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DETERMINING THE PLASMA LYSOZYME ACTIVITY IN *OREOCHROMIS NILOTICUS* L. INFECTED BY *LACTOCOCCUS GARVIEAE*

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Summary

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In this study the plasma lysozyme activity, as an indicator of non-specific immune response, and haematocrit values were evaluated in 4 groups (Group L: experimentally infected fish via intraperitonal injection of *L. garvieae* at a sublethal dose; Group LK: cohabitated fish with experimentally infected fish; Group P: control fish injected with PBS; Group PK: control fish without any application) of 5 tilapia (*Oreochromis niloticus* L.) fish each, experimentally infected with sublethal concentration of *Lactococcus garvieae* or healthy tilapia cohabitated with infected tilapia. Plasma lysozyme activity and haematocrit values on experimental days 3, 5 and 7 were as followed: Group L – 11.5 ± 3.03 U/mL, 19.3 ± 3.83 U/mL and 6.1 ± 0.95 U/mL ($P<0.05$); 21.20 ± 0.50%, 18.24 ± 0.80% and 10.59 ± 0.83% ($P<0.001$); for Group LK – 17.4 ± 2.83 U/mL, 7.4 ± 1.54 U/mL and 8.1 ± 2.67 U/mL ($P<0.05$); 22.64 ± 0.46%, 18.88 ± 0.53% and 12.14 ± 0.66% ($P<0.001$); for Group P – 14.6 ± 4.86 U/mL, 21.0 ± 9.30 U/mL and 9.8 ± 1.53 U/mL ($P<0.05$); 22.42 ± 0.50%, 13.84 ± 0.45% and 11.54 ± 0.49% ($P<0.001$) and for Group PK – 9.2 ± 1.32 U/mL, 5.8 ± 1.86 U/mL and 7.6 ± 1.29 U/mL ($P<0.05$); 22.13 ± 0.68%, 19.92 ± 0.52% and 14.37 ± 0.45% ($P<0.001$) respectively.

Key words: cohabitation, haematocrit, *Lactococcus garvieae*, Nile tilapia, *Oreochromis niloticus*, plasma lysozyme activity

INTRODUCTION

Infections are known as main causes of economic loss in intensive fish farms. In prophylactic terms, haematological parameters are increasingly becoming important to prevent economic loss. *Tilapia* is the second aquacultured genus after *Cyprinus* around the world particularly in subtropic and tropic areas. The Nile Tilapia (*Oreochromis niloticus)* has distinctive, regular, vertical stripes extending as far down the body as the bottom edge of the caudal fin, of variable colour. Adults reach up to 60 cm (24 inch) in length and up to 4.3 kg (9.5 lb). They live for up to 9 years, tolerate brackish water and survive temperatures between 8 and 42 °C (46 and 108 °F). Tilapia is one of the most important fishery products of 21st century. All around the world, 85 countries produce tilapia and more than 90% of their produce is *Oreochromis niloticus*. China is the first and The Philippines is the second world producer. The fish has been variously reported in the literature to be a plankton feeder, an omnivore, and to feed on higher plants to the extent it may be used in control of aquatic weeds. Also hybrid varieties *O. niloticus x O. mos-*

sambicus were used for culture (Anonymous, 2006).

It's known that in intensive culture conditions *Tilapia* could be affected by certain bacterial agents. One of the most common infective pathogens are *Streptococcus*. *Lactococcus garvieae* belongs to *Streptococcoceae* family. *L. garviea* is facultative anaerobic non-motile, non-spore forming, Gram positive, ovoid, encountered in pairs and short chains and produces α-hemolysis on blood agar. It also grows rapidly in Tripticase-soy agar and Tripticase-soy broth. Conversely, it does not growth on McConkey agar or *Enterococcus* agar (Venderell, 2006).

It can cause infections in other animals than fish – mastitis in cows and buffaloes; endocarditis, liver abscess, osteomyelitis. It is also isolated from human skin, blood and urine. The first isolation report is in the USA (1991) than from Japan and Taiwan from consumed or contacted with infected raw fish (Alfonso *et al*. 2003; Wang *et al*. 2006).

Lactococcosis (streptococcosis), is known as a systemic disease of fresh water, brackish water and marine fishes. Lactococcosis is reported from different fish species such as *Seriola quinqueradiata*, *Paralichtys olivaceus*, *Scophtalmus maximus*, *Mugil cephalus*, *Sparus aurata*, *Oncorhynchus mykiss*, *Acipenser naccarii*, *Tilapia niloticus*, *Anguilla japonica* (Kusuda *et al*., 1978; Kusuda & Kawai, 1982; Sakai *et al*., 1989; 1995; Ceschia *et al*., 1992; Carson *et al*., 1993; Kim & Lee 1994; Toranzo *et al*., 1994; Salati *et al*. ,1996; Kusuda & Salati, 1999; Gonzalez *et al*., 2000). Tilapias have a high market value with better consumer acceptance in the world. High water temperature and poor water quality influence lactococcal mortality in infected tilapia even that they can tolerate poor water quality. There are some data about the tilapia disease in the literature (Watts *et al*., 2001). However the plasma lysozyme activity and haematocrit values in tilapia have not been examined in experimental conditions.

The objective of this study was to evaluate the plasma lysozyme activity, haematocrit value in experimentally infected and cohabitated groups of tilapia, as an indicator of non-specific immune response.

MATERIAL AND METHODS

Experimental design

In this experiment 16.95±0.70 cm total length and 80.59±11.61 g live weight *Oreochromis niloticus,* 65×40×50 cm disinfected glass tanks (100 L) were used. Water temperature, pH and dissolved oxygen were 27±1 °C, 7.38 and 7.8 mg/L respectively. The experimental study were carried out in four groups: 1st Group (L): experimentally infected fish via intraperitonal injection of *Lactococcus garvieae* at a sublethal dose; 2nd Group (LK): cohabitated fish with experimentally infected fish; 3rd Group (P): control fish injected with PBS; 4th Group (PK): control fish without any application. Stocking rate was 10 fish/100 L tank (5 fish treated, 5 fish non-treated). Infected groups were duplicated in the experimental design.

LC_{50} of L. garvieae

LC_{50} values of the bacterial pathogen were determined from the equation of Kärber method. Intraperitoneal (i.p.) injection was used in all experimental infection (Brunt & Austin, 2005). LC_{50} value of *L. garvieae* was determined as 1×10^{10} cfU/mL and calculated using the equation:

$$\text{Log titre} = m\text{-}d\ (\Sigma p/n - 0.5)$$

where: m – logarithm of the last dilution that 100% death has occurred; d – fold dilutions between the logarithm of the number; Σp – sum of positive reactions; n – number of animals; 0.5 – fixed value.

Blood sampling, haematocrit value and plasma lysozyme activity

Blood samples were collected directly by cardiac puncture with 5 mL heparinised injectors on the 3rd, 5th and 7th days of the treatment (Yıldız *et al.,* 1997). Haematocrit value was determined through the microhaematocrit method (Siwicki & Anderson, 1993). Plasma samples were collected and kept at –20 °C until the turbidimetric assay for plasma lysozyme activity were performed (Ellis, 1989; Kubilay, 2002; Yıldız, 2006).

L. garvieae isolation and identification

Samplings were made on the 3rd, 5th and 7th days. Fish were observed three times a day and if any mortality were occurred, died fish were collected and necropsy were performed. At the end of experiment the fish were dissected.

TSA were used for bacterial growth. Serological tests (Oxidase, Catalase, Haemolysis, Motility, Nitrate Reduction, Indole, Citrate, Urea Hydrolysis, Voges-Proskauer, Aesculin Hydrolysis, Oxidative Fermentative, L-Arabinose Fermentation, Methyl Red, Glucose Fermentation, Inositol Fermentation, Maltose Fermentation, Mannose Fermentation, Salicin Fermentation, Sorbitol Fermentation, Sucrose Fermentation, Xylose Fermentation, Production of Hydrogen Sulphide, Trehalose Fermentation Hippurate Hydrolysis) were made for confirmation of the strain (Holt *et al*., 2000; Buller, 2004).

Lactococcus garvieae are Gram-positive cocci, of circular or elliptical shape, not spore forming, facultative anaerobic bacteria, with a diameter between 0.5–1.2 × 0.5–1.5 µm. In broth media, it produces twin or short chains. *L. garvieae* is non-motile, catalase and oxidase negative, and encapsulated. Optimum reproduction of the bacteria is at 30 °C (<10 °C no colonisation occurred) (Holt *et al*., 2000).

Statistical analysis

For statistical analysis Kruskal Walis test and Friedman test were performed in SPSS 14.0.

RESULTS AND DISCUSSION

LC_{50} of the *L. garvieae* in tilapia was detected as 1×10^{10} cfU/mL with Kärber and the sublethal dose calculated as 1×10^{9} cfU/mL (i.p). After experimental infection mortality rates and re-isolation of bacteria results were given in Table 1.

Plasma lysozyme activity of Group L (Table 2) was increased on day 5 and decreased on day 7 compared to day 3. Ly-

Table 1. Mortality rates and re-isolation of *L.garvieae*

Groups	Number of fish	Number of dead fish	*L. garvieae* isolation and identification	Alive	*L. garvieae* isolation and identification
Group L	10	7	6	3	3
Group LC	10	3	3	7	3
Group P	5	–	–	5	–
Group PC	5	–	–	5	–

Table 2. Plasma lysozyme activity of tilapia infected with sublethal dose of *L. garvieae* ($\times 10^3$ U/mL)

Experimental days	Experimental groups			
	Group L	Group LC	Group P	Group PC
3	$11.5 \pm 3.03^{Aa*}$	17.4 ± 2.83^{Aa}	14.6 ± 4.86^{Aa}	9.2 ± 1.32^{Aa}
5	19.3 ± 3.83^{ABa}	7.4 ± 1.54^{ACb}	21.0 ± 9.30^{Ba}	5.8 ± 1.86^{Ca}
7	6.1 ± 0.95^{Ab}	8.1 ± 2.67^{Ab}	9.8 ± 1.53^{Aa}	7.6 ± 1.29^{Aa}

*Different superscript capital letters in the same line and small letters in the same column indicate statistical significance (P<0.05).

Table 3. Haematocrit value of tilapia infected with sublethal dose of *L. garvieae* (%)

Experimental days	Experimental groups			
	Group L	Group LC	Group P	Group PC
3	$21.20 \pm 0.50^{Aa**}$	22.64 ± 0.46^{Aa}	22.42 ± 0.50^{Aa}	22.13 ± 0.68^{Aa}
5	18.24 ± 0.80^{Ba}	18.88 ± 0.53^{Ba}	13.84 ± 0.45^{Bb}	19.92 ± 0.52^{Bc}
7	10.59 ± 0.83^{Ca}	12.14 ± 0.66^{Ca}	11.54 ± 0.49^{Ca}	14.37 ± 0.45^{Cb}

*Different superscript capital letters in the same line and small letters in the same column indicate statistical significance (P<0.001).

sozyme activity on the day 7 was decreased compared to day 3 (P<0.05). Plasma lysozyme activity of Group LC was lower on the day 5 compared to day 3 and higher on day 7 vs day 5 but decreased compared to day 3 (P<0.05). Lyzozyme concentrations of group P were increased on day 5 and decreased on day 7 compared to day 3 (P<0.05). In Group PC, lysozyme activity decreased on day 5 and increased on day 7 compared to day 3 (P<0.05). Haematocrit value of tilapia infected with sublethal dose of *L. garvieae* ispresented in Table 3. Haematocrit values of Group L, Group LC, Group P and Group PC were reduced on day 5 compared to day 3, on day 7 compared to day 5 and on day 7 compared to day 3 (P<0.001).

Plasma lysozyme activity and haematocrit values on experimental days 3, 5 and 7 were as followed: Group L – 11.5 ± 3.03 U/mL, 19.3 ± 3.83 U/mL and 6.1 ± 0.95 U/mL (P<0.05); 21.20 ± 0.50%, 18.24 ± 0.80% and 10.59 ± 0.83% (P<0.001); for Group LK – 17.4 ± 2.83 U/mL, 7.4 ± 1.54 U/mL and 8.1 ± 2.67 U/mL (P<0.05); 22.64 ± 0.46%, 18.88 ± 0.53% and 12.14 ± 0.66% (P<0.001); for Group P – 14.6 ± 4.86 U/mL, 21.0 ± 9.30 U/mL and 9.8 ± 1.53 U/mL (P<0.05); 22.42 ± 0.50%, 13.84 ± 0.45% and 11.54 ± 0.49% (P<0.001) and for Group PK – 9.2 ± 1.32 U/mL, 5.8 ± 1.86 U/mL and 7.6 ± 1.29 U/mL (P<0.05); 22.13 ± 0.68%, 19.92 ± 0.52% and 14.37 ± 0.45% (P<0.001) respectively.

Clinically darkness and skin haemorrhages, slower movement, exophthalmos, and fin rot were observed in infected and cohabited group individuals compared with the healthy control group (Fig. 1). Lack of appetite was also detected in injected and cohabitated group.

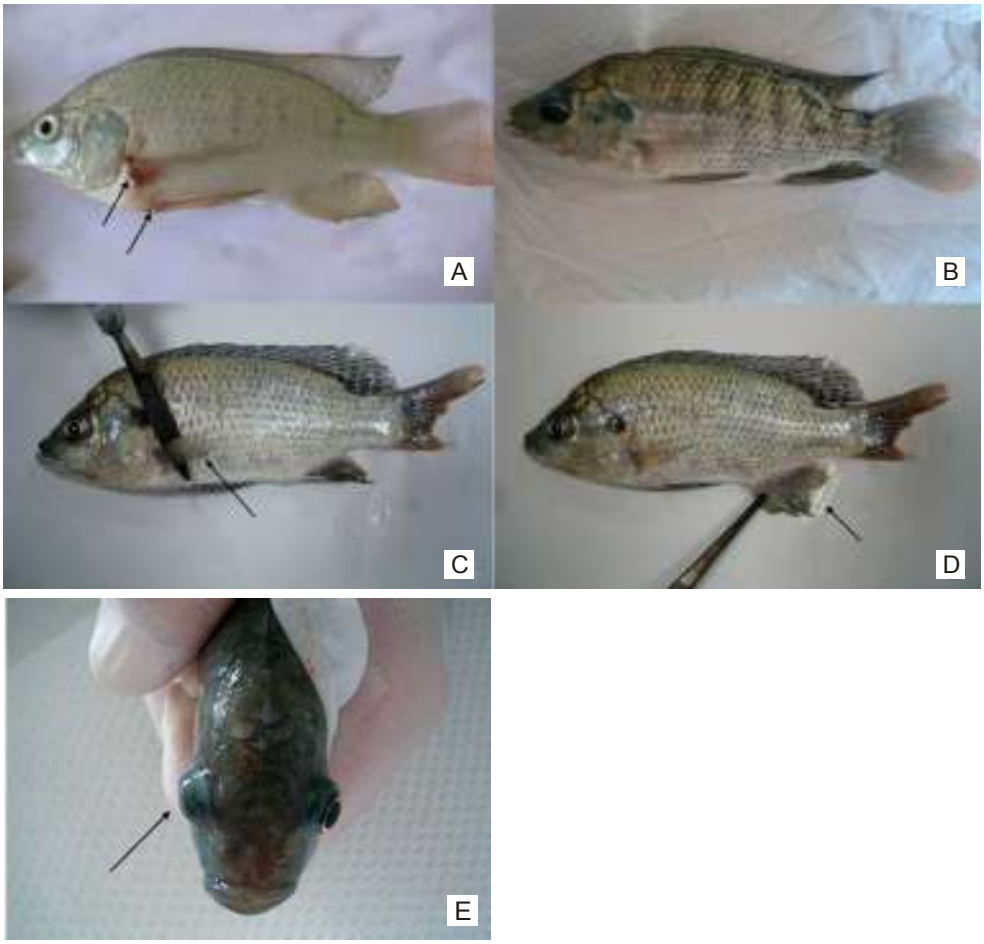

Fig. 1. Clinical pathology after experimental infection with *L. garviae*. **A**: Fin haemorrhage, **B**: Skin darkness, **C, D**: Fin rot, **E**: Exophthalmos.

The horizontal contagious infection was detected after the cohabitation challenge. The significant differences on the plasma lysozyme activity ($P<0.05$) and on the haematocrit values ($P<0.001$) were detected in cohabitated group.

Obviously the present experiment's blood parameters results were consistent with previous studies (Barham *et al.,* 1980; Siwicki & Studnicka, 1987; Lie *et al*., 1989; Demers & Boyne, 1997; Yıldız, 1998; Caruso *et al*., 2002; Chen *et al*., 2002; Ogut & Reno, 2004: Benli & Yıldız, 2004; Cnaani *et al*., 2004; Yıldız, 2006).

In conclusion the blood parameters including the plasma lysozyme activity values are occasionally used for evaluation of health status of the vertebrates. However in the present study it could be stated that these blood parameters were not successful indicators for *O. niloticus* health. One possible reason could be that *O. niloticus* is not a sensitive fish species, but it pos-

sesses high tolerance to different environmental conditions.

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