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Effects of GH on immune and endocrine responses of channel catfish challenged with *Edwardsiella ictaluri*

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Abstract

The effects of GH on immune and endocrine responses to channel catfish challenged with the bacterium *Edwardsiella ictaluri* were examined. Catfish (11.7±1.0 g) treated with recombinant bovine growth hormone (rbGH) and challenged with *E. ictaluri* experienced similar mortality as control-exposed fish. Plasma activity of lysozyme was higher ($P<0.01$) in rbGH-exposed fish. Compared to day 0 controls (non-exposed fish), IGF-I levels decreased ($P<0.05$) in challenged fish while levels were similar ($P>0.10$) between treatments. Abundance of GH receptor (GHR) mRNA tended to decrease ($P=0.055$) in liver of challenged fish while toll like receptor 5 (TLR5) mRNA increased ($P<0.05$) in liver compared to d 0 controls. An increase in lysozyme may suggest GH enhances a nonspecific immune response. A decrease in GHR mRNA and plasma IGF-I suggests a downregulation of the somatotropic axis in response to disease. The increase in TLR5 mRNA suggests that TLR5 may play a role in host response to bacterial challenge. While exogenous rbGH may play a stimulatory role to increase lysozyme levels, there was no apparent effect of rbGH on mortality to *E. ictaluri*.

Keywords: GH; TLR-5; GHR; IGF-I; Lysozyme; *Edwardsiella ictaluri*; Catfish

1. Introduction

Growth hormone is an important hypophyseal hormone that is primarily involved in body growth and metabolism. In mammals, GH also functions as an important modulator of the immune system (Kelley, 1990) and there is evidence for a similar effect of GH in fish (Sakai et al., 1996a,b,c; Narnaware et al., 1997; Yada et al., 2004). Growth hormone has been shown to stimulate phagocytosis and lymphopoiesis in gilthead sea bream (*Sparus aurata*) (Calduch-Giner et al., 1995, 1997) and silver sea bream (*Sparus sarba*) (Narnaware et al., 1997) as well as enhance natural killer cell activity (Kajita et al., 1992; Sakai et al., 1995), serum haemolytic activity (Sakai et al., 1996a), and antibody production in rainbow trout (Yada et al., 1999). Growth hormone also enhances leukocyte mitogenesis in chum salmon (*Oncorhynchus keta*) (Sakai et al., 1996b), respiratory burst activity of leukocytes in rainbow trout (*Oncorhynchus mykiss*) (Sakai et al., 1996c; Kitlen et al., 1997) and Mediterranean sea bass (*Dicentrarchus labrax*) (Munoz et al., 1998), and superoxide anion production in head kidney leukocytes in a dose-dependent manner in tilapia (*Oreochromis mossambicus*) (Yada et al., 2002). In addition, Sakai et al. (1997) demonstrated that GH primes macrophages and enhances the resistance of rainbow trout to *Vibrio anguillarum*. One study actually found that GH suppresses immune function in GH transgenic Coho salmon (*Oncorhynchus kisutch*) through the time-dependent suppression of lysozyme activity, influence of parr-smolt transformation on the resistance to pathogen, and enhanced response to cold-shock stress (Jhingan et al., 2003).

Although most fish studies demonstrate positive effects of GH on immune function *in vitro*, Sakai et al. (1997) is the only study to examine the effects of GH on mortality of the fish. This study showed that GH administration increased the mean number of days to death in rainbow trout. However, the serum bactericidal activity against *V. anguillarum* was not increased by injection with GH (Sakai et al., 1997).

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In mammals, it is becoming evident that toll-like receptors (TLRs) are important in both innate and adaptive immune responses (Barton and Medzhitov, 2002; Werling and Jungi, 2003). Toll-like receptors play a critical role in the early immune response to invading pathogens by sensing microorganisms. These receptors recognize structural motifs only expressed by microbial pathogens, called pathogen-associated molecular patterns (PAMPs) (Medzhitov and Janeway, 1997; Werling and Jungi, 2003). To date, 14 TLRs have been described in various teleosts; zebra fish (*Danio rerio*) (Meijer et al., 2004), Pufferfish (*Fugu rubripes)* (Oshiumi et al., 2003), goldfish (*Carrassius auratus*) (Stafford et al., 2003), flounder (*Paralichthys olivaceus*) (Hirono et al., 2004), rainbow trout (*O. mykiss)* (Tsujita et al., 2004), Atlantic salmon (*Salmo salar*) (Tsoi et al., 2006), and channel catfish (*Ictalurus punctatus*) (Bilodeau and Waldbieser, 2005). Information regarding the functional roles of TLRs is beginning to emerge. In catfish, it has been demonstrated that both TLR3 and TLR5 mRNA increase during embryogenesis and early larval development (Peterson et al., 2005a) and in fingerlings after exposure to *Edwardsiella ictaluri* (Bilodeau and Waldbieser, 2005; Bilodeau et al., 2005). In response to exposure to virulent bacteria, TLR5 expression appears to be significantly higher than that of TLR3 (Bilodeau and Waldbieser, 2005; Bilodeau et al., 2005). Results of these studies suggest that both TLR3 and TLR5 may be important in the host response to bacterial challenge.

A study using 3-day-old dairy calves (time when calves are most susceptible to diseases) examined the effects of GH on abundance of TLR2 and TLR4 mRNA (Eicher et al., 2004). Abundance of TLR2 and TLR4 mRNA in blood leukocytes decreased in GH treated calves compared to controls at day 14 of age suggesting these calves may be more susceptible to disease. The role, if any, GH has on regulating TLRs is not clear.

While many studies have shown positive effects of GH on the immune system in a variety of species of fish, the immunoregulatory role(s) of GH in channel catfish have not been investigated. Furthermore, the effects of GH in disease-challenged catfish in stimulating an immune response through TLRs, a nonspecific response, or affecting the somatotropic axis are not known.

Enteric septicemia of catfish is caused by the bacterium *E. ictaluri* and is the most prevalent disease affecting commercial catfish farms in the southern United States (USDA, 1997). Defining the mechanisms involved in the immune response to *E. ictaluri* will be key in understanding how to protect catfish from this devastating disease. The present study investigated the possible immunoregulatory properties of recombinant bovine growth hormone (rbGH) in catfish experimentally challenged with *E. ictaluri*.

2. Materials and methods

2.1. Research animals

The channel catfish used in the study were from the National Warmwater Aquaculture Center (NWAC103) strain that originated from broodstock maintained at the USDA-ARS Catfish Genetics Research Unit, Stoneville, MS, aquaculture facility. Prior to randomization into tanks, approximately 800 fish from four different catfish families were placed into a holding tank. Four hundred and eighty fish averaging 11.7±1.0 g were randomly assigned to two treatments with six replicate tanks each. The treatments were (1) control-exposed (one needle puncture per week for 3 weeks and challenged with *E. ictaluri*) and (2) rbGH-exposed (recombinant bovine growth hormone, Posilac (Monsanto, St. Louis, MO), injected at 30 μg/g body weight per week for 3 weeks and challenged with *E. ictaluri*). After the last injection and weighing, the fish were allowed to recover for 2 days. The fish were then challenged with *E. ictaluri* (day 0=*E. ictaluri* challenge). To ensure that negligible splashing occurred from tank to tank, three tanks (10 fish per tank) were randomly placed among the other 48 tanks and served as non-exposed controls. Fish were fed daily a 36% CP diet (Melick Aquafeed Inc., Catawissa, PA) at 6.0% of their body weight to ensure that rbGH-treated fish did not consume more feed than controls. Research has shown that rbGH-injected fish consume more feed (Silverstein et al., 2000), while others studies have shown no difference in intake between controls and rbGH-treated fish (Peterson et al., 2004, 2005a,b). Fish were sampled on days 1, 4, 8, or 14 (5 fish/tank; $N=30$). Non-exposed groups (day 0 controls) of fish were sampled on d 0 (5 fish/tank; $N=15$). On the days of sampling, fish were euthanized with tricaine methanesulfonate (TMS; Argent Chemical Laboratories, Redmond, WA), bled from the caudal vasculature into syringes coated with heparin, and kidney, liver, spleen, and gut tissue were excised and flash-frozen in liquid nitrogen and stored at −80 °C. Mortality was recorded daily for 21 days. Water quality (pH ~ 8.5 and dissolved oxygen levels > 5.0 mg/L), temperature (26.0 °C), and flow rates were similar between tanks. The study was conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, USDA/ARS Catfish Genetics Research Unit.

2.2. Specific growth rates

Specific growth rates (SGR) were calculated from the formula $((\ln(\mathrm{WT}) - \ln(\mathrm{wt})/T - t)) \times 100$ where WT and wt are initial and final weights, respectively, and T and t are initial and final times (days), respectively.

2.3. Disease challenge

An *E. ictaluri* isolate from a natural outbreak (confirmed by the Fish Diagnostic Laboratory at the Delta Research and Extension Center, Stoneville, MS, USA) was used for the challenge. Fish were challenged with virulent *E. ictaluri* by a bath immersion for 30 min (Wolters and Johnson, 1994).

Genomic DNA was extracted from all heparin-treated blood samples using the High Pure PCR Template Preparation Kit (Roche Diagnostics Corp., Indianapolis, IN, USA) with a final elution volume of 100 μl filtered deionized water. An *E. ictaluri*-specific target sequence was then amplified using a validated real-time PCR assay that enabled direct quantification of bacterial DNA (Bilodeau et al., 2003). Although this assay has been validated against standard plate counts of viable *E.*

ictaluri, target DNA fragments from dead bacteria may also be included in the quantification. Hence, the data are expressed in cell equivalents.

2.4. Sample preparation and RNA isolation

Total RNA was isolated with Trizol (Molecular Research Center, Inc., Cincinnati, OH, USA) according to the manufacturer's recommendations and utilized for analysis of TLR5 and GH receptor (GHR) mRNA from kidney, liver, spleen, and gut tissues. The real-time PCR assays for TLR5 and GHR mRNA have been previously described (Bilodeau and Waldbieser, 2005; Small et al., in press). The integrity of the RNA preparations was verified by visualization of the 18S and 28S ribosomal bands stained with ethidium bromide after electrophoresis on 2.0% agarose gels. Total RNA was quantified by measuring the absorbance at 260 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE).

2.5. Real-time PCR

RNA (1 μg) from tissues was reverse-transcribed in 10-μl reactions using the iScript cDNA Synthesis Kit (BioRad, Hercules, CA). Real-time PCR was performed using the iCycler iQ (BioRad) to quantify TLR5 and GHR mRNA as previously described (Bilodeau et al., 2005; Small et al., in press). All specific quantities were normalized against the amount of alpha tubulin amplified. Probe and primer sequences, concentrations, and amplification profile used for alpha tubulin has also been previously described (Bilodeau et al., 2005).

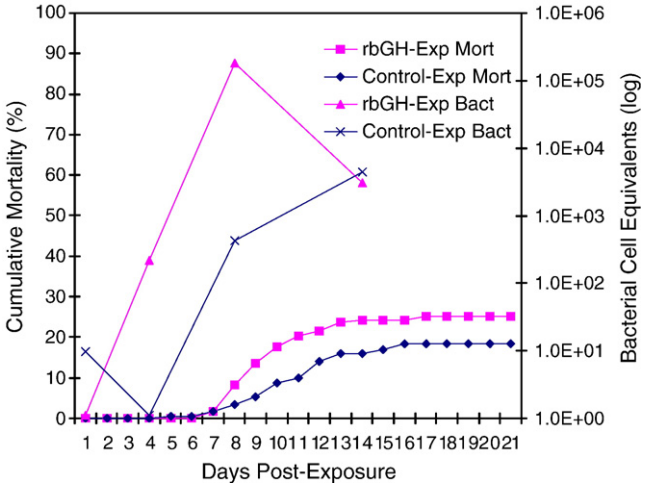

Fig. 1. Levels of *E. ictaluri* in whole blood samples of channel catfish after challenge with virulent *E. ictaluri*. Cell-equivalent values were calculated per 100 μl whole blood and based on estimated genome size of *E. ictaluri*. rbGH samples were from fish injected with rbGH 2 days prior to challenge. Control samples were from fish sham injected with a needle puncture 2 days prior to challenge. Cell equivalent values were higher ($P<0.05$) on days 4 and 8 in rbGH-exposed fish but were similar on d 14 compared to control-exposed fish. Cumulative mortality of channel catfish following exposure to virulent *E. ictaluri*. rbGH-treated fish were injected 2 days prior to challenge. Control samples were from fish sham injected with a needle puncture 2 days prior to challenge. Mean cumulative mortality was similar ($P>0.05$) in rbGH-exposed fish compared to control-exposed fish.

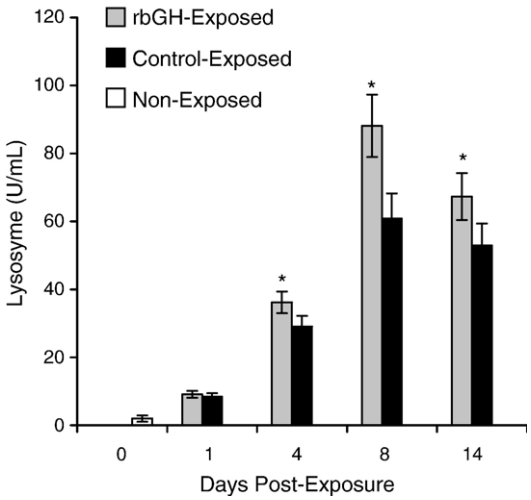

Fig. 2. Plasma lysozyme activity of channel catfish following exposure to virulent *E. ictaluri*. Day 0 represents non-exposed fish ($N=15$). Values represent means ± S.E.M. $N=30$/treatment at each time point (day 1, 4, 8, and 14). Plasma lysozyme activity was higher ($P<0.01$) in rbGH-exposed fish on days 4, 8, and 14 compared to control-exposed fish.

2.6. IGF-I fluoroimmunoassay

Plasma IGF-I levels were measured using a competitive time-resolved fluoroimmunoassay validated for channel catfish (Small and Peterson, 2005). Plasma samples were acid–ethanol extracted prior to assaying and standards were run in triplicate while samples were run in duplicate.

2.7. Lysozyme assay

Levels of lysozyme activity were determined using the Enz Chek Lysozyme Assay Kit (E-22013; Molecular Probes, Eugene, OR, USA). Briefly, 25 μl of plasma was diluted with 25 μl reaction buffer (0.1 M sodium phosphate, 0.1 M NaCl, pH, 7.5) and incubated with 50 μl 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. The lysozyme activity of the samples was calculated from a standard curve prepared with lysozyme from chicken egg white. All samples were run in duplicate.

2.8. Statistical analysis

Bacterial levels (cell equivalents) and the gene expression data that initially failed Levene's test of homogeneity were then subjected to a reciprocal square root transformation and subjected to ANOVA using SAS Version 9.1 software (SAS Institute, Inc., Cary, NC, USA). All expression data were analyzed as copy number and normalized as the ratio of the expression level of the gene of interest/housekeeping gene. Alpha-tubulin was used as the housekeeping gene. There was no significant ($P>0.10$) effect of time for the housekeeping

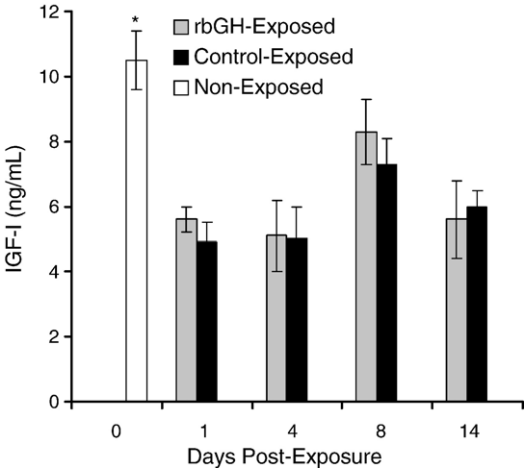

Fig. 3. Plasma IGF-I concentrations of channel catfish following exposure to virulent *E. ictaluri*. Day 0 represents non-exposed fish. Values represent means±S.E.M. (n=30/treatment). Compared to non-exposed fish, IGF-I levels were lower (P<0.05) while levels were similar (P>0.10) between treatments throughout the study.

gene data for all tissues. Data are reported as fold change, which indicates exposed fish relative to non-exposed fish. Plasma IGF-I and lysozyme concentrations were subjected to ANOVA followed by a Duncan's multiple range test. Differences were considered different at P<0.05. Tank served as the experimental unit for each variable measured.

3. Results

3.1. Growth rate, mortality, and E. ictaluri levels

During the 3-week period prior to challenge, SGR of rbGH-treated fish was higher (P=0.009) compared to sham-injected controls (3.9±0.1 v. 3.4±0.1) prior to challenge. Mean cumulative mortality was similar (P=0.105) in rbGH-exposed fish (25.2%) compared to control-exposed fish (18.5%) (Fig. 1). No mortalities were observed in the non-exposed fish. Using the validated real-time PCR assay, all non-exposed fish tested negative for the presence of *E. ictaluri*. Bacterial DNA levels

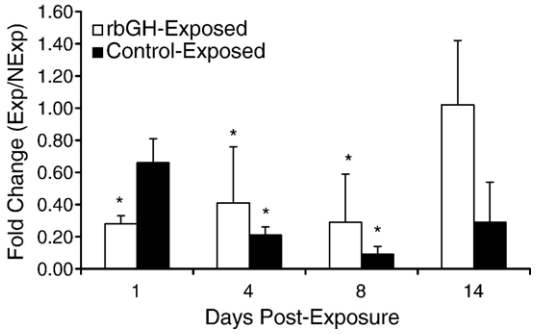

Fig. 4. Abundance of GH receptor (GHR) mRNA of channel catfish liver as determined by real-time PCR following exposure to virulent *E. ictaluri*. Fold change indicates exposed fish relative to non-exposed fish. Values represent means±S.E.M. (6 replicate tanks for each time period sampled). Asterisks represent comparisons (fold change) between exposed and non-exposed fish and each day that are different at P<0.06.

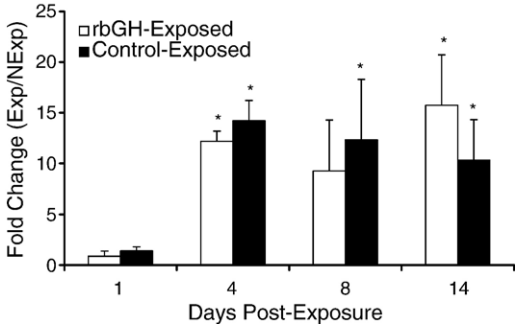

Fig. 5. Abundance of toll like receptor 5 (TLR5) mRNA of channel catfish liver as determined by real-time PCR following exposure to virulent *E. ictaluri*. Fold change indicates exposed fish relative to non-exposed fish. Values represent means±S.E.M. (6 replicate tanks for each time period sampled). Asterisks represent comparisons (fold change) between exposed and non-exposed fish and each day that are different at P<0.05.

were higher (P<0.05) on days 4 and 8 in rbGH-exposed fish but were similar on d 14 compared to control-exposed fish (Fig. 1).

3.2. Plasma lysozyme and IGF-I

Plasma lysozyme activity was higher in rbGH-exposed fish on days 4 (P=0.042), 8 (P=0.008), and 14 (P=0.007) compared to control-exposed fish (Fig. 2). In non-exposed controls, lysozyme activity was low (less than 5 U/ml) and not detected in all samples. Circulating levels of IGF-I were higher (P<0.05) in non-exposed fish (day 0) while levels were similar (P>0.10) between treatments throughout the study (Fig. 3).

3.3. GHR and TLR mRNA

Abundance of GH receptor (GHR) mRNA decreased in liver of challenged fish on days 1 (P<0.06), 4 (P<0.05), and 8 (P<0.05) (Fig. 4) while levels were similar (P>0.10) between treatments in the spleen, kidney, liver, and gut throughout the study (data not shown). Abundance of toll-like receptor 5 (TLR5) mRNA increased (P<0.05) in the liver of fish challenged with bacteria compared to d 0 controls on days 4, 8, and 14 (Fig. 5), while levels were similar (P>0.10) between treatments in the spleen, kidney, liver, and gut throughout the study (data not shown).

4. Discussion

In mammals and fish, a coordinated regulation of endocrine and immune responses to disease is essential to maintain homeostasis. *In vitro* studies in fish showing an immunostimulating effect of GH have provided the impetus for further investigation into the relationship between the endocrine and immune system. The present study provides information towards our understanding of a possible relationship between GH and the immune system in channel catfish.

Fish were injected with rbGH for 3 weeks prior to bacterial challenge. Fish that were administered rbGH gained approximately 19% more weight. The increase in weight is less than what has been previously reported for channel catfish injected

with rbGH (48% compared to non-injected catfish) (Peterson et al., 2005b). The discrepancy may be explained by the fish in the current study being fed at 6% of their body weight. This amount was chosen for two reasons: to ensure that rbGH treatment did not have any effect on feed intake and that copious amounts of feed were not being fed to the fish once they were exposed to virulent *E. ictaluri*. In previous studies with catfish exposed to virulent bacteria, feed intake was noticeably reduced during the study (Bilodeau et al., 2005). It should be acknowledged that the actual feed intake after the fish were exposed to bacteria was not at 6% of their body weight. The only time that the fish consumed 6% of their body weight is during the 3 weeks prior to challenge and 2 days after the challenge. The actual amount of feed after that time period was not directly measured and so may have been affected by treatment.

Bacterial levels were higher in rbGH-exposed fish compared to control-exposed fish on days 4 and 8; however, mortality was similar between treatments. Activity of lysozyme was also higher on days 4 and 8 in rbGH-exposed fish. In rainbow trout experimentally infected with virulent *V. anguillarum*, the mean number of days to death was 7.2 days for fish administered GH and 4.1 days for control (injected with bovine serum albumin) fish (Sakai et al., 1997). The overall mortality level was not reported. The current study and the study reported by Sakai et al. (1997) are the only two *in vivo* studies that have examined the relationship between GH, the immune system, and mortality in fish. These studies do not support a role for exogenous GH in decreasing overall mortality in experimentally infected fish.

One possible explanation of why bacterial levels were higher in rbGH-exposed fish compared to control-exposed fish is that the immune response was decreased in rbGH-treated fish. Biga et al. (2005) reported specific antibody production to rbGH when rainbow trout were administered rbGH. It is likely that bovine GH would also stimulate an immune response in channel catfish due to the low amino acid sequence between bovine and catfish GH. Thus, immune function may have been altered.

Activity of lysozyme was higher in rbGH-exposed fish compared to control-exposed fish on days 4, 8, and 14. This is a similar pattern to bacterial DNA levels except for day 14 where bacterial levels were similar between treatments. Activity of lysozyme has been shown to track levels of *E. ictaluri* in two previous catfish studies (Bilodeau et al., 2005; Small and Bilodeau, 2005). Others have also demonstrated a relationship between exogenous GH and increasing lysozyme activity. For example, administration of salmon GH to rainbow trout increased plasma lysozyme activity (Yada et al., 2001). In brown trout (*Salmo trutta*), plasma lysozyme activity was increased after transfer from fresh water to seawater and there were positive correlations between plasma GH level and lysozyme activity (Marc et al., 1995). More recently in GH transgenic common carp (*Cyprinus carpio*), lysozyme activity was higher in the transgenic fish serum compared to non-transgenic control fish (Wang et al., in press). Lysozyme plays an important role in the non-specific immune response by attacking bacterial cell walls, thereby causing lysis, and stimulating phagocytosis of the bacteria (Bowden et al., 2004). The significance of higher activity of lysozyme in rbGH-exposed fish is not clear but may suggest GH is stimulating a non-specific immune response, a conclusion that is consistent with studies in mammals (Blalock, 1994; Kelley, 1989) and other fish species (Sakai et al., 1996a,b,c; Narnaware et al., 1997; Kajita et al., 1992; Sakai et al., 1995; Leedom et al., 2002). The higher levels of lysozyme in rbGH-exposed fish may also reflect higher levels of bacteria in the blood.

It was hypothesized that components of the somatotropic axis would be down-regulated in disease-challenged fish. We found that circulating levels of IGF-I and abundance of GHR mRNA were lower in disease challenged fish compared to non-exposed fish. Levels of plasma IGF-I and GHR mRNA were similar between rbGH-exposed and control-exposed fish throughout the study. It was surprising that levels of IGF-I and GHR were not higher in GH-injected catfish after 3 weeks of treatment. Peterson et al. (2005b) reported higher levels of circulating IGF-I levels after 2 weeks of administering rbGH to catfish. However, levels were similar at 3 weeks (Peterson et al., 2005b). In the current study, the amount of food was restricted to 6% of their body weight. Perhaps one of the reasons we did not observe an increase in GHR or IGF-I is because we were restricting the amount of food the fish would eat.

The decrease in levels of IGF-I in disease challenged fish compared to non-exposed controls is similar to what has been previously reported in catfish exposed to *E. ictaluri* (Bilodeau et al., 2005). Bilodeau et al. (2005) demonstrated that circulating levels of IGF-I decreased approximately 3-fold on days 2, 5, 8, and 14 after exposure to *E. ictaluri* compared to non-exposed fish. Similarly, pigs infected with *Salmonella typhimurium* showed reduced concentrations of IGF-I compared to non-infected animals (Jenkins et al., 2004). In other research, levels of IGF-I were reduced during the acute infection phase of *Sarcocystis suicanis* in a group of growing swine (Barrows et al., 1982). It is well documented that circulating concentrations of IGF-I are associated with nutritional status (McCusker et al., 1989). In this regard, the changes in IGF-I observed in the disease-induced swine studies, the current study, and the above-mentioned catfish study (Bilodeau et al., 2005), are likely a result of reduced feed intake. Although feed intake was not directly measured in this study or the previous catfish study (Bilodeau et al., 2005), the fish exposed to virulent bacteria consumed noticeably less feed compared to the time period when the fish were being acclimated and compared to the non-exposed controls. The reduction in feed intake would also explain the decrease in abundance of GHR. Small et al. (in press) demonstrated that GHR mRNA was approximately 2.8-fold lower in fasted channel catfish compared to fed controls.

The effects of GH treatment on TLRs are not well understood. In the current study, abundance of TLR5 mRNA increased in the liver of fish challenged with bacteria compared to non-exposed fish on days 4, 8, and 14. However, levels of TLR5 mRNA were similar between rbGH-exposed and control-exposed fish in the spleen, kidney, liver, and gut. A recent study using 3-day-old dairy calves (time when calves are most susceptible to diseases) examined the effects of GH on abundance of TLR2 and TLR4 mRNA (Eicher et al., 2004). Abundance of TLR2 and TLR4 mRNA in blood leukocytes decreased in GH treated calves compared to controls at day 14

of age suggesting these calves may be more susceptible to disease; however, this was never directly tested (Eicher et al., 2004). No other studies have examined the effects of GH treatment on TLR regulation in disease infected animals. The current study as well as the two previous studies (Bilodeau and Waldbieser, 2005; Bilodeau et al., 2005) has clearly shown that expression of TLR5 mRNA is elevated in catfish exposed to *E. ictaluri*; however, there is no evidence to suggest any interaction between exogenous GH and TLR5 expression.

Our understanding of the role(s) of TLRs in catfish immune function is at an infancy stage. The timing in activation of TLR5 and lysozyme suggests that the innate response may be activated and maintained until the adaptive response is mounted. The pattern of lysozyme activity in response to *E. ictaluri* reported in the current study as well as other catfish studies (Bilodeau et al., 2005, 2006) suggest that lysozyme may play a role in protecting catfish against this bacterium.

An increase in lysozyme due to rbGH treatment in catfish challenged with virulent *E. ictaluri* suggests a relationship exists between GH and the immune system. A decrease in GHR mRNA and plasma IGF-I suggests a downregulation of the somatotropic axis in response to disease. The increase in TLR5 mRNA suggests that TLR5 may play a role in host response to bacterial challenge. While exogenous rbGH may play a stimulatory role to increase lysozyme levels, there was no apparent effect of rbGH on mortality to *E. ictaluri*. Examining other endocrine factors such as insulin like growth factor binding proteins and IGF-II as well as cytokines may provide further insight into how *E. ictaluri* affects the immune system of catfish. Defining these mechanisms will be crucial in developing more effective methods for enhancing the immune system through selective breeding and in developing treatment and management protocols to control the disease.

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