Available online at www.sciencedirect.com
SCIENCE DIRECT®
General and Comparative Endocrinology 142 (2005) 256–262
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

Effects of cortisol and stress on channel catfish (*Ictalurus punctatus*) pathogen susceptibility and lysozyme activity following exposure to *Edwardsiella ictaluri*

Brian C. Small*, A. Lelania Bilodeau

Thad Cochran National Warmwater Aquaculture Center, USDA/ARS Catfish Genetics Research Unit, P.O. Box 38, Stoneville, MS 38776, USA

Received 17 September 2004; revised 3 December 2004; accepted 13 December 2004
Available online 26 January 2005

Abstract

Periods of stress are often associated with disease outbreaks in cultured fish, and stress is often characterized by the secretion of cortisol. Although stress and cortisol secretion are highly correlated in fish, the role of cortisol in affecting channel catfish (*Ictalurus punctatus*) pathogen susceptibility is unclear. The effects of short-term stress and exogenous cortisol administration on channel catfish susceptibility to *Edwardsiella ictaluri*, the etiologic agent of enteric septicemia of catfish (ESC), were investigated. Channel catfish were exposed to virulent *E. ictaluri* following a standardized 30-min low-water stress or administration of dietary cortisol (100 mg/kg feed) and compared to a pathogen-challenged control group of catfish. Pathogen susceptibility increased in stressed catfish (43.3% mortality) when compared to cortisol-fed catfish (26.7%) and controls (26.7%). A greater ($P < 0.05$) percentage of stressed catfish (25.9%) tested positive for *E. ictaluri* relative to cortisol-fed catfish (13.0%) over the course of the study, however, average levels of circulating bacteria were not different ($P > 0.05$) among the treatments. Catfish challenged by the low-water stress event had elevated ($P < 0.05$) circulating levels of cortisol 1-day post-pathogen exposure and elevated ($P < 0.05$) lysozyme activity 4 and 14 days post-pathogen exposure when compared to cortisol-fed and control-challenged catfish. Cortisol concentrations were not correlated ($P > 0.05$) to either lysozyme activity or bacterial levels; however, lysozyme activity was positively correlated ($P = 0.0197$) to blood bacterial concentrations. These results implicate other stress factors or pathways, separate from or possibly in conjunction with cortisol, in the stress-associated immunosuppression of channel catfish as it relates to ESC susceptibility.
Published by Elsevier Inc.

Keywords: Cortisol; Channel catfish; ESC; Infection; Lysozyme; Disease

1. Introduction

Channel catfish (*Ictalurus punctatus*) are an important aquacultural species in the United States. Like many cultured species, channel catfish are subject to intensive rearing conditions and associated stressors, potentially increasing the risk of pathogen exposure and disease outbreaks. Enteric septicemia of catfish (ESC), caused by the bacterium *Edwardsiella ictaluri* (Hawke, 1979), is the most prevalent disease affecting farm-raised channel catfish in the United States, and is responsible for as much as 50% of total losses to catfish farmers each year (USDA, 1997). The pathology of ESC is well defined (Baldwin and Newton, 1993; Newton et al., 1989; Shotts et al., 1986), however, the immune mechanisms involved in resistance to *E. ictaluri* are only beginning to be elucidated (Bilodeau et al., 2003a,b; Camp et al., 2000).

The effects of stress on immune function in fishes have received much attention (see Schreck and Maule, 2001; Schreck et al., 1993; Weyts et al., 1999). In channel

* Corresponding author. Fax: +1 662 686 3567.
E-mail address: bsmall@ars.usda.gov (B.C. Small).

0016-6480/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.ygcen.2004.12.004

catfish, Davis et al. (2002) demonstrated that handling stress increased susceptibility to the protozoan parasite *Ichthyophthirius multifiliis*, but had no effect on susceptibility to channel catfish virus (CCV). Davis et al. (2002) speculated that the stress-induced susceptibility to *I. multifiliis* may have been due to suppression of the innate immune system and that the lack of effect of stress on CCV may have been due to protection afforded by an inducible immune system unaffected by stress.

A convenient and perhaps the most common measure of fish stress is plasma cortisol concentration. Fish rearing conditions, such as water quality, handling, and stocking density, can have a significant effect on circulating levels of cortisol (Patiño et al., 1986; Small, 2004; Tomasso et al., 1981a,b). Several studies have demonstrated the effects of handling, crowding, social, and behavioral stressors on fish health and immune function (see Barton and Iwama, 1991; Ellis, 1981; Schreck, 1996; Wendelaar Bonga, 1997). Prolonged elevations of plasma cortisol associated with chronic stress are generally considered to be detrimental to immune function. Although fewer studies have dealt with the immediate effects of acute stressors on fish immune function, instances of enhanced immunity and altered hematology following stress have been reported (Demers and Bayne, 1997; Dhabhar et al., 1996; Möck and Peters, 1990; Peters and Schwarzer, 1985; Peters et al., 1991). Demers and Bayne (1997) suggested that the flight-or-flight response prepares an organism for coping with alarming situations, which might include injury and pathogen exposure, and their research demonstrated a short-term enhancement in lysozyme (a plasma protein central to innate defenses) activity following acute stressors in rainbow trout (*Oncorhynchus mykiss*). Their results suggest that the well-established long-term immunosuppressive effects of stress may be preceded by short-term enhancement.

There is increasing evidence that supports a bi-directional relationship between the endocrine and immune systems in fish (see Schreck and Maule, 2001). Perhaps the best understood is the interaction between the hypothalamo–pituitary–interrenal (HPI) axis and the immune system (see Buckingham et al., 1996; Dhabhar and McEwen, 2001; Engelsma et al., 2002; Weyts et al., 1999). Weyts et al. (1999) suggests that maintenance of physiological homeostasis is dependent on the communication between endocrine and immune systems. The key to this bi-directional relationship may be that the two systems share receptors to react to mutual signals (Engelsma et al., 2002). "Trafficking" of immune system cells during the stress response (Dhabhar and McEwen, 2001) is one of the more interesting aspects of this relationship. Stress hormones such as cortisol cause the redistribution of immune system cells, and elements of the innate immune system appear to be redistributed to peripheral sites where they constitute a front line of defense (Schreck and Maule, 2001). On the contrary, chronic stress results in the maladaptive consequences of downregulation of acquired immunity (Schreck and Maule, 2001).

The aim of this study was to investigate the effects of an acute stressor and exogenous cortisol on the immune response in channel catfish following exposure to virulent *E. ictaluri*, the etiological agent of ESC. The effects of low-water-induced stress and dietary cortisol administration on mortality, circulating cortisol, lysozyme activity, and bacterial load were investigated. The functional significance of administering exogenous cortisol was to assess whether cortisol is the single upstream signal causing increased susceptibility to *E. ictaluri* in channel catfish.

2. Materials and methods

2.1. Animals and husbandry procedures

A total of 470 juvenile (15 g) USDA103-line channel catfish (male and female) from a mixed-family population were maintained indoors at the Thad Cochran National Warmwater Aquaculture facilities at a density of 10 fish/aquarium in forty-seven 76-L aquaria with a single-pass flow-rate of 8 L/min, at 26 °C, and on a 14:10 light:dark photoperiod. Fish were acclimated for 10 days and fed daily in the morning to satiety using a commercial floating catfish feed (36% crude protein; Land O'Lakes Farmland Feed, Fort Dodge, IA). All animal procedures followed accepted standards of animal care, approved by the Institutional Animal Care and Use Committee (IACUC) according to United States Department of Agriculture, Agricultural Research Service policies and procedures.

2.2. Cortisol feed

The cortisol-treated feed was prepared by dissolving crystalline cortisol (Sigma Chemical, St. Louis, MO) in 100% ethanol, and spraying the commercial catfish feed using an atomizer while turning in a small concrete mixer to produce a concentration of 100 mg cortisol/kg feed, a concentration sufficient to cause a physiological increase in plasma cortisol within 4 h of feeding (Davis et al., 2003; B.C. Small and B.C. Peterson, unpublished). The feed was air-dried and stored at 4 °C until fed.

2.3. Experimental design

Forty-five of the aquaria were randomly assigned (15/treatment) to one of three treatments: control; cortisol-fed; and stressed. At 09:00 on day 10 of acclimation, catfish in the 15 cortisol-fed treatment aquaria were fed the cortisol-laden feed to satiety and the remaining aquaria

were fed the commercial feed to satiety. At 12:15, catfish in the 15 stressed treatment aquaria were subjected to a low-water stress event. Water flow to the aquaria was stopped, and the water in each aquarium was rapidly drained to fish eye-level to induce a stress response. After 30 min of low-water stress, flow of water was resumed and the aquaria allowed to fill. At 13:00, fish in the 45 aquaria were exposed to virulent *E. ictaluri* using a 30-min static exposure (Wolters and Johnson, 1994). All the fish in three replicate aquaria per treatment were bled at the following times throughout the experiment: time 0, 1 day, 4 days, 8 days, and 14 days post-exposure. Fish in a given aquarium were sampled only once during the experiment. Fish in all treatments were fed the commercial feed daily to satiety beginning 1-day post-pathogen exposure until the end of the experiment.

2.4. Blood collection

Whole blood was collected from the caudal vasculature into syringes coated with heparin. Fish were initially anesthetized with 0.6 mg/L metomidate hydrochloride prior to blood collection, then euthanized in a 200 mg/L solution of tricaine methanesulfonate (TMS, MS-222). Metomidate hydrochloride blocks the handling-related release of cortisol into circulation, thus decreasing plasma cortisol variability due to sampling (Small, 2003). An aliquot of whole blood (100 µl) was stored at −80 °C until pathogen detection and quantification were conducted. Plasma was collected from the remaining heparinized blood following centrifugation, and stored at −80 °C until cortisol and lysozyme analyses were conducted. All the fish in the remaining two aquaria (negative controls) were bled on days 0 and 14 of the challenge, respectively, and tested to verify no presence of *E. ictaluri*.

2.5. Cortisol and lysozyme analyses

Plasma cortisol concentrations were determined using a time-resolved fluoroimmunoassay (TR-FIA) kit (R060-101; Perkin–Elmer Life Sciences, Akron, OH) modified and validated for channel catfish (Small and Davis, 2002). The TR-FIA satisfied strict criteria of precision (intra-assay CV < 7%) and reproducibility (inter-assay CV < 10%). Accuracy of the TR-FIA, calculated as the percent of exogenous cortisol recovered from spiked catfish plasma, averaged 99.5%. Assay sensitivity (minimum detection limit) in catfish plasma was 1.2 ng/ml, and the displacement curve for serially diluted channel catfish plasma paralleled the cortisol standard curve.

Plasma lysozyme activity was determined using the EnzChek lysozyme assay kit (E22013; Molecular Probes, Eugene, OR). Briefly, 25 µl of plasma was diluted with 25 µl of reaction buffer (0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5) and incubated with 50 µl of fluorescein labeled *Micrococcus lysodeikticus* (50 µg/ml) for 30 min at 37 °C. The fluorescence was measured in a fluorescence microplate reader using excitation/emission wavelengths of 485/535 nm. Background fluorescence, determined for a no-enzyme control, was subtracted from each value. Lysozyme activity of the experimental samples was calculated from a standard curve prepared with lysozyme from chicken egg white.

2.6. Pathogen detection and quantification

Genomic DNA was extracted from 100 µl of each blood sample using the High-Pure PCR Template Preparation kit (Roche Applied Science, Indianapolis, IN) with the addition of 5 µl lysozyme (10 mg/ml) and incubation at 37 °C for 15 min prior to the addition of binding buffer. All samples were eluted in 100 µl prewarmed (70 °C) elution buffer. An *E. ictaluri*-specific target sequence was then amplified using a validated real-time PCR assay that enabled direct quantification of bacterial DNA/cell-equivalents (Bilodeau et al., 2003b). All real-time PCRs were carried out on a Bio-Rad iQ iCycler. The data were expressed in bacterial cell-equivalents based on validation against standard plate counts of viable *E. ictaluri*.

2.7. Statistical analyses

Experimental data were subjected to analysis of variance (ANOVA) mixed-model procedures using the SAS software system version 8.00 (SAS Institute, Cary, NC) with treatment (control, cortisol-fed, and stressed) as the fixed effect and aquarium within treatment as the random effect. Percentage data and bacterial levels (cell-equivalents) were log-transformed prior to ANOVA to meet the assumptions for homogeneity and normality. When significant differences were found using ANOVA, pairwise contrasts were made using an LSD test to identify significant differences at the 5% level. Correlations between bacterial levels, cortisol concentrations, and lysozyme activity were determined using the CORR procedure (SAS).

3. Results

3.1. Disease susceptibility

Mortalities were first recorded on day 6 post-pathogen exposure and continued for approximately 12 days in the control and cortisol-fed treatments and for the entire 14 days in the stressed treatment (Fig. 1). Fish in the control and cortisol-fed treatments had lower ($P < 0.05$) mean cumulative mortalities (26.7%) than did stressed treatment fish (43.3%).

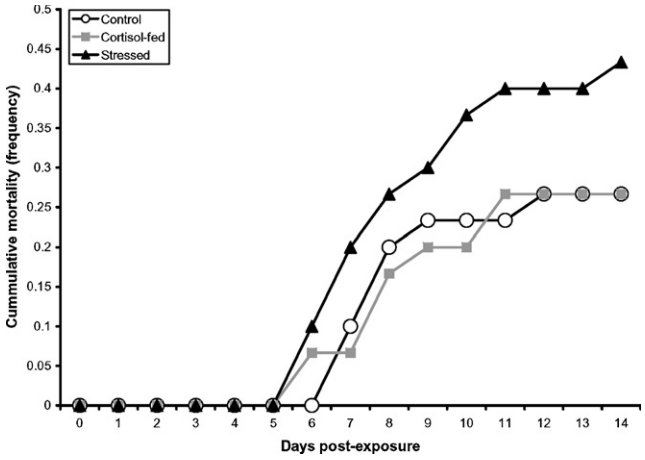

Fig. 1. Cumulative mortality of channel catfish following exposure to virulent *Edwardsiella ictaluri* after not being handled (Control), being fed a cortisol-laden feed (Cortisol-fed), or being stressed for 30 min (Stressed) the day of exposure.

3.2. Plasma cortisol levels

Circulating levels of cortisol were significantly ($P < 0.05$) different between treatments at the time of pathogen exposure (Fig. 2). Channel catfish fed the cortisol-laden feed had plasma cortisol levels of 104 ± 6 ng/ml compared to 29.4 ± 4 for stressed catfish and 2.7 ± 0 ng/ml for the unhandled controls. By 1-day post-pathogen exposure, plasma cortisol in cortisol-fed catfish had cleared and was similar ($P > 0.05$) to controls. Catfish in the stressed treatment maintained elevated ($P < 0.05$) cortisol levels 1-day post-exposure. By the fourth day, fish in all three treatments demonstrated circulating cortisol levels above pre-exposure control concentrations, with levels being equally reduced in all three treatments by day 8. There was no correlation ($P > 0.05$) between cortisol and either blood pathogen levels or lysozyme activity.

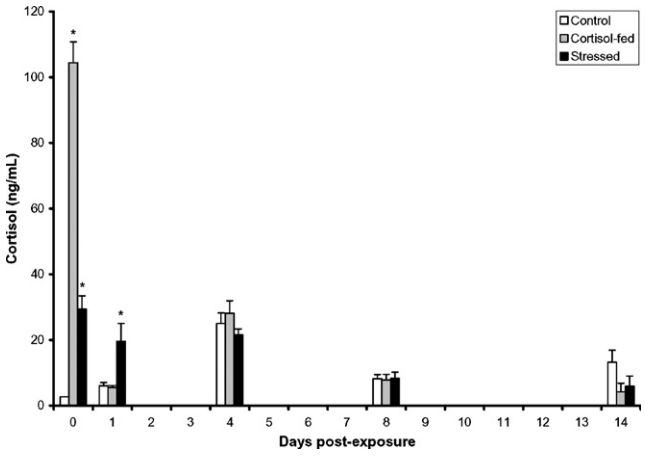

Fig. 2. Plasma cortisol concentrations of channel catfish following exposure to virulent *Edwardsiella ictaluri* after not being handled (Control), being fed a cortisol-laden feed (Cortisol-fed), or being stressed for 30 min (Stressed) the day of exposure. An asterisk (∗) indicates mean (±SE) cortisol concentrations are statistically different ($P < 0.05$) between treatments within time.

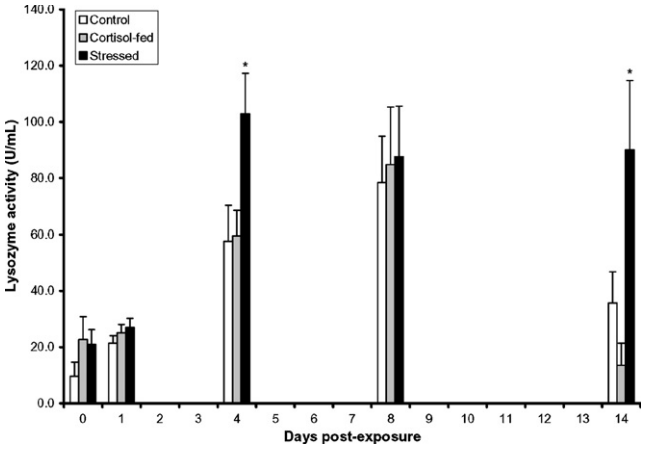

Fig. 3. Plasma lysozyme activity of channel catfish following exposure to virulent *Edwardsiella ictaluri* after not being handled (Control), being fed a cortisol-laden feed (Cortisol-fed), or being stressed for 30 min (Stressed) the day of exposure. An asterisk (∗) indicates mean (±SE) lysozyme activities are statistically different ($P < 0.05$) between treatments within time.

3.3. Plasma lysozyme activity

Plasma lysozyme activity was not different ($P > 0.05$) among treatments at the start of the experiment, nor were there any differences 1-day post-pathogen exposure (Fig. 3). On day 4 post-exposure, lysozyme activity increased in all three treatments and was highest ($P < 0.05$) for fish in the stressed treatment. Lysozyme activity remained elevated on day 14 in stressed treatment fish, but was reduced significantly ($P < 0.05$) on day 14 in control and cortisol-fed treatments. Lysozyme activity was positively correlated ($P = 0.0197$) with blood bacterial levels.

3.4. Bacterial load

Blood bacterial levels of individual fish were highly variable and were not different ($P > 0.05$) between treatments (Fig. 4; Table 1). A higher ($P < 0.05$) percentage of stressed fish, however, were found to be positive for bacterial DNA when compared to cortisol-fed catfish over the length of the experiment (Table 1). The percentage of PCR-positive control fish was intermediate between the other two treatments.

4. Discussion

The immunosuppressive effects of an acute stressor (low-water) on channel catfish exposed to the bacterial pathogen *E. ictaluri* were demonstrated in the present

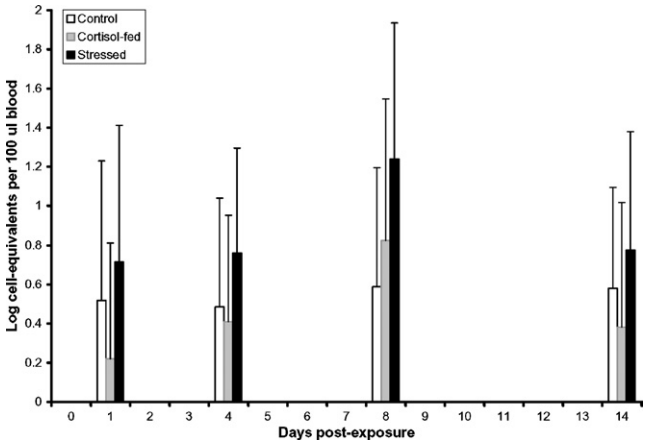

Fig. 4. Bacterial load in channel catfish blood following exposure to virulent *Edwardsiella ictaluri* after not being handled (Control), being fed a cortisol-laden feed (Cortisol-fed), or being stressed for 30 min (Stressed) the day of exposure. Mean (±SE) bacterial cell-equivalents within time are not statistically different ($P > 0.05$) between treatments.

Table 1
Effect of exogenous cortisol and stress on mean (±SE) percentage of channel catfish having bacterial DNA present in the blood and mean (±SE) circulating bacterial load of PCR-positive fish following *E. ictaluri* exposure ($n = 12$)[A]

Treatment	PCR-positive fish (%)	Bacterial load (log cell-equivalents/100 μl)
Control	16.1 ± 2.5^{ab}	3.19 ± 0.26
Cortisol-fed	13.0 ± 2.6^{a}	3.38 ± 0.32
Stressed	25.9 ± 5.4^{b}	3.35 ± 0.28

[ab] Means within a column having different superscripts are statistically different ($P < 0.05$).
[A] Fish were sampled from triplicate aquaria on days 1, 4, 8, and 14 post-pathogen exposure.

study. A greater percentage of these stress-challenged catfish tested positive for blood-borne *E. ictaluri* and had higher mortality rates over the course of the experiment. Observations of stress-induced alterations in the capacity of fishes to mount an immune response or to resist disease are common in the literature. Walters and Plumb (1980) found stressed catfish had 56% higher concentrations of *Aeromonas hydrophila* in trunk kidneys and 36% higher concentrations of *Edwardsiella tarda* than non-stressed fish. Wise et al. (1993) reported higher cumulative mortality in catfish subjected to confinement stress prior to *E. ictaluri* exposure, and Davis et al. (2002) demonstrated increased susceptibility of channel catfish to *I. multifiliis* following confinement stress. The present study is unique in that it is the first to describe differential effects of an acute stressor and exogenous cortisol on *E. ictaluri* susceptibility (uptake and mortality) and the profiles of cortisol and lysozyme in circulation.

Plasma cortisol levels in the stressed catfish at the time of pathogen exposure were moderately elevated (29.4 ng/ml) in this study, and are typical of a stress response in channel catfish associated with moderate environmental and behavioral stress events (Small, 2004; Small and Davis, 2002). Circulating cortisol levels generally peak in catfish after approximately 30 min of stress (Small, 2004). The timing of the stress challenge in the present study (45 min prior to pathogen exposure) was chosen to ensure elevated cortisol levels at the time of pathogen exposure. Following pathogen exposure, plasma cortisol remained elevated in stressed catfish until day 8 post-exposure. Exposure to *E. ictaluri* has been previously demonstrated to cause an increase in circulating cortisol in catfish (Bilodeau et al., 2003a). Increased concentrations of circulating cortisol have also been observed in rainbow trout following *Vibrio anguillarium* infection (Ackerman and Iwama, 2001).

The cortisol profile of catfish in both control and cortisol-fed treatments was similar, except at time zero. Channel catfish fed cortisol 4 h prior to pathogen exposure had elevated circulating cortisol concentrations (104 ng/ml). Similar levels have been observed in catfish exposed to ammonia and nitrite (Tomasso et al., 1981a,b). Administration of the cortisol-laden feed 4 h prior to pathogen exposure was done in an attempt to expose the fish to pathogen during peak circulating cortisol concentrations. Davis et al. (2003) demonstrated elevated plasma cortisol levels in channel catfish between 4 and 6 h after administering dietary cortisol. A pathogen-associated cortisol response was observed in both control and cortisol-fed treatment fish 4 days post-exposure. Unlike in the stressed treatment, fish in control and cortisol-fed treatments had lower mortality, and the cortisol-fed fish demonstrated a lower incidence of bacteria in the blood. These results demonstrate that cortisol in itself does not increase susceptibility to ESC in channel catfish, but that an acute stressor does increase susceptibility.

The profile of plasma lysozyme activity was also different among the treatments in the present study. Catfish in all three treatments demonstrated an increase in plasma lysozyme activity 4 days post-pathogen exposure, and activities were highest among previously stressed fish. Lysozyme activities in fish from control and cortisol-fed treatments returned to basal levels by day 14, but activities remained elevated in the stressed treatment fish. Since lysozyme activity was positively correlated to blood bacterial levels in the present study, these results might be explained as an innate immune response to the bacterial infection. Lysozyme is an effective bacteriolytic agent against both Gram-positive and Gram-negative fish pathogens (Grinde, 1989; Yousif et al., 1994). Demers and Bayne (1997) reported an immediate positive response in lysozyme activity to stress in trout, suggesting an early enhancement of innate immunity. Our results indicate no direct effect, positive or negative, of stress or cortisol on lysozyme activity following exposure to *E. ictaluri*, but suggest treatment differences are a

result of stress-induced bacterial susceptibility correlated to an innate immune response characterized by increased lysozyme activity.

In conclusion, the present data indicate that a single low-water stress event or administration of exogenous cortisol prior to *E. ictaluri* exposure differentially affect disease susceptibility in channel catfish. Stressed catfish were more susceptible than both controls and cortisol-fed catfish. Exogenous cortisol appeared to have no effect, immunosuppressive or otherwise, on susceptibility to ESC. Together these results implicate other stress factors or pathways, separate from or possibly in conjunction with cortisol, in the stress-associated immunosuppression of channel catfish as it relates to ESC susceptibility.

Acknowledgments

The authors express their thanks to Mr. Terry Bates, Ms. La-Chanda Brown, Mrs. Myrtis Ford-Bailey, Ms. Kira Johnson, and Mr. Jimmie Warren for there technical assistance in the conduct of this experiment. Mention of trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply approval to the exclusion of other products that may be suitable.

References

Ackerman, P.A., Iwama, G.K., 2001. Physiological and cellular stress responses of juvenile rainbow trout to vibriosis. J. Aquat. Anim. Health 13, 173–180.

Baldwin, T.J., Newton, J.C., 1993. Pathogenesis of enteric septicemia of channel catfish caused by *Edwardsiella ictaluri*: bacteriologic and light electron microscopic findings. J. Aquat. Anim. Health 5, 189–198.

Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish Dis. 1, 3–26.

Bilodeau, A.L., Small, B.C., Wolters, W.R., 2003a. Pathogen loads, clearance and plasma cortisol response in channel catfish, *Ictalurus punctatus* (Rafinesque), following challenge with *Edwardsiella ictaluri*. J. Fish. Dis. 26, 433–437.

Bilodeau, A.L., Waldbieser, G.C., Terhune, J., Wise, D.J., Wolters, W.R., 2003b. A real-time PCR assay of the bacterium *Edwardsiella ictaluri* for use in a selective breeding program for disease resistance in channel catfish. J. Aquat. Anim. Health 15, 80–86.

Buckingham, J.C., Loxley, H.D., Christian, H.C., Philip, J.G., 1996. Activation of the HPA axis by immune insults: roles and interactions of cytokines, eicosanoids, glucocorticoids. Pharmacol. Biochem. Behav. 54, 285–298.

Camp, K.L., Wolters, W.R., Rice, C.D., 2000. Survivability and immune responses after challenge with *Edwardsiella ictaluri* in susceptible and resistant families of channel catfish, *Ictalurus punctatus*. Fish Shellfish Immunol. 10, 475–487.

Davis, K.B., Griffin, B.R., Gray, W.L., 2002. Effect of handling stress on susceptibility of channel catfish *Ictalurus punctatus* to *Ichthyophthirius multifiliis* and channel catfish virus infection. Aquaculture 214, 55–66.

Davis, K.B., Griffin, B.R., Gray, W.L., 2003. Effect of dietary cortisol on resistance of channel catfish to infection by *Ichthyophthirius multifiliis* and channel catfish virus disease. Aquaculture 218, 121–130.

Demers, N.E., Bayne, C.J., 1997. The immediate effects of stress on hormones and plasma lysozyme in rainbow trout. Dev. Comp. Immunol. 21, 363–373.

Dhabhar, F.S., McEwen, B.S., 2001. Bidirectional effects of stress and glucocorticoid hormone on immune function: possible explanations for paradoxical observations. In: Ader, R., Feltem, D.L., Cohen, N. (Eds.), Psychoneuroimmunology, vol. 1. Academic Press, San Diego, pp. 301–338.

Dhabhar, F.S., Miller, A.H., McEwen, B.S., Spencer, R.L., 1996. Stress-induced changes in blood leukocyte distribution. Role of adrenal steroid hormones. J. Immunol. 157, 1638–1644.

Ellis, A.E., 1981. Stress and modulation of defense mechanisms in fish. In: Pickering, A.D. (Ed.), Stress in Fish. Academic Press, London, pp. 147–169.

Engelsma, M.Y., Huising, M.O., van Muiswinkel, W.B., Flik, G., Kwang, J., Savelkoul, H.F., Verburg-van Kemenade, B.M., 2002. Neuroendocrine-immune interactions in fish: a role for interleukin-1. Vet. Immunol. Immunopathol. 87, 467–479.

Grinde, B., 1989. Lysozyme from rainbow trout, *Salmo gairdnerii* Richardson, as an antibacterial agent against fish pathogens. J. Fish. Dis. 12, 95–104.

Hawke, J.P., 1979. A bacterium associated with disease of pond cultured channel catfish, *Ictalurus punctatus*. J. Fish. Res. Board Can. 36, 1508–1512.

Möck, A., Peters, G., 1990. Lysozyme activity in rainbow trout, *Oncorhynchus mykiss* (Walbaum), stressed by handling, transport and water pollution. J. Fish. Biol. 37, 873–885.

Newton, J.C., Wolfe, L.G., Grizzle, J.M., Plumb, J.A., 1989. Pathology of experimental enteric septicaemia in channel catfish, *Ictalurus punctatus* (Rafinesque), following immersion-exposure to *Edwardsiella ictaluri*. J. Fish. Dis. 12, 335–347.

Patiño, R., Schreck, C.B., Banks, J.L., Zaugg, W.S., 1986. Effects of rearing conditions on the developmental physiology of smolting coho salmon. Trans. Am. Fish. Soc. 115, 828–837.

Peters, G., Schwarzer, R., 1985. Changes in hemopoietic tissue of rainbow trout under influence of stress. Dis. Aquat. Org. 1, 1–10.

Peters, G., Nuessgen, A., Raabe, A, Möck, A., 1991. Social stress induces structural and functional alterations of phagocytes in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 1, 17–31.

Schreck, C.B., 1996. Immunomodulation: endogenous factors. In: Iwama, G., Nakanishi, T. (Eds.), The Fish Immune System: Organism, Pathogen and Environment. Academic Press, London, pp. 311–337.

Schreck, C. Maule, A.G., 2001. Are the endocrine and immune systems really the same thing? In: Goos, H.J.T., Rastogi, R.K., Vaudry, H., Pierantoni, R. (Eds.), Perspective in Comparative Endocrinology: Unity and Diversity. Proceedings of the 14th International Congress of Comparative Endocrinology, Sorrento, Italy. Monduzzi Editore, Bologna, pp. 351–357.

Schreck, C., Maule, A.G., Kaattari, S.L., 1993. Stress and disease resistance. In: Muir, J.F., Roberts, R.J. (Eds.), Recent Advances in Aquaculture, vol. 4. Blackwell Scientific, Oxford, pp. 170–175.

Shotts, E.B., Blazer, V.S., Waltman, W.D., 1986. Pathogenesis of experimental *Edwardsiella ictaluri* infections in channel catfish (*Ictalurus punctatus*). Can. J. Fish. Aquat. Sci. 43, 36–42.

Small, B.C., 2003. Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinoldine and clove oil anesthetized channel catfish *Ictalurus punctatus*. Aquaculture 218, 177–185.

Small, B.C., 2004. Effect of isoeugenol sedation on plasma cortisol, glucose, and lactate dynamics in channel catfish *Ictalurus punctatus* exposed to three stressors. Aquaculture 238, 469–481.

Small, B.C., Davis, K.B., 2002. Validation of a time-resolved fluoroimmunoassay for measuring plasma cortisol in channel catfish *Ictalurus punctatus*. J. World Aquacult. Soc. 184, 33–187.

Tomasso, J.R., Davis, K.B., Parker, N.C., 1981a. Plasma corticosteroid dynamics in channel catfish, *Ictalurus punctatus* (Rafinesque), during and after oxygen depletion. J. Fish. Biol. 18, 519–526.

Tomasso, J.R., Davis, K.B., Simco, B.A., 1981b. Plasma corticosteroid dynamics in channel catfish (*Ictalurus punctatus*) exposed to ammonia and nitrite. Can. J. Fish. Aquat. Sci. 38, 1106–1112.

USDA, 1997. Reference of 1996 U.S. catfish health and production practices: part 1. United States Department of Agriculture, Animal and Plant Health Inspection Services, Fort Collins, Colorado, USA, 1997.

Walters, G.R., Plumb, J.A., 1980. Environmental stress and bacterial infection in channel catfish, *Ictalurus punctatus* Rafinesque. J. Fish. Biol. 17, 177–185.

Wendelaar Bonga, S.E., 1997. The stress response in fish. Physiol. Rev. 77, 591–625.

Weyts, F.A.A., Cohen, N., Flik, G., Verburg-van Kemenade, B.M.L., 1999. Interactions between the immune system and the hypothalamo–pituitary–interrenal axis in fish. Fish Shellfish Immunol. 9, 1–20.

Wise, D.J., Schwedler, T.E., Otis, D.L., 1993. Effects of stress on susceptibility of naive channel catfish in immersion challenge with *Edwardsiella ictaluri*. J. Aquat. Anim. Health 5, 92–97.

Wolters, W.R., Johnson, M.R., 1994. Enteric septicemia resistance in blue catfish and three channel catfish strains. J. Aquat. Anim. Health 6, 329–334.

Yousif, A.N., Albright, L.J., Evelyn, T.P.T., 1994. In vitro evidence for the antibacterial role lysozyme in salmonid eggs. Dis. Aquat. Org. 19, 15–19.