1. Groups of sick and healthy carps used for the studies.

Experimental group	Number of fishes used for the studies	Group of cultured carps and their symbol	Clinical status	Age (months)	Body weight (g \pm 10%)
1	32	fru-K ₁	sick - brianchiomycosis acute form	2	8
2	20	fru-K ₁	healthy - control group	2	9
3	45	older fry-K ₂	sick - brianchonecrosis chronic form	12	90
4	20	older fry-K ₂	healthy - control group	12	80
5	30	commercial carps-K ₃	sick - erythrodermatitis acute form	23	250
6	20	commercial carps-K ₃	healthy - control group	23	240
7	27	commercial carps-K ₃	sick - erythrodermatitis acute form	23	250
8	20	commercial carps-K ₃	healthy - control group	23	250
9	30	commercial carps-K ₃	sick - erythrodermatitis acute form	28	750
10	20	commercial carps-K ₃	healthy - control group	28	760

- commercial carp, aged 23 months, showing signs of acute erythrodermatitis - group I, or of chronic erythrodermatitis - group III,
controls: healthy carp of the same age - groups II and IV;

- commercial carp, aged 28 months, with signs of acute erythrodermatitis - group V, controls: healthy carp of the same age - group VI.

A. *Non-specific cell-mediated resistance.* 1. The ability to ingest *Staphylococcus aureus* strain 209P by neutrophilic granulocytes was examined as described by Bruchowska (1966), as adapted for fishes (Stosik, 1991). The results are presented as the index of ingestion by neutrophilic granulocytes (lig) and the percent of ingesting neutrophilic granulocytes (%ig).

2. Metabolic activity of neutrophilic granulocytes was determined by:

2a. The nitrotetrazolium blue spontaneous reduction test (NBT), using the cytochemical technique of Park et al. (1968), as adapted for fishes (Stosik, 1987). The results are presented as the percentage of neutrophilic granulocytes which contained deposits of reduced NBT, i. e. formazan (NBT + cells).

2b. Spontaneous NB reduction employing the microquantitative technique of Raman and Poland (1975) and Sychlowy and Lucas (1978), as adapted for fishes (Siwicki et al., 1985) and expressed as the NBT index and formazan level (g/L blood).

B. *Non-specific humoral resistance.* 1. The level of lysozyme (LZM) in the serum was determined by the plate technique against *Micrococcus luteus*, according to Hankiewicz and Swierze (1974).

2. activity of myeloperoxidase (MPO) of neutrophilic granulocytes was determined cytochemically according to Graham (Zbwistowski, 1986), as adapted to fishes (Stosik, 1990). The results are expressed as MPO activity index.

C. *Serum proteins.* 1. Serum globulin level was determined according to Stone and Gitt R (1969).

2. Total serum protein, was determined by the biuret technique Pinkiewicz, 1971. The results were subjected to statistical evaluation using Student's t test for $p = 0,05$. Arithmetical means and standard deviations were calculated and the significance of differences between results obtained for healthy and sick carps.

RESULTS AND DISCUSSION

The index of ingestion by granulocytes (lig) and the percent of ingesting granulocytes (%ig) (Table 2) were significantly higher in sick carp than in healthy carp, independently of their age and the form of the disease. The highest values of the lig were found in carp with chronic erythrodermatitis. The results are consistent with those of Siwicki et al. (1985) obtained in 2 year-old carps with branchionecrosis and with our earlier studies on 2 year-old (Stosik, 1987) and 3 year-old (Stosik, 1991) carp suffering from erythrodermatitis.

Metabolic activity of granulocytes (Table 3), expressed as the percentage of NBT + cells, showed no changes in carp with the acute form of the disease as compared to the control fishes. On the other hand, in carp suffering from the chronic form of the disease, a significant decrease in number of cells was observed. Metabolic activity of granulocytes, expressed by the NBT index and formazan level, was lower in carp with the acute disease. The observations

confirm the tendencies and direction of changes noted earlier in 2- and 3 year-old carp (Stosik, 1987 and 1991, respectively), suffering from erythrodermatitis and in one year-old fishes experimentally infected with *Pseudomonas* and *Aeromonas* bacteria (Siwicki and Studnicka, 1987). In carp with the chronic form of erythrodermatitis the change in granulocyte metabolic activity, manifested by the increase in NBT index and in formazan level was consistent with the results of studies conducted on 2 year-old carp with the chronic form of bracionecrosis (Siwicki et al., 1985) and in 2- or 3-year-old carp suffering from erythrodermatitis (Stosik, 1987 and 1991, respectively). Summing up the results of studies on the metabolic activity of granulocytes, it should be stressed that the absence of correlation between results obtained by the cytochemical technique (% NBT + cells) and those obtained in the microquantitative NBT reduction test (NBT index, formazon level) which has been noted at different stages of individual development of the fish, confirms earlier results obtained in carp (Stosik, 1987, 1991) as well as in humans and "higher" animals (Raman, and Poland, 1975; Sychlowya and Lucas, 1978; Salwa, 1980). Also the absence of correlation between ingesting ability of granulocytes and metabolic activity of granulocytes in fishes with acute forms of the disease observed in our studies confirms earlier studies in carp with bacterial infection (Stosik 1987, 1991) and in humans (Mackiewicz, and Somik, 1976; Svobodova, and Vykusova, 1989).

Table 2. Chemical tests on water samples from ponds, in which carps were cultured.

Ponds, from which water samples were taken	Season of the year (months)	Groups of experimental fishes	number of tested water samples	water temp.* (°C)	chemical tests*					
					pH (mg/l)	O ₂ (mg/l)	NH ₄ ⁺ (mg/l)	NH ₃ (mg/l)	NH ₄ ⁺ + NH ₃ (mg/l)	PO ₄ (mg/l)
I	VII	2	33	24,1	7,02	6,85	0,55	nd	0,55	0,20
II	V	4	3	14,3	7,00	8,30	1,76	nd	1,76	0,40
III	IV	6, 8	6	12,3	7,43	7,30	1,40	nd	1,40	0,30
IV	IX	10	3	11,3	7,35	7,40	1,52	nd	1,52	0,20
V	VII	1	3	23,3	7,82	5,70	1,94	nd	1,94	0,70
VI	V	3	6	20,1	8,89	4,30	2,60	0,038	2,638	0,90
VII	IV	5	6	10,8	7,47	8,20	1,34	nd	1,34	0,40
VIII	IV	7	3	12,3	7,53	7,50	1,38	nd	1,38	0,30
IX	IX	9	6	10,8	7,28	7,30	1,51	nd	1,51	0,30

Explanation: * - the established parameters were given in the form of arithmetical means.
nd - no NH₃ was detected.

The lysozyme level in carp of different ages affected with an acute disease process was significantly higher than in healthy fishes (Table 4). The results confirm data of Siwicki and Studnicka (1987), who noted similar changes in one year-old carp experimentally infected with *Pseudomonas alcaligenes* and *Aeromonas punctata* bacteria. On the other hand, no changes, compared to the control carp were observed in lysozym levels in 23 month-old carps suffering from a chronic form of erythrodermatitis. A similar trend of changes in LZM levels was demonstrated in carp chronically intoxicated with zinc sulfate (Svobodova and

Vykusova, 1989) and the in rainbow trout (*Oncorhynchus mykiss*) (Mock, Peters, 1990). The recorded changes in the level and activity of LZM in fishes reflect the reactivity of resistance systems in the fishes or alterations in the reactivity. As indicated by the studies of Mock and Peters (1990), situations weakly stressing the fish may induce either an increase or a decrease in the level and activity of the enzyme. Interpretation of the findings is difficult, particularly since the mechanism of stress effects on the amount and activity of lysozyme in fishes has not been clarified yet.

Table 3. Number and ingesting ability of thrombocytes in sick and healthy carps.

Experimental group	Type and form of the disease	Age (months)	Thrombocytes $\times 10^9/L$	Thrombocytes	
				lit	%it
1	branchiomycosis - acute form	2	$18,24 \pm 4,82^*$	$0,98 \pm 0,18^*$	$11,43 \pm 1,96^*$
2	healthy	2	$23,46 \pm 2,34$	$1,48 \pm 0,32$	$15,24 \pm 2,98$
3	branchionecrosis - chronic form	12	$34,63 \pm 5,27^{**}$	$1,69 \pm 0,24$	$17,13 \pm 2,19$
4	healthy	12	$28,68 \pm 3,31$	$1,78 \pm 0,26$	$17,43 \pm 3,11$
5	erythrodermatitis - acute form	23	$22,32 \pm 4,81^*$	$1,24 \pm 0,21^*$	$9,24 \pm 2,07^*$
6	healthy	23	$31,48 \pm 5,51$	$2,00 \pm 0,24$	$17,16 \pm 3,14$
7	erythrodermatitis - chronic form	23	$49,23 \pm 5,92^{**}$	$1,86 \pm 0,29$	$16,97 \pm 2,86$
8	healthy	23	$31,48 \pm 5,51$	$2,00 \pm 0,24$	$17,16 \pm 3,14$
9	erythrodermatitis - acute form	28	$24,98 \pm 6,12^*$	$1,22 \pm 0,19^*$	$10,17 \pm 3,11^*$
10	healthy	28	$31,63 \pm 7,25$	$1,87 \pm 0,21$	$17,09 \pm 2,41$

Explanation: lit - index of ingesting ability of thrombocytes.

%it - % of ingesting thrombocytes.

* - statistically significant decrease in value as compared to the control group.

** - statistically significant increase in value as compared to the control group.

Activity of myeloperoxidase in carp with acute erythrodermatitis (Table 4) was higher than in healthy carps. A similar trend in MPO activity changes was observed in carp affected by the chronic form of the disease. Thus, our own earlier observations on two year-old carp, in which acute or chronic erythrodermatitis was diagnosed (Stosik, 1990; Stosik, 1991) and the data of Studnicka and Siwicki (1990) recorded on one year-old carp invaded by *Eimeria subepithelialis*, confirm the importance of non-specific resistance in this species. The higher MPO activity in the sick carp documents the stimulatory effect of bacterial and parasitic factors on neutrophilic granulocytes and indicates that the MPO hydrogen peroxide system plays significant role in elimination of pathogenic factors in carp. The direction of changes found in both indices of non-specific humoral immunity (LZM level and MPO activity) confirms the significant role of non-specific immunity in fishes.

Levels of the markers which indirectly also reflect the condition of specific humoral immunity, i. e. levels of globulin and total protein, behaved in a distinct manner. The typical decrease in globulin level and in total serum protein, observed in our studies in sick carp, was independent of fish age and form of the disease (Table 5). It should, however, be stressed that in carp affected by the

chronic form of erythrodermatitis, the decrease in total globulins, even if significant, was two-to three-fold less pronounced than in the fishes affected by the acute form of the disease. The results correspond to the observations of Svobodova and Vykusova (1989) in carp subjected to chronic intoxication with Anc sulphate in which decreases in globulin level and in total protein were found. Also the studies of Evenberg et al. (1986) on *Aeromonas salmonicida* infected carp followed the trend noted in the present studies. It should be added that the reasons for the decreased total protein and the lowered globulins in bacterial infected fish are far from clear (Evenberg et al. 1986). The decreases may be linked to increased permeability of blood vessels due to augmented histamine release (Ellis et al., 1981), to inhibited protein synthesis and to the lowered or absent appetite in the sick fishes (Evenberg et al., 1986).

According to the latter authors, most probably the phenomenon results first of all from nonspecific proteolysis of serum proteins, as earlier remarked by Ellis (cited by Evenberg et al., 1986). The hypothesis was confirmed by Sakai (1985) who demonstrated that *Aeromonas salmonicida* protease destroys serum proteins in fishes and by Duswald cited by Evenberg et al., 1986), who noted that proteolysis of almost all serum proteins in mammals was associated with the presence of Gram-negative bacteria (and this group includes microbes capable of inducing erythrodermatitis in the fish). Summing up our own results we may state that, irrespectively of the disease form, similar changes take place in globulins and in total protein in the sick carp. It should also be stressed that the phenomenon develops independently of the age and body mass of the fish. It seems that the findings emphasize the role and significance of non-specific resistance mechanisms in fish.

CONCLUSION

From the results of studies on carp suffering from acute or chronic erythrodermatitis it may be concluded that the age of the sick fishes did not affect the pattern of resistance found in the carp. The observed differences seemed to be associated with the chronic or acute course of the disease. The acute or chronic course of the disease mainly affected the metabolic activity of neutrophilic granulocytes (%NTB + cells, MT index, amount of formazan) (Table 3) and the serum lysozyme level (Table 4).

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OTPORNOST ŠARANA (*Cyprinus carpio* L.) NA PRIRODNU BAKTERIJSKU INFEKCIJU

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SADRŽAJ

U radu je izučavana bolest šarana zahvaćenih akutnim ili hroničnim formama erythrodermatita (CE). U krvi obolelih riba ispitivana je metabolička aktivnost neutrofilnih granulocita, kvantitativna i kvalitativna zastupljenost proteina u krvnoj plazmi. Utvrđeno je da su nivoi ukupnih proteina i serum globulina bili kod obolelih riba značajno niži nego kod zdravih koji su predstavljali kontrolnu grupu.