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Changes in Susceptibility of Channel Catfish, *Ictalurus punctatus*, to Enteric Septicemia of Catfish by Hormonal Altering of the Hypothalamo-Pituitary- Interrenal Axis

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Abstract: Diagnostic tests using hormones in fisheries sciences are lacking when compared to other animal production industries. The goals of this study were to:1) examine how manipulation of the hypothalamic-pituitary-interrenal axis using dexamethasone (Dex) and adrenocorticotropic hormone (ACTH) changes disease susceptibility of channel catfish to ESC, 2) evaluate Dex as a possible way to reduce the negative effects of stress during confinement and transport and 3) determine the effects of the synthetic hormones dexamethasone and Cortrosyn® (ACTH) on blood plasma cortisol concentrations of stressed and unstressed channel catfish fingerlings in addition to evaluation of the effects Dex and ACTH have on blood plasma mineral concentrations in channel catfish. A series of disease challenges using Edwardsiella ictaluri in combination with hormonal treatments were used to assess these goals. Manipulation of the hypothalamo-pituitary-interrenal axis using Dex and ACTH does change disease susceptibility of channel catfish. Cortisol concentrations were assumed to be correlated with mortality in fish and mortalities for control treatments were low as expected. Other treatments demonstrated a lack of differentiation in mortalities and could not be explained by cortisol. Dex treatments exhibited low cortisol (7.4±1.2 ng mL^{-1}) concentrations and had the greatest mortality (78.3%±11.7). Dex was not different from ACTH (66.7%±6.0) and ACTH stress 30 min (51.7%±1.7) which were expected to exhibit the greatest mortality. Mineral analysis revealed that there were no differences in blood plasma concentrations of sodium, potassium and chloride in any treatment. Dex was effective at suppressing the stress hormone cortisol in both stressed (17.5±2.3 ng mL^{-1}) and unstressed (7.4±1.2 ng mL^{-1}) catfish. Although Dex is capable of suppressing ACTH and cortisol, its use as a stress preventative, especially for the reduction of disease susceptibility is unfounded based on this study due to increased mortality rates associated with its use (78.3%±11.7).

Key words: Cortisol, dexamethasone, disease, *Edwardsiella ictaluri*, ESC, ACTH, interrenal

INTRODUCTION

Channel catfish, *Ictalurus punctatus Rafinesque*, farming is the largest aquaculture industry in the United States with production reaching 317 million food size fish and 1.3 billion fingerlings as of July 1, 2004[1]. Channel Catfish are more susceptible to infections when they are subjected to stressful conditions such as handling, improper nutrition, poor water quality and close confinement[2]. Channel catfish have been shown to be more susceptible to Enteric Septicemia of Catfish (ESC) when subjected to confinement stress[3,4].

ESC, caused by the bacteria *Edwardsiella ictaluri*, is the most serious disease of commercially reared channel catfish. The United States Department of Agriculture (USDA) in conjunction with the Animal and Plant Health Inspection Service (APHIS) recently published a fact sheet of compiled survey materials, Catfish 2003. This survey found that 57% of large fingerling operations experienced losses to ESC in the previous 2 years. ESC was the leading cause of all fry and fingerling loss at 27.3%[2].

Treatment of ESC is limited with only one antibiotic, Ormetoprim combined with sulfadimethoxine (Romet-30® by Alpharma Inc.), approved for ESC treatment of channel catfish[2]. Extra-use by veterinarians [5]does occur for the catfish approved antibiotic oxytetracycline HCL (Terramycin® by Pfizer, Inc.)[6].

The American Society of Microbiologists claim antibiotic use in disease treatment is becoming a concern due to increasing antibiotic resistance of bacteria and environmental contamination of antibiotics[7]. Isolates of *E. ictaluri* have been exhibiting antibiotic resistance to Romet-30® since the early 1990's[8,9].

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The ASM also states that aquaculture treatment practices are a large focal point due to non-standardized and unregulated treatment practices[7]. Total antibiotic use for the treatment of ESC is estimated at 126,000 to 252,000 pounds annually[10]. Yet with substantial drug use, losses in catfish production remain as high as 60%, with diseases accounting for by far the largest portion[11].

New treatment methods and preventions are currently being sought due to the current shortcomings of antibiotic use. The efficacy of previous vaccinations for ESC has proved to be inconsistent and the use of vaccines has produced mortality in channel catfish[12]. The first approved ESC vaccine for use in channel catfish was developed by the USDA's Agriculture Research Service in 1999 and marketed in 2000 by Intervet, Inc[13]. The current vaccine still has shortcomings due to a limited window of age that it can be administered (7-31 d after hatching). The vaccine also contains live bacteria, so it presents storage, shipping and shelf life problems, in addition to creating an exposure source to catfish stocks already in commercial facilities. Although a vaccine was commercially available, only 18.1% of all fry were vaccinated in 2002[2].

Physiological evidence indicates that another option in ESC prevention is the limitation of stress, or possibly reducing the physiological effects of stress. Wise *et al.* (1993) found channel catfish exposed to stress events are more susceptible to ESC than nonstressed fish (81 and 55% mortality, respectively). Wise also indicated increased exposure time to *E. ictaluri* after stress resulted in increased mortality rates. [14]Confirmed that stressed fish were more susceptible to infection by ESC. Unstressed control groups exhibited lower infection rates than stressed fish exposed to an *E. ictaluri* bath and intraperitoneal injected fish (15.7, 52.7% and 46.6%, respectively).

Sink and Strange[3] confirmed Wise *et al.* and Ciembor *et al.*'s results (mean±SE: nonstressed 22.5%, stressed 30 min 47.5% and stressed 60 min 81.7% mortality), but linked infection rates to the hormone cortisol, a physiological indicator of stress in fish. As stress increased, blood cortisol and infection rates increased proportionally when fish were exposed to *E. ictaluri* baths post confinement stress. Cortisol increases have long been associated with stress events in channel catfish[15,16]. It is unclear whether cortisol directly causes immune suppression, or whether it plays a secondary role.

Apoptosis of leukocytes was found to be lower in confinement stressed channel catfish than in non-stressed fish[17]. Alford's study could indicate a direct effect of stress on individual leukocyte cells, causing them to remain active for a longer period of time. Another study found that handling and transport stress produces a decrease of lymphocytes and an increase of circulating neutrophils in the blood[18]. These studies indicated cortisol alone does not cause suppression of phagocyte function but that elevated physiological concentrations of cortisol can initiate phagocyte suppression. Determined that cortisol directly inhibits proliferation of peripheral blood lymphocytes and induces apoptosis of lymphocytes in carp, *Cyprinus carpio*.

Cortisol concentrations have been manipulated in fish to create simulated stress responses by either feeding or injecting cortisol, dexamethasone (Dex), or adrenocorticotropin Hormone (ACTH)[19]. Pickering and Duston[20] fed cortisol to brown trout, *Salmo trutta*, in order to evaluate susceptibility to *Saprolegnia* infection. Although they fed cortisol in order to evaluate disease susceptibility, only blood cortisol concentrations would have been elevated.

The use of ACTH would both increase blood ACTH and cortisol concentrations as the use of Dex would decrease blood cortisol concentrations. It is also appropriate to note that the half life of Dex in much greater than that of cortisol. The bioactivity of Dex is greater than 48 h, whereas cortiso bioactivity is less than 12 h. The hypothalamo-pituitary-interrenal axis needs to be thoroughly manipulated hormonally in order to begin to examine the effects that stress hormones may play on disease susceptibility. Physiological rationale in this research is that inhibition of the renal axis by the synthetic glucocorticoid dexamethasone will inhibit secretion of corticotrophin releasing hormone, adrenocorticotropin hormone and cortisol and possibly may prevent some of the negative effects of stress response on disease susceptibility.

This study had three main objectives: 1) to examine how manipulation of the hypothalamo-pituitary-interrenal axis using Dex and ACTH changes disease susceptibility of channel catfish to ESC, 2) to evaluate Dex as a possible way to reduce the negative effects of stress during confinement and transport and 3) to determine the effects of the synthetic hormones dexamethasone and Cortrosyn® (adrenocorticotropin ACTH) (Amphastar Pharmaceuticals, Inc) on blood plasma cortisol concentrations of stressed and unstressed channel catfish fingerlings in addition to evaluate the effect Dex and ACTH have on blood plasma mineral concentrations in channel catfish.

MATERIALS AND METHODS

Fish: Fingerling channel catfish, *Ictalurus punctatus*, (N = 1000) were obtained from commercial aquaculture ponds at Greenwater Fish Farm (Gibson County, Tennessee). Fingerlings (N = 450) were hand graded

for weight, with weights ranging from 86-111 g (mean 98.2, SD 5.3 g). The fingerlings were hatched on-site at Greenwater Fish Farm from eggs of broodstock maintained at the facility and stocked into commercial grow-out ponds with no previous outbreaks of *E. ictaluri*. The fingerlings did not exhibit clinical signs of infection (unusual swimming, hemorrhaging, pustules, lesions, hole in head syndrome) and appeared healthy upon inspection. A sample swipe from the abdominal cavity, liver and brain cavity of seven fish was plated on brain-heart infusion (BHI) agar. Samples from all seven fish failed to produce any colonies of *E. ictaluri* after incubation at 30°C for 72 hrs. Additional samples (10 fish) failed to generate any *E. ictaluri* cells in BHI broth after incubation at 30°C for 48 h. All samples were verified using analytical profile index 20E (API 20E).

Edwarsiella ictaluri: An *E. ictaluri* unknown strain isolate was obtained from a naturally occurring outbreak at a commercial catfish operation (Auburn University Fish Health Laboratory). The identity of this isolate as *E. ictaluri* was verified by biochemical isolation tests described by Hawke *et al.*[21] and produced API 20E analytical profile index codes of 4004000. This isolate was taken from frozen aliquots used in previous experiments by Sink and Strange [3] and Sink *et al.*[22].

To assure virulence, one aliquot of the isolate was propagated in BHI broth in a shaking bath at 27°C for 48 h. Ten fish (97-105 g) were exposed by immersion bath to 7.1 Log colony-forming units per L (Log CFU L^{-1}) in 70-L tanks. The exposed fish were reared for 10 d with all exhibiting one or more clinical signs of ESC infection (red pustules, hemorrhaging and ulcers were the most common). The posterior kidney of five fish was swabbed and inoculated into BHI broth tubes for reisolation of *E. ictaluri*. The identity of the isolate was verified using the API 20E. Seven of the ten inoculated tubes produced API 20E codes of 4004000. The BHI broth containing the isolate was then frozen in six aliquots of 1 mL for future verification and use in other challenges. The one remaining aliquot was used to inoculate two, 500 mL samples of BHI broth for use in the challenge. The 500 mL BHI samples were then cultured at 30°C for 48 h. Spectrophotometer readings were taken in association with plate counts to allow comparison with future challenges.

Experimental system: The experimental system contained 24 independently maintained 85-L tanks with four banks of six tanks. Each tank was operated in a flow-through mode with filtered city water supplied at 2.2 L min^{-1}. The water was filtered with activated carbon and total chlorine content was less than 0.002 mg L^{-1}. Water temperature was maintained at 27±2°C. An air stone was used in each aquarium for supplemental aeration to maintain dissolved oxygen levels. Water quality was monitored daily for dissolved oxygen (>6 mg L^{-1}), temperature (27±2°C) and total chlorine (<0.0003 mg L^{-1}). Water quality was tested weekly for pH (7.0-7.3) and total ammonia (<1.0 mg L^{-1}). Lighting was automatically controlled by timer with a 16 h light/8 h dark cycle. The fingerlings were fed Zeigler floating finfish bronze (35% protein, 5% fat) 3mm pellets at a rate of 2% of body weight daily.

Hormone level determination: Hormone dosage was calculated based on an average fish weight of 100 g. Hormones were dissolved in sterile physiological saline to a desired concentration. Fish received intramuscular injections of Dex 20 μg/fish (approximately 200 mg kg^{-1}) and Cortrosyn® (Amphastar Pharmaceuticals, Inc) (cosyntropin a synthetic ACTH) 5.0 μg/fish (approximately 50 μg kg^{-1}) in 0.5 cc saline.

Selection of dosage was arbitrary, taking into consideration that suppression of cortisol secretion (inhibition of ACTH secretion) by Dex is usually attained with Dex doses between 10-100 μg kg^{-1} (intravenously) in dogs and 40-100 μg kg^{-1} (intravenously) in horses. Dosage of Dex for therapy of different conditions in different species often varies between 0.5 to 6.0 mg kg^{-1}. Dose of synthetic ACTH used for adrenal stimulation varies between 1.0 and 20 μg kg^{-1} (intravenously) depending on specie[23]. In summary, dosages of Dex and ACTH injected fish were compatible with diagnostic dosages used in mammals. To our knowledge, optimization of dose-response and time-response relationships for Dex and ACTh are not available in channel catfish.

In order to test these dosages, six fish (100-108 g) were given doses of Dex or ACTH (3 each) by intramuscular injection. Fish were quickly anaesthetized previous to injection using 100 mg L^{-1} of MS-222. Fish were then returned to their tank to recover immediately after the injection was administered. Fish receiving Dex were bled (under anesthetic 150 mg L^{-1} MS-222) by vivisection of the tail at the caudal peduncle for cortisol analysis at time 0, time 180 and time 240 min after receiving the injection. The time 0 min fish had a cortisol concentration of 18 ng mL^{-1}, similar control levels for channel catfish in Sink and Strange[22]. Time 180 (3 ng mL^{-1}) and time 240 min (5 ng mL^{-1}) fish exhibited suppressed cortisol concentrations. Twenty ug 100 g of Dex was deemed sufficient to suppress cortisol response for use in the main challenge. Fish receiving ACTH anaesthetized and bled in the same manner. Bleeding times for ACTH fish were times 0.60 and

120 min. Once again, cortisol concentrations of the time 0 min fish (22 ng mL^{-1}) were similar to the controls (23.63 ng mL^{-1}) in Sink and Strange[22]. Time 60 (77 ng mL^{-1}) and time 120 (81 ng mL^{-1}) min fish were similar to the time 30 min (77.70 ng mL^{-1}) confinement stress fish in Sink and Strange[3]. The cortisol concentrations for the ACTH injections were elevated enough to be comparable to an elevated stress level, so they were considered sufficient for use in the main challenge.

HORMONAL DISEASE CHALLENGE

Twenty-five graded (86-111 g, mean 98.2 g, SD 5.3 g) fingerling catfish (total N = 450) were stocked into each of 18 experimental tanks. The fish were allowed to acclimate for 14 d. Six treatments were applied to three tanks each for replication.

Treatment 1

Control/baseline: The objective of this treatment was to establish a baseline for cortisol and mortality of unstressed and non-injected fish for control comparisons of other treatments. Twenty five fish were removed from the tank and placed in 100 mg L^{-1} MS-222. All fish achieved stage IV anesthesia (total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes) in less than 3 min. Once the fish achieved stage IV anesthesia, each was injected with 0.3 cc sterile saline intramuscularly to simulate hormone injection and placed back in the tank to recover. All fish were injected in less than 5 min. Once all fish were replaced in the tank, 5 fish were randomly selected and placed in 150 mg L^{-1} MS-222. Once the fish achieved stage V anesthesia (total loss of reactivity; opercular movements slow and irregular; heart rate very slow; loss of all reflexes), they were bled using 370 μL heparinized Caraway tubes by vivisection of the tail at the caudal peduncle for analysis of cortisol. Water flow to the tank was then shut off with aeration remaining constant. The remaining 20 fish per tank were exposed to 1.4 Log colony forming units/L (Log CFU L^{-1}) of *E. ictaluri* (20 mL of inoculated BHI@ 1×10^{6} CFU) verified by spectrophotometer readings of 0.390 for 650 nm and 0.346 for 700 nm. Water flow was restored allowing the bacteria to flush from the system at the end of the 8 h exposure period. Fish were then monitored for mortalities daily for 21 days.

Treatment 2

Confinement stress 30 min: The objective if this treatment was to establish mortality and cortisol concentrations of a stress event similar to handling or transport stress for comparison with hormonally treated fish. Twenty five fish were anesthetized and injected with saline as in treatment I. The fish were placed back into the tanks in confinement baskets (12.5×15×10-cm) constructed of rigid vinyl netting. The baskets caused the fish to be restricted in movement and to be in contact with other fish as well as the basket at all times. Five fish were then removed from each tank for bleeding and cortisol analysis as in treatment I. The remaining 20 fish per tank were released into the tanks at the conclusion of the 30 min confinement stress period. Water flow and bacteria administration was completed as in the previous treatment. The fish were monitored for mortalities for 21 d.

Treatment 3

Dexamethasone injection: The purpose of this treatment was to evaluate Dex's cortsiol suppression capabilities and evaluate its effect on mortality in channel catfish exposed to ESC. Twenty five fish were anesthetized as in the previous treatments and injected intramuscularly with 20 μg of Dex. All fish were then placed back into the tanks. Five fish from each tank were randomly removed for bleeding and cortisol analysis after 180 min, allowing the Dex time to suppress cortisol concentrations. Water flow manipulation and bacteria administration was then completed as in previous treatments. Mortalities were monitored for 21 d.

Treatment 4

Adrenocorticotropin injection: The objective of this treatment was to examine if cortisol concentrations brought about by ACTH injection were similar to those caused by stress events and if elevated cortisol concentrations caused by ACTH injection would produce similar mortality rates to stress treatments. Twenty five fish were anesthetized as in the previous treatments and injected intramuscularly with 5 μg of ACTH. All fish were then placed back into the tanks. Five fish from each tank were randomly removed for bleeding and cortisol analysis after 60 min, allowing the ACTH time to stimulate cortisol production. Water flow manipulation and bacteria administration was then completed as in previous treatments. Mortalities were monitored for 21 d.

Treatment 5

Dexamethasone injection and confinement stress 30 min: The objective of this treatment was similar to treatment III, but was used to examine whether stress stimuli can overcome Dex suppression and what the overall effect on cortisol concentration and mortality was. Twenty five fish were anesthetized as in the previous treatments and

injected intramuscularly with 20 μg of Dex. All fish were then placed back into the tanks. The 25 fish in each tank were netted and placed in confinement baskets (12.5×15×10-cm) at time 150 min. At the conclusion of the confinement stress (time = 180 min), the fish were released into the tank and 5 fish from each tank were removed and bled for cortisol analysis as in previous treatments. Water flow manipulation and bacteria administration was then completed as in previous treatments. Mortalities were monitored for 21 d.

Treatment 6

Adrenocorticotropin injection and confinement stress 30 min: The objective of this treatment was to determine what the combined effects of stress and ACTH injection were on cortisol concentration and mortality. Twenty five fish were anesthetized as in the previous treatments and injected intramuscularly with 5 μg of ACTH. All fish were then placed back into the tanks. The 25 fish in each tank were netted and placed in confinement baskets (12.5×15×10-cm) at time 30 min. At the conclusion of the confinement stress (time = 60 min), the fish were released into the tank and 5 fish from each tank were removed and bled for cortisol analysis as in previous treatments. Water flow manipulation and bacteria administration was then completed as in previous treatments. Mortalities were monitored for 21 d.

Cortisol analysis: The 370 μL heparinized Caraway tubes containing collected blood were blown into 1.5 mL centrifuge tubes. The plasma was separated from the red blood cells by centrifugation. The plasma was removed by disposable pipettes and placed into 1.5-mL cryotubes and frozen until cortisol assays were performed. Plasma was later thawed at room temperature for 30 min and used in conjunction with Coat-A-Count® solid-phase ^{125}I radioimmunoassay. The competitive assay was used for quantitative measure of cortisol (hydrocortisone, compound F) in the blood plasma. The assay was modified from 25 μL to 10 μL of plasma and calibrators in order to accommodate the small blood volumes received from the fish. Validation of the assay was done with known plasma pools from previous experiments done with 25 μL plasma volumes randomly conducted during the assay procedure. All samples and calibrators were done in duplicate.

Mineral analysis: Blood plasma mineral analysis was conducted by the Clinical Pathology Laboratory, College of Veterinary Medicine, The University of Tennessee. The technique used was based on the use of ion selective electrodes and a Hitachi 911 chemistry analyzer. Plasma from fish for each treatment was measured for sodium, potassium and chloride concentrations.

Statistical analysis: Differences in cortisol concentrations, mortality rates and plasma mineral concentrations among treatments were tested using one way analysis of variance (ANOVA) and Tukey's post hoc tests (SPSS 11.0 2000). Differences were considered significant at $p<0.05$. Pearson correlation was used to measure the relationship between cortisol level and mortality.

RESULTS

Effects of hormones and stress on blood plasma cortisol concentration: A one-way ANOVA of cortisol for the six treatments showed that cortisol concentrations for treatments were different ($p<0.05$). Tukey's HSD post hoc test demonstrated three treatment groupings that were not different from each other. Control/Baseline cortisol concentrations were not different from Dex and Dex stress 30 min treatments ($p = 0.973$ and 0.971, respectively). Stress 30 min cortisol concentrations did not differ from ACTH concentrations ($p = 0.468$) and ACTH treated cortisol concentrations were not different from ACTH stress 30 min treatments ($p = 0.129$). However, stress 30 min cortisol concentrations were different ($p = 0.001$) from ACTH stress 30 min. Treatments were divided into three separate groups from lowest cortisol concentration to highest: 1-control/baseline, Dex and Dex stress 30 min, 2-stress 30 min and ACTH and 3- ACTH and ACTH stress 30 min. Mean cortisol concentrations for each replicate in each treatment may be found in Table 1.

Effect of hormones and stress on mortality: A one-way ANOVA of percent mortality for channel catfish in the six treatments showed that treatments were different. A Tukey's HSD post hoc test revealed many overlapping treatment groups (Table 2). The control/baseline treatment mortalities were different ($p<0.05$, mean±SE: 8.3%±1.7) than the other five treatments (mortality - Table 3). Dex (78.3%±11.7) was not different from ACTH treatments (66.7%±6.0). ACTH treated mortalities were also similar to ACTH stress 30 min (51.7%±1.7). ACTH stress 30 min mortalities could not be differentiated from Dex stress 30 min (40.0%±0.0) and stress 30 min (36.7%±1.7). Only the control/baseline treatment mortalities could be distinguished as a separate treatment group, with all other treatment means exhibiting overlap with at least two other treatments.

Table 1: Mean cortisol concentrations (ng mL^{-1}) of channel catfish for each replicate of hormonal treatment

Mean (±SE) cortisol concentrations (ng mL^{-1})						
Treatment						
Tank	Control/Baseline	Dexamethasone	Dexamethasone stress 30 min	Stress 30 min	ACTH	ACTH stress 30 min
1	10.3±1.6	9.0±3.5	13.5±5.0	82.0±21.0	81.7±15.3	60.5±6.8
2	13.8±1.6	7.2±0.9	16.9±2.5	85.9±10.8	71.5±9.7	64.9±4.0
3	13.2±1.5	6.0±0.8	22.1±4.1	98.2±11.0	77.4±9.1	55.7±7.9

Table 2: Statistically overlapping treatment groups for percent mortality means (±SE) of channel catfish in hormonal disease challenge

Treatment	Mean±SE	Group
Control	8.3%±1.7	A
Dexamethasone	78.3%±11.7	B
Adrenocorticotropin	66.7%±6.0	B C
ACTH Stress 30 min	51.7%±1.7	B C D
Dex Stress 30 min	40.0%±0.0	C D
Stress 30 min	36.7%±1.7	C D

Effects of treatments on plasma concentrations of sodium, potassium and chloride: One-way ANOVAs of sodium ($p = 0.251$), potassium ($p = 0.457$) and chloride ($p = 0.390$) revealed that all three were not statistically different for any treatment. Sodium had a range of 15.0 mEq L^{-1} (max:148, min:133 mEq L^{-1}) and a mean of 139.9±1.3 mEq L^{-1} for all treatments combined. Potassium's range was 2.4 mEq L^{-1} (max: 6.2, min: 3.8 mEq L^{-1}) and a mean of 5.3±0.2 mEq L^{-1}. Chloride possessed a range of 11.0 mEq L^{-1} (max: 114, min: 103 mEq L^{-1}) and a mean of 109.4±1.2 mEq L^{-1} for all six treatments.

DISCUSSION

Channel catfish are often exposed to various physical and environmental stressors during production that often occur in the presence of one or more pathogens. Stress often facilitates disease outbreaks in commercial fish rearing operations. These stressors increase the susceptibility of channel catfish fingerlings to ESC in experimental situations[4]. Disease outbreak intensity, frequency and duration can be reduced by minimizing stress[24]. This study examined the hormonal manipulation of the hypothalamo-pituitary-interrenal axis, or stress axis, in an attempt to evaluate changes in the stress response and disease susceptibility of channel catfish to ESC.

The first objective was to examine how manipulation of the hypothalamo-pituitary-interrenal axis using Dex and ACTH changes disease susceptibility of channel catfish to ESC. The results were unexpected as cortisol concentrations are assumed to be correlated with immune suppression in fish. Mortalities for the baseline/control treatments (Table 3) were low and comparable to control mortalities in Sink and Strange[3]. Slightly elevated mortalities in the control/baseline treatment would not have been surprising due to the fact that their skin was punctured by injection previous to exposure creating a new route of infection.

The lack of differentiation in mortality between other treatments (Table 2) could not be explained by cortisol concentrations as we expected those with lower concentrations to be less susceptible to ESC. However, we determined that Dex exhibited the greatest mortality (78.3%±11.7) with the lowest mean cortisol concentration (7.4±1.2 ng mL^{-1}). Dex was not different from ACTH (66.7%±6.0) and ACTH stress 30 min (51.7%±1.7) which were expected to exhibit the greatest mortality.

Mineral imbalance or depletion due to hormonal overdose was thought to be a possible culprit in the unexplained mortality. This led us to evaluate the effect Dex and ACTH have on blood plasma mineral concentrations in channel catfish. However, the results from the mineral analysis revealed that there were no differences in blood plasma concentrations of sodium, potassium and chloride in any treatment. This does not mean that a mineral imbalance was not occurring at the cellular level, but this was not the focus of this study. Mineral analysis at the cellular level should be considered in future studies.

The second objective of this study was to evaluate Dex as a possible way to reduce the negative effects of stress during confinement and transport. Dex was effective at suppressing the primary stress hormone cortisol in fish, in both stressed (17.5±2.3 ng mL^{-1}) and unstressed (7.4±1.2 ng mL^{-1}) channel catfish fingerlings. Although Dex is capable of suppressing ACTH and cortisol, its use as a stress preventative, especially for the reduction of disease susceptibility is unfounded based on this study. Dex is a synthetic glucocorticoid that competes with naturally produced cortisol by binding receptors. It is possible that some or all of the receptors in fish could be occupied by Dex enforcing the stress response in the absence of cortisol.

The final objective was to determine the effects of the synthetic hormones Dex and ACTH on blood plasma cortisol concentrations of stressed and unstressed channel catfish fingerlings. Results from the study were consistent with those from the hormone level determination pre-study. Control/baseline cortisol

Table 3: Percent mortality rates for channel catfish in each replicate of six hormonal treatments

Percent mortalities by treatment						
Treatment						
Tank	Control/Baseline	Dexamethasone	Dexamethasone stress 30 min	Stress 30 min	ACTH	ACTH stress 30 min
1	5	100	75	35	40	50
2	10	60	55	40	40	50
3	10	75	70	35	40	55

concentrations (mean±SE: 12.4±0.9 ng mL^{-1}) were low and comparable to controls in Sink and Strange[3]. Dex (7.4±1.2 ng mL^{-1}) and Dex stress 30 min (17.5±2.3 ng mL^{-1}) were also low and not different from the controls as expected. The Dex stress 30 min cortisol mean concentration was slightly higher than both the Dex and control/baseline concentrations, but Dex was still capable of cortisol suppression under induced stress conditions.

ACTH treated cortisol concentrations (76.9±6.4 ng mL^{-1}) were expected to be similar to stress 30 min concentrations (88.7±8.2 ng mL^{-1}) based upon results of the hormonal level determination study. Our assumption was proven to be correct by the cortisol concentration ANOVA. However, ACTH stress 30 min (60.3±3.6) mean concentrations were lower than stress 30 min concentrations. This was not as expected as stress compiled with ACTH injections should theoretically push cortisol concentrations beyond what stress alone can produce. We however know that there must be a maximum rate of production for cortisol after which further stimulation has no effect, but we expect that cortisol concentrations of ACTH stress 30 min would equal those of the ACTH treated fish.

Two possible explanations are: 1) the fish placed in the confinement baskets struggled and moved constantly increasing metabolic rate. This could have lead to greater metabolic breakdown and faster use of the ACTH, causing faster recovery times for cortisol concentrations, 2) blood plasma cortisol profiles could have still been in transition and sampling at time = 60 min was not the best stage to measure cortisol concentrations. Time response studies for the duration of each treatment are needed to remedy this possible problem. From this study, we can see that Dex is capable of suppressing cortisol concentrations to baseline/control concentrations and that ACTH injections can create cortisol concentration stress responses similar to confinement stress.

Mortality increased in all treatments above control/baseline rates. All treatments included stress components: dexamethasone, adrenocorticotropin hormone and/or confinement. The common denominator was increased glucocorticoid concentrations: either Dex or cortisol. Lethal effect of ACTH injection was probably mediated by cortisol in part, not due to ACTH itself. However the fact that mortality remained high when cortisol was low (but Dex was high) indicate that elevated glucocorticoid activity, rather than specific cortisol is a lethal factor in disease susceptibility of catfish.

What is so bad in glucocorticoids to increase susceptibility to enteric septicemia and become a lethal factor? There is not a final answer. The following effects of glucocorticoids should be considered. Dexamethasone is a potent anti-inflammatory steroid (inflammation is a defensive response) that may cause gastrointestinal complications including septicemia and endotoxic shock. It does block the acquisition and expression of cell-mediated immunity and inhibit the protective mechanism by the white blood cells in mammals.

New treatment and prevention methods must be devised as prevalence of disease continues to increase in aquaculture industries. Current unregulated use and overuse of the few antibiotics available for treatment[10] must be alleviated by new technologies. Development of new vaccines, such as the ESC vaccine available from Intervet, Inc[7]. must be complimented with reduction of pathogen exposure and reduction of stressors at the source of outbreaks. Further understanding of stress, cortisol and their relationship to disease susceptibility is still needed. This study helps to formulate an understanding of the underlying causes of infection and their relationship to stress in fish.

REFERENCES

1. U.S. Department of Agriculture, 2004. Catfish Production 2004. U.S. Department of Agriculture, National Agriculture Statistics Service.
2. U.S. Department of Agriculture, 2003. Part II: Reference of foodsize catfish health and production practices in the United States, 2003 U.S. Department of Agriculture, Animal and Plant Health Inspection Service, National Animal Health Monitoring System, Ft. Collins, Colorado #N407.1103.
3. Sink, T.D., R.J. Strange, H.G. Kattesh and A.G. Mathew, 2004. Linking Stress to Increased Mortality of Channel Catfish at Varying Concentrations of Edwardsiella ictaluri. Aquaculture Research. In Press.

4. Wise, D.J., T.E. Schwedler and D.L. Otis, 1993. Effects of stress on susceptibility of naive channel catfish in immersion challenge with Edwardsiella ictaluri. J. Aquatic Anim. Health, 5: 92-97.
5. Food and Drug Administration, 2001. Food and Drug Administration. Chapter 22: Aquaculture Drugs, in Fish and Fishery Products Hazards and Controls Guide. (2nd Edn). FDA: Washington, DC: http://www-seafood.ucdavis.edu/haccp/Compendium/Chapt22.htm#Approved.
6. Food and Drug Administration, 2003. Reference of drugs approved for use in aquaculture (poikilothermic food species), 2003, Food and Drug Administration, Center for Veterinary Medicine, Washington, D.C.
7. American Society of Microbiologists (ASM)., 1994. American Society of Microbiology. Report of the ASM task force on antibiotic resistance.
8. Cooper, R.K., C.E. Starliper, E.B. Shotts and P.W. Taylor, 1993. Comparison of plasmids isolated from Romet-30-resistant Edwardsiella ictaluri and Tribrissen-resistant Escherichia coli. J. Aquatic Anim. Health, 5: 9-15.
9. Starliper, C.E., R.K. Cooper, E.B. Shotts and P.W. Taylor, 1993. Plasmid-Mediated Romet Resistance of Edwardsiella ictaluri. J. Aquatic Anim. Health, 5: 1-8.
10. Benbrook, C., 2002. Antibiotic drug use in U.S. aquaculture. The Northwest Science and Environmental Policy Center. Sandpoint, Idaho.
11. U.S. Department of Agriculture, 1997. Reference of National Animal Health Monitoring System: Catfish 1997. National Animal Health Monitoring System, Animal and Plant Health Inspection Service/USDA. Washington, D.C.
12. Thune, R.L., 1997. Attenuation, persistence and vaccine potential of an Edwardsiella ictaluri purA mutant. Infection and Immunity, 65: 4642-4651.
13. Agriculture Research Service, 1999. Agency develops and licenses new catfish vaccine. U.S. Department of Agriculture, Agriculture Research Service news and events press release.
14. Ciembor, P.G., V.S. Blazer, D. Dawe and E.B. Shotts, 1995. Susceptibility of channel catfish to infection with Edwardsiella ictaluri: Effect of exposure method. J. Aquatic Anim. Health, 7: 132-140.
15. Strange, R.J., 1980. Acclimation temperature influences cortisol and glucose concentrations in stressed channel catfish. Transactions of the Am. Fisheries Soc., 109: 298-303.
16. Tomasso, J.R., K.B. Davis and N.C. Parker, 1981. Plasma corticosteroid dynamics in channel catfish, Ictalurus punctatus (Rafinesque), during and after oxygen depletion. North Am. J. Aquac., 65: 162-164.
18. Ainsworth, A.J., C. Dexiang and P.R. Waterstrat, 1991. Changes in peripheral blood leukocyte percentages and function of neutrophils in stressed channel catfish. J. Aquatic Anim. Health, 3: 41-47.
17. Alford III, P.B., J.R. Tomasso, A.B. Bodine and C. Kendall, 1994. Apoptotic death of peripheral leukocytes in channel catfish: Effect of confinement-induced stress. J. Aquatic Animal Health, 6: 64-69.
19. Pottinger, T.G. and T.R. Carrick, 2001. ACTH does not mediate divergent stress responsiveness in rainbow trout. Comparative Biochem. Physiol., 129: 399-404.
20. Pickering, A.D. and J. Duston, 1983. Administration of cortisol to brown trout, *Salmo trutta* L. and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. J. Fish Biol., 23: 163-175.
21. Hawke, J.P., R.M. Durborow, R.L. Thune and A.C. Camus, 1998. ESC-Enteric septicemia of catfish. Southern Regional Aquaculture Center fact sheet. Publication No. 477.
22. Sink, T.D. and R.J. Strange, 2004. Linking stress to the increased susceptibility of channel catfish to enteric septicemia using cortisol. J. Aquatic Anim. Health, 16: 93-98.
23. Plumb, D.C., 2002. Veterinary Drug Handbook. Iowa State Press. Blackwell Publishing. St. Paul, Minnesota., 213: 238.
24. Lewis, G.W. and J.L. Shelton, 2003. Channel catfish production. Warnell School of Natural Resources, University of Georgia, Cooperative Extension Service Technical Publication.