第 30 卷第 4 期
2005 年 8 月

水 产 学 报
JOURNAL OF FISHERIES OF CHINA

Vol.30, No.4
Aug., 2006

文章编号：1000 – 0615(2006)04 – 0495 – 07

Recovery of earth-pond-reared *Pelteobagrus fulvidraco* from transport stress in acclimatization of laboratory system

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Abstract: Earth-pond-reared adult yellow catfish (*Pelteobagrus fulvidraco* R.) were transported to the indoor laboratory recycle system. Feeding and responsiveness to daily management, blood cortisol and glucose, percentage and phagocytic function of circulating leukocytes of the fish were monitored immediately after transport (day 0) and in acclimatization of the laboratory system for a period of 35 days. Cessation of feeding was apparent during the first 4 to 7 days after transport. All fish resumed feeding by day 8 and adapted to daily management by day 15. Blood cortisol and glucose concentrations were significantly high at day 0, 1, 2 and 3. Elevated blood cortisol and glucose significantly decreased by day 7 and maintained at low levels at day 21, 28 and 35. No significant differences were found in circulating erythrocyte numbers. Compared to values of each parameter for circulating leukocytes at day 21, 28 and 35, lymphocytopenia was found at day 3 and 7, neutrophilia was detected at day 0, 1, 2 and 3, significantly decreased phagocytosis and phagocytic index of peripheral leukocytes were found at day 7 and 14. The results of the present study indicate (1) that transport induced characteristic stress responses in the adult yellow catfish, (2) that transport resulted in significant alterations in the percentage of circulating lymphocytes and neutrophils and suppression in the phagocytic function of circulating leukocytes in the adult yellow catfish, (3) that a minimum of three weeks needed for the earth-pond-reared adult yellow catfish to completely recover from transport stress in acclimatization of the laboratory system.

Key words: *Pelteobagrus fulvidraco*; transport stress; recovery

CLC number: S 917; S 962.3+9 **Document code**: A

1 Introduction

For many centuries yellow catfish (*Pelteobagrus fulvidraco* R.) has been one of the popular small-sized food species in China, found in quantity in rivers and lakes in the middle and lower reaches of the Yangtze River. As the market demand for yellow catfish increases and the resources decrease, the culture of yellow catfish has gained attention and become an economically viable industry in China. In recent years various pathogens and diseases of yellow catfish in both natural and cultured conditions have been reported[1 – 5], which necessitate studies on disease control for further intensive yellow catfish farming.

Farmed fish, particularly in intensive culture, encounter a variety of stressful situations. Stress is known to increase the susceptibility of fish to infectious diseases and it is well established that stress-induced immunosuppression in fish is cortisol-dependent[6,7]. Although a couple of studies put emphasis on investigating the immune system of yellow catfish[8,9], little is known about the stress responses and the effects of stress on the various immunological parameters in this newly developed commercial species.

In an attempt to investigate the effects of stress hormone cortisol on the immunological cells in yellow catfish, earth-pond-raised fish were seined and transported to the indoor laboratory recycle system. Transport is a common practice both in experimental investigations and in aquaculture. It contains a combination of stressful components, including

Received date: 2005-10-08

Foundation item: This research was funded by the Key Laboratory of Freshwater Fish Germplasm Resources & Biotechnology to Ministry of Agriculture, the Yangtze River Fishery Research Institute (YRFRI) (SHI2001-01)

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capture in pond, loading and unloading, and transportation itself, which can result in extensive and intensive changes in fish behavior and physiology[10-14]. Therefore, a thorough recovery of yellow catfish from transport stress is vital for making better experimental design and explanation of experimental results. The aim of the present paper is to provide the minimum time needed for the experimental fish to recover completely from stress and well adapt to the laboratory system.

2 Materials and methods

2.1 Source and maintenance of fish

Yellow catfish of both sexes [weight: (84.6 ± 22.7) g; (mean ± S.D.)] were seined from an earth-pond and transported to the indoor laboratory recycle system. The seining and transportation took about two hours. Six fish were anaesthetized in MS222 (150 mg·L^{-1}) and sampled immediately after transport (day 0). Another 56 fish were randomly distributed into the eight rectangular concrete tanks of the laboratory system, seven fish per tank. Each tank maintained a water volume of 120 L and was supplied with a constant charcoal-filtered and well-aerated water flow. Fish were fed daily at 1% of the body weight on a commercial chow. Uneaten feed was netted out 1 h after offering the diet. At day 1, 2, 3, 7, 14, 21, 28 and 35 respectively, six fish within one of the eight tanks were sampled. The order of tanks for sampling was randomly determined. During the present investigation, temperature ranged from 22 to 28 ℃ (ambient temperature), oxygen was above 6 mg·L^{-1}, and unionized ammonia was below 0.01 mg·L^{-1}.

2.2 Sampling

Individual fish was gently netted out and anaesthetized in MS222 (150 mg·L^{-1}). Blood was immediately collected from the caudal vessel into the syringe, then a drop was applied to the glucose kit for plasma glucose concentration reading and two blood smears were made for differential leukocyte counting (DLC). The remaining extracted blood was allocated into two tubes, the heparinized one for red blood cell counting and phagocytosis test, and the non-heparinized one for clotting and serum collection. All tubes were kept on ice during the sampling process. Determinations of the number of blood cells and the phagocytosis assay were performed immediately on fresh blood. Blood in the non-heparinized tubes was left to clot at 4 ℃ for 4 h, then centrifuged at 2500 rpm for 10 min to obtain serum. The serum was stored at −20 ℃ until needed.

2.3 Blood cortisol and glucose determination

Serum cortisol was determined by radio immuno assay (RIA) using a commercial kit (Shanghai Nuclear Technique Development Co., Ltd.) and γ-Scintillation Counter (B5002-01, COBRA-PACK-ARD). Plasma glucose concentrations were determined using a commercial kit (Yicheng Sentest JPS-Ⅲ, Yicheng Bioelectronics Technology Co. Ltd.).

2.4 Hematological measurements

The erythrocyte number was counted in a Neubauer chamber after diluting 20 μL blood by 200 times in 0.6% saline solution. Air-dried blood smears were fixed in absolute methanol for 5 min at room temperature, stained with Wright's and Giemsa compound stain, and then examined at 1000 × magnification with oil immersion. DLC were based on counting a minimum of 300 leucocytes by the battlement method for each fish.

2.5 Phagocytic assay

Phagocytosis of the peripheral leucocytes was evaluated by the uptake of *Staphylococcus aureus in vitro* in whole blood. Briefly the bacteria were grown in Ordinary Broth Agar for 24 – 48 h and harvested in sterile saline. After being washed three times by centrifugation, the bacteria were killed in 0.5% formaldehyde in sterile saline at room temperature for 2 h. After washing and verification of no further bacterial growth, the bacteria were adjusted to a final concentration of about 10^8 cell·mL^{-1}. Then 0.2 ml of bacterial suspension and the same volume of whole fish blood were mixed up and incubated at 28 ℃ for half an hour. Smears were made and examined in oil immersion. The percent phagocytosis (PP) was

determined by counting a combined total of 100 neutrophils and monocytes and the result expressed as percent positive phagocytic cells. The phagocytic index (PI) was determined by counting 100 neutrophils and monocytes (both phagocytic and nonphagocytic) and the total number of bacteria engulfed. The total number of the bacteria was then divided by 100 to yield an average number of bacteria per cell. A minimum counting of 300 neutrophils and monocytes for each fish was made and the means were used for further statistical analysis.

2.6 Data analysis

Data were analyzed by a one-way ANOVA followed by Tukey's test with STATISTICA v6.0. Results are reported as means ± S.E., and significance level of 0.05 was chosen.

3 Results

3.1 Behavioral changes

Throughout the experiment, no mortality of fish occurred. None of fish fed during the first four days in the laboratory system. By day 8 all fish resumed feeding. Fish were highly alert to daily management for the first 9 days in the laboratory system and well adapted to it by day 15.

3.2 Temporal changes of blood cortisol and glucose

Changes of serum cortisol and plasma glucose displayed a similar progressive decrease pattern after transport and in acclimatization of laboratory system (Fig. 1). Both serum cortisol and plasma glucose concentrations at day 0, 1, 2, 3 were significantly higher than values at day 7, 14, 21, 28 and 35. Maximum serum cortisol level occurred immediately after transportation (day 0). By day 7 serum cortisol had reduced significantly and was about 73 nmol·L^{-1} at day 21, 28 and 35. While plasma glucose reached the highest value at day 1, reduced a point below the measurement limit (< 40 mg·dL^{-1}) at day 7, and stabilized at day 14, 21, 28 and 35 in the range of 56 ~ 60 mg·dL^{-1}.

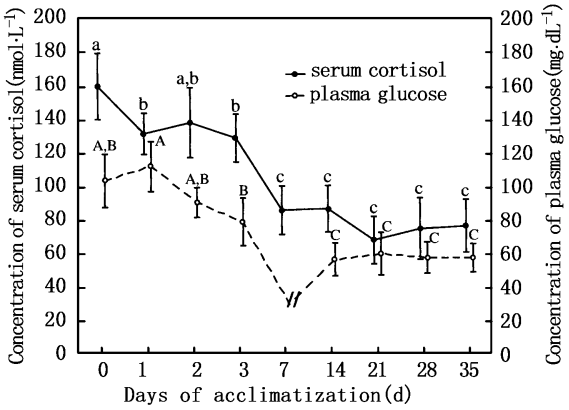

Fig.1　Changes in concentrations of serum cortisol and plasma glucose (mg·L^{-1})

Values are represented as mean ± S.E. (n = 6 for each sampling time). Statistical differences ($P < 0.05$) are indicated by lower case letters (a, b, c), or capital letters (A, B, C). No significant differences appear among values marked with the same letter

3.3 Temporal changes of erythrocyte numbers and percent leukocytes in differential leukocyte counting (DLC)

Five main cell types have been identified based on characteristics observed under electron transmission microscope: erythrocytes, lymphocytes, thrombocytes, neutrophils and monocytes (reported in a separate paper). They can be easily identified in the traditional blood smear preparations, except the difficulties sometimes caused by differentiating small lymphocytes and circular thrombocytes. No significant difference was found in erythrocyte numbers throughout the experiment, although means at day 0, 1, 2, 3 and 7 showed a progressive decrease and were lower than those at day 14, 21, 28 and 35 (Fig. 2).

Overall the percentages of lymphocytes in DLC at day 0, 1, 2, 3 and 7 were lower than those at day 14, 21, 28 and 35 (Fig. 2). The lowest percent lymphocytes were found at day 3 and 7. In contrast to percent lymphocytes, the percentages of neutrophils in DLC at day 0, 1, 2, 3 and 7 were higher than those at day 14, 21, 28 and 35 (Fig. 2).

3.4 Temporal changes in phagocytic ability of peripheral leukocytes

Both neutrophils and monocytes showed phagocytic ability. Significantly lower percent

phagocytosis occurred at day 7 and 14, which was accompanied by the lowest phagocytic index (Fig. 3). Besides, phagocytic index at day 3 was significantly lower than that at day 0, 1, 21, 28, and 35.

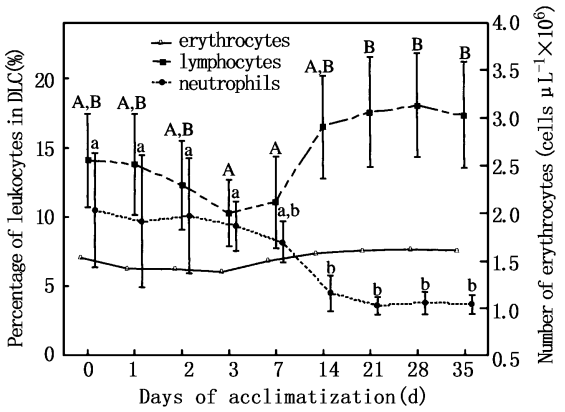

Fig.2 Changes in erythrocyte numbers, the percentage of lymphocytes and neutrophils

Values are represented as mean ± S.E. (n = 6 for each sampling time). No significant difference was found in erythrocyte numbers. Statistical differences ($P < 0.05$) in the percentage of leukocytes in DLC are indicated by lower case letters (a, b, c), or capital letters (A, B, C). No significant differences appear among values marked with the same letter

4 Discussion

Cortisol is the most widely used quantitative stress indicator in fish and is sensitive to almost all forms of stressors in aquaculture[6]. Studies on transport stress showed that successive operations of capture, loading and transportation resulted in stepwise rise in blood cortisol concentrations, which reduced the adaptability and survivor rate of the fish after transport[10,11,14]. Time needed for the elevated blood cortisol levels to return to the pre-stress values varied greatly, being 3 h in yearling *Oncorhynchus kisutch* after 2 h transport[10], within 24 h in fingerling *Stizostedion vitreum* after 5 h transport[14], but one week in immature *Perca fluviatilis* after 4 h transport[13]. It is generally accepted that the magnitude and duration of the elevated blood cortisol reflect the severity and duration of stress or the adaptability of fish[6,14]. In the present study significantly high levels of cortisol occurred immediately after transport, indicating that transport had resulted in the typical hormonal stress responses in the adult yellow catfish. Blood cortisol levels at day 21, 28 and 35 were significantly lower than values at day 1, 2, 3, and fell within the range of unstressed levels for many other fishes, i.e. < 100 nmol·L^{-1}[6]. The sustained high levels of blood cortisol during the first three days in acclimatization of laboratory system may suggest a relatively slow degree of adaptation of yellow catfish to the laboratory system.

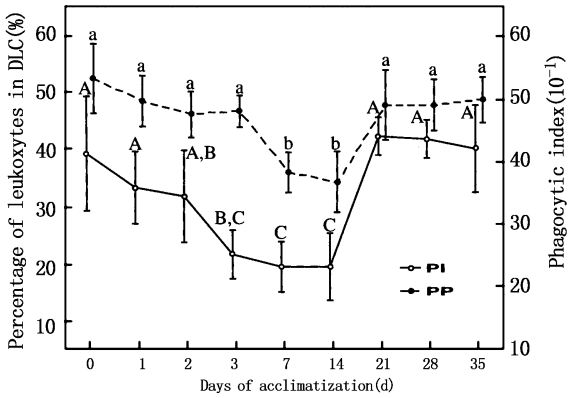

Fig.3 Changes in percent phagocytosis and phagocytic index of peripheral leucocytes

Values are represented as mean ± S.E. (n = 6 for each sampling time). Statistical differences ($P < 0.05$) are indicated by lower case letters (a, b, c), or capital letters (A, B, C). No significant differences appear among values marked with the same letter

The elevation in blood glucose, or hyperglycemia, is an important aspect of the integrated stress responses in fish. It reflects the increased energetic needs for fish to cope with stress situations, and is attributed to the effects of stress hormones on both glycogenolysis and gluconeogenesis[6,7]. In this study hyperglycemia was concomitant with elevations in blood cortisol concentrations, indicating the typical metabolic stress response in the adult yellow catfish. The lowest blood glucose value at day 7 could be explained by the significant reduction in cortisol concentrations and the prolonged cessation of feeding during the first four to seven days in acclimatization of the laboratory system.

A single 2 min handling led to 3 days cessation of feeding in brown trout (*Salmo trutta* L.) in experimental tanks[15]. Behavioral changes after

transport, such as lethargy, disorientation, reduced activity in moving and feeding were observed and tested in *Stizostedion vitreum*[14]. These changes had important impacts on survival rate of the releasing stock population in the wild. In the present study, the prolonged stop of feeding in the fish seemed to be a combined effect of transport stress and changes of living environment and food. Since yellow catfish are omnivorous and mainly feed on natural food in pond.

The elevation of red blood cell numbers or haematocrit is a common stress response in fish, which indicates an increased oxygen supply to the major organs in response to the higher metabolic demand of fish during stress[13]. As the erythrocyte numbers did not rise after transport and in acclimatization of the laboratory system, it can be assumed that the adult yellow catfish did not need to raise the oxygen carrying capacity just as the case in common carp[16].

Both numbers and function of circulating leukocytes can be altered in stressed fish. The characteristic changes include reduction in the number of circulating lymphocytes (lymphocytopenia)[15,17-19] and increase in neutrophils (neutrophilia)[17,18]. The function of immunological cells were often suppressed during stress[6,7]. It is proposed that the elevation of blood cortisol plays an important role in the immunosuppression effect of stress[20]. Recent studies have demonstrated that cortisol can directly affect the function of leukocytes through glucocorticoid receptors[21,22]. However, the mechanisms of cortisol-induced suppression of the fish's immune system are complex. For example, stress differentially affected the distribution of leukocyte subpopulations between peripheral blood and lymphoid tissues[17,18]. There were also reports on the suppressive effects of stress on the antibody response of B lymphocytes[18,19]. Cortisol induced apoptosis of fish lymphocytes both *in vivo*[21] and *in vitro*[22], and differentially affected the apoptosis and proliferation of B lymphocytes from different tissues of common carp[22]. Experiments also showed that high physiological concentration of cortisol *in vivo* can initiate phagocytic suppression, but cortisol did not act alone to induce suppression of the phagocytic function[18]. Considerable attention has been given in recent years to the modulation of immune responses by hormones[23].

In this study, the percentages of peripheral lymphocytes and neutrophils and the function of circulating phagocytic leucocytes were significantly altered in yellow catfish after transport. Based on the behavioral adaptation to daily management and the significant reduction in blood cortisol and glucose concentrations, the earth-pond-reared yellow catfish had recovered from transport stress in acclimatization of laboratory system by day 14. However, significantly low percent phagocytosis and phagocytic index of peripheral leukocytes were found at day 7 and 14 in fish after transport. Therefore two weeks are not enough for the transport-stressed adult yellow catfish to be used in studies on immune-endocrine interactions. The lymphocyte percentage in differential leukocyte counting (DLC) at day 21, 28 and 35 in fish after transport maintained at 17.4%. Compared to this value lymphocytopenia was found at day 3 and 7. The stabilized neutrophil percentage in DLC at day 21, 28 and 35 was about 4%. Compared to it, neutrophilia was detected immediately after transport and at day 1, 2 and 3.

The effects of cortisol on the function of phagocytic cells in both circulating blood and lymphoid tissues of yellow catfish will be the subjects of further studies. Based on the above data, the recommended minimum time period for the adult yellow catfish to completely recover from transport stress in acclimatization of the laboratory system is three weeks.

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池养黄颡鱼运输应激后在实验循环系统中的恢复和适应过程

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摘要:本文将土池养殖的黄颡鱼成鱼运往室内实验循环系统驯养,并对鱼的摄食及鱼对日常管理活动的反应、血液皮质醇和血糖、外周血红细胞数量、外周血白细胞百分比和吞噬功能进行了 35 d 的观察或监测。运输后的黄颡鱼在最初 4 ~ 7 d 内,明显的行为变化是停止摄食。所有的鱼从第 8 天开始恢复了摄食,从第 15 天开始适应了日常管理活动。血液皮质醇和血糖浓度在刚刚运抵实验循环系统(0 d)及驯养 1、2、3 d 的鱼体内显著升高,至第 7 天已显著下降,并在第 21、28、35 天稳定在低水平上。外周血红细胞数量没有显著变化。与第 21、28、35 天外周血白细胞的各参数值相比,淋巴细胞百分比在第 3、7 天显著减少,嗜中粒细胞百分比在 0、1、2、3 天显著升高,吞噬细胞吞噬百分比和吞噬指数在第 7、14 天显著降低。以上结果显示(1)运输使黄颡鱼成鱼产生典型的应激反应,(2)运输应激导致黄颡鱼成鱼外周血淋巴细胞和嗜中粒细胞百分比的显著改变和外周血白细胞吞噬功能的抑制,(3)运输应激后的池养黄颡鱼成鱼在本文使用的实验循环系统中充分恢复和驯化所需的最少时间是 3 周。

关键词:黄颡鱼;运输应激;恢复

中图分类号:S 917;S 962.3+9　　**文献标识码**:A

收稿日期:2005-10-08

资助项目:长江水产研究所农业部淡水鱼类资源与生物技术重点开放实验室开放课题(实 2001-01)

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