Lysozyme activity of grass carp (*Ctenopharingodon idella*) following exposure to sublethal concentrations of organophosphate, diazinon

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Abstract: Lysozyme activity of grass carp (*Ctenopharingodon idella*) weighing 850 g were assessed following exposing fish to various concentrations of diazinon at 1, 2 and 4mg/L provided as a bath for 12 hours at 20–2"C. Compared with the control fish there was no significant difference in levels of lysozyme in tissues of spleen and kidney of experimental fish after one day post-exposing to the toxicant ($p>0.05$), whilst lysozyme contents in sera of experimental fish were significantly lower than control fish ($p<0.05$). After 7 day-post-exposing fish to toxicant, the lysozyme contents in both spleen and kidney of fish exposed to 2 and 4 mg/L of toxicant were significantly higher than control fish ($p<0.05$). Also, compared with control group, no significant difference was observed in lysozyme content in sera and tissues of kidney and spleen of experimental fish 15, 30 and 45 days post-exposure ($p>0.05$). These results show that diazinon at sublethal concentrations stimulate some non-specific immune defence mechanisms of grass carp through enhancing of lysozyme content in heamatopoeitic tissues and blood serum. *J.Vet.Res. 62,2:49-52,2007.*

Key words: Diazinon, grass carp, lysozyme.

Introduction

Nonspecific defence mechanisms are eminently important in fish because of possessing fewer complex specific immune capabilities than higher vertebrates (Ingram, 1980; Rijkers, 1982; Anderson and Zeeman, 1995; Kozinenko *et al.,* 1999; Rice, 2001). Adequate works have been carried out to study the antibacterial and opsonin stimulation properties of lysozyme as a major factor of natural resistance or innate immunity in fish (Fletcher and White, 1976; Murrary and Fletcher, 1976; Studnicka *et al.,* 1986; Siwicki and Studnicka, 1987; Grinde, 1988; Grinde *et al.,* ,1988; Mock and Peters, 1990; Itami *et al.,* 1992; Paulsen *et al.,* 2001; Mikryakov *et al.,* 2002). However, limited data are available concerning the role of this enzyme to unfavourable environmental conditions or in pathological and adaptive processes under such stresses. Diazinon is one of major herbicidal/insecticidal chemicals currently used in both North and South Iran where grass carp are mainly grown. The aim of this study was to determine the effect of various concentrations of diazinon on the lysozyme activity of grass carp in order to evaluate the fish immunity after exposing to such toxic chemical.

Materials and Methods

Fish: One hundred twenty grass carp (*Ctenopharingodon idella*) weighting 850 g from Mazandran province fish farms were used for the experiments. Fish were held in 1200 L tanks containing 15 fish each tank with continuing water flow at 4L/minute at 20–2"C, dissolved oxygen of 7.7 mg/L and pH 7.5. Fish were acclimated to new conditions one week prior to the experiments. Fish were fed with fresh

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vegetables consisting of lucerne, clover and lettuce.

Application of Diazinon: Diazinon (Maccidal EC 600) in the form of 60% emulsion was applied to the water of each tank at concentrations of 1, 2 and 4 mg /L as a bath for 12 hours at 20–2"C. Fish were then transferred to clean water and were kept separately for 45 days. Control group were kept in clean water separately.

Sample Collection and Assay: Samples were collected after 1, 7, 15, 30 and 45 days post exposure to the toxicant. Five fish per treatment were used at each sampling time. Blood was obtained by cutting fish tail after stunning with a sharp blow to the head and removing the scales and scraping the tail with alcohol.

In order to assay lysozyme content, 3-4 ml blood was collected into strile tubes, after 1 hour at room temperature, blood samples were centrifuged at 7000g for 10 minutes and separated sera were frozen at -20"C until used for lysozyme assay within 3 days post sampling. Weighted amounts of <1.0 g of tissue samples consisting of spleen and kidney were immediately obtained, homogenized with one part (W/V) of strile sodium phosphate buffer (PBS) (0.004M, pH 6.2), incubated at room temperature for one hour and subjected to three cycles of freezethaw to extract additional lysozyme from the samples. The tissue samples were then centrifuged at 7000g for 30 minutes and the supernatants were used for lysozyme assay. Lysozyme level in blood and tissue samples was determined by the turbidimetric assay according to the method described by Ellis (1990) with slight modifications. Briefly, aliquots (1.75 ml) of *Micrococcus lysodeikticus* suspension (Sigma) (0.375 mg/ml, 0.05 M sodium phosphate buffer, pH 6.2) was mixed with 250 L of each sample and the optical density was measured after 15 and 180 seconds by spectrophotometer (Biophotometer Eppendorf) at 670 nm wavelength. PBS was used as the blank and results were expressed in amounts of lysozyme (g) per one milligram of sample calibrated using a standard cure determined with hens egg white lysozyme (Sigma) in PBS.

Data were statistically analysed using Spss and Anova software (t-test) and values were significant as $p<0.05$.

Results

Results of lysozyme contents in sera and tissues of spleen and kidney samples of fish exposed to various concentrations of diazinon are shown in Table 1. Compared with control fish the level of lysozyme in tissues of spleen and kidney was significantly higher in fish exposed to 2 and 4 mg/L diazinon 7 days post-exposure ($p<0.05$). Also, significantly lower level of lysozyme was detected in sera of fish exposed to all concentrations of toxicant only one day post-exposure ($p<0.05$). Also, compared with control group, no significant difference was observed in lysozyme content in sera and tissues of kidney and spleen of experimental fish 15, 30 and 45 days post-exposure ($p>0.05$). The highest and the lowest reactions to diazinon on the average level of lysozyme were recorded in spleen and sera samples of fish exposed to 4 mg/L of toxicant 7 and 1 day post-exposure, respectively.

Discussion

Lysozyme available in the lysosomes of neutrophils and macrophages, is secreted into the blood by these cells (Goldstein *et al.,* 1975; Murray *et al.,* 1976; Gallin, 1982; Mock *et al.,* 1990) and can function in the blood or intercellular spaces of the tissue following their destruction possibly under the influence of stressor-localized factors as reported in mammals (Fange, 1984; 1986; Weeks and Warinner,1984; Florensov and Pestova, 1990; Zapata *et al.,* 1996, Kozinenko *et al.,* 1999). The highest level of lysozyme in spleen and kidney tissues following 7 days of exposing grass carp to sublethal concentrations of diazinon may be indicative of an increase in the percentage of segmented forms of neutrophils in the blood as shown by differential counts of leucocytes (data not shown). The strong reaction in spleen tissue in response to the toxicants was considered in studies on the influence of sublethal concentrations of some heavy metal salts consisting of Hg, Cd and Cu on the contents of

Table 1. Lysozyme contents (µg/mg or ml) in spleen, kidney and serum of grass carp exposed to diazinon a 20±2°C, (n=5, Mean±SEM).

Organ sampled	Sampling time (day)	Diazinon concentration (mg/l)			Control
		1	2	4	
Spleen	1	6.15 – 0.06	6.22 – 0.13	5.95 – 0.07	6 – 0.28
	7	7.95 – 0.06	8.6 – 0.08 *	9.35 – 0.11 *	7.09 – 0.2
	15	6.4 – 0.15	6.74 – 0.05	6.8 – 0.02	6.9 – 0.05
	30	5.5 – 0.03	6.1 – 0.04	6 – 0.04	6.1 – 0.16
	45	6.5 – 0.16	6.4 – 0.02	6.5–0.03	6.5 – 0.05
Kidney	1	3.9 – 0.04	3.8 – 0.02	3.8 – 0.02	4 – 0.28
	7	4.9 – 0.06	6.1 – 0.08 *	6.1 – 0.13 *	4 – 0.28
	15	4.8 – 0.04	4.9 – 0.02	4.9 – 0.03	5 – 0.04
	30	5.02 – 0.03	4.5 – 0.04	4.8 – 0.1	5 – 0.1
	45	6 – 0.04	5 – 0.24	6.2–.05	6 – 0.05
Serum	1	2 – 0.2 *	1.47 – 0.02 *	1.07 – 0.04 *	10.54 – 1.2
	7	12.25 – 0.02	12 – 0.05	11.77 – 0.1	12.34 – 0.06
	15	13.94 – 0.13	14.4 – 0.07	14.6 – 0.07	14.56 – 0.98
	30	12.2 – 0.02	11.72 – 0.25	12.07 – 0.16	12.32 – 0.06
	45	12.07 – 0.04	12.1 – 0.04	12.13 – 0.03	12.2 – 0.05

*** Indicating values are significant at p< 0.05.**

lysozyme in the tissues of sturgenon fingerlings (*Acipenser baeri*) by Mikryakov *et al.,* (2002). Moreover, the rise in the levels of lysozyme in spleen tissues of fish may be due to an increasing number of lysozyme producing cells of the myeloid tissues, in particular non-segmented and segmented neutrophils as were demonstrated by other authors (e.g. Mikryakov and Lapirova (1997). It is assumed that this enzyme participates in the support of homeostasis during adaptation to unexpected environmental changes (Marc *et al.,* 1995; Charles and Rice, 2001). A high activity of this enzyme has been found in lymphomyeloid tissue of cartilaginous fish as well as in lymph and plasma of bony fishes (Fange, 1984; lysein *et al.,* 1989; Anderson and Zeeman, 1995). In mammals, during periods of high stress, lysosome exocytosis from macrophages and neutrophils can be altered by action of the stress hormones, in particular hydrocortisone as mentioned by Mock *et al.,* (1990). The present results propose that the discharge of different amounts of lysozyme in response to toxic stress, possibly mediated via hydrocortisone, may also occur in fish. Also, Mock *et al.,* (1990) concluded that the activity of this enzyme presents the modulatory action of the defence system of the organism, relying not only on the nature, but also concentration or strength of stress factor. Therefore, weak influences occur, for example 30 minute handling can result in both decrease and increase in the level of serum lysozyme, while a strong stress, for example the acute exposure of fish to ammonia ions, decreases the level of this enzyme. Therefore, the comparison of these data on concentrations of lysozyme in tissues and serum of grass carp shows that lysozyme level depends on the effect of various factors including diazinon concentrations. Also, these data show that the lysozyme content in serum and tissues of grass carp were variable and the fluctuations were defined by duration of sampling and concentration of toxicant.

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