Effect of fasting on nychthemeral concentrations of plasma growth hormone (GH), insulin-like growth factor I (IGF-I), and cortisol in channel catfish (Ictalurus punctatus)

Brian C. Small*

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

Received 7 April 2005; received in revised form 18 July 2005; accepted 19 July 2005

Available online 26 August 2005

Abstract

This experiment was conducted to characterize the effect of fasting versus satiety feeding on plasma concentrations of GH, IGF-I, and cortisol over a nychthemeron. Channel catfish fingerlings were acclimated for two weeks under a 12L:12D photoperiod, then fed or fasted for 21 d. On day 21, blood samples were collected every 2 h for 24 h. Weight of fed fish increased an average of 66.2% and fasted fish lost 21.7% of body weight on average. Average nychthemeral concentrations of plasma GH were not significantly different between fed (24.7 ng/mL) and fasted (26.8 ng/mL) fish, but average nychthemeral IGF-I concentrations were higher in fed (23.4 ng/mL) versus fasted (17.8 ng/mL) fish. An increase in plasma IGF-I concentrations was observed in fasted fish 2 h after a peak in plasma GH, but not in fed fish. Average nychthemeral plasma cortisol concentrations were higher in fed (14.5 ng/mL) versus fasted (11.0 ng/mL) fish after 21 d. Significant fluctuations and a postprandial increase in plasma cortisol were observed in fed fish and there was an overall increase in plasma cortisol of both fasted and fed fish during the scotophase. The present experiment indicates little or no effect of 21-d fasting on plasma GH levels but demonstrates fasting-induced suppression of plasma IGF-I and cortisol levels in channel catfish.

Published by Elsevier Inc.

Keywords: IGF-I; GH; Cortisol; Fasting; Channel catfish

1. Introduction

In many vertebrate species, catabolic states such as fasting result in elevated circulating growth hormone (GH) concentrations (for a review, see Harvey et al. (1995)). Several studies with fish have demonstrated increased concentrations of plasma GH during food deprivation (Wagner and McKeown, 1986; Barrett and McKeown, 1989; Sumpter et al., 1991; Kakisawa et al., 1995; Rand-Weaver et al., 1995; Johnsson et al., 1996; Small et al., 2002). In contrast, fasting typically has the opposite effect on circulating concentrations of insulin-like growth factor I (IGF-I) in fish and mammals (Duan et al., 1994; Thissen et al., 1994). The apparent paradox between increased concentrations of circulating GH and decreased concentrations of circulating IGF-I has been explained for fish as tissue resistance to GH (Duan and Plisetskaya, 1993; Duan et al., 1995) and a reduction in hepatic GH-receptors (GHR) during starvation (Gray et al., 1992; Pérez-Sánchez et al., 1994, 1995). The effects of fasting on nychthemeral plasma GH and IGF-I concentrations have not been characterized in channel catfish, Ictalurus punctatus. In fact, much of the characterization of GH and IGF-I nychthemeral and seasonal plasma profiles in fish has focused on salmonid species (Boujard and Leatherland, 1992; Pérez-Sánchez et al., 1994, 1996). Circulating basal glucocorticoid levels exhibit significant fluctuations over a nychthemeron in many vertebrate species (Krieger, 1979; Dauphin-Villemant and Xavier, 1987; Summers and Norman, 1988; Breuner et al., 1999).
In rats and humans, glucocorticoid levels often peak right before or after feeding (for review, see Krieger (1979)). Dallman et al. (1993) suggest that this modulation of glucocorticoids by feeding is a bi-directional relationship, with both fasting and feeding, in some cases, stimulating plasma glucocorticoids. In fish, feed intake and circulating glucocorticoid levels also appear to have a bi-directional relationship (for review, see Mommsen et al. (1999)) and the effects of feed intake are not fully understood. Kelley et al. (2001) reported a six-fold increase in circulating cortisol levels in gobies fasted for 20 d; however, Peterson and Small (2004) observed that the effect of fasting on plasma cortisol levels in channel catfish was dependent on the length of the fast. In several salmonid species, temporal rhythms with higher nocturnal plasma cortisol levels have been observed (Rance et al., 1982; Pickering and Pottinger, 1983; Nichols and Weisbart, 1984).

Channel catfish are an economically important species in the Southern United States. In culture, channel catfish are often subjected to periods of restricted feeding and fasting as management tools for water quality and disease (Hawke et al., 1998; Robinson and Li, 1999). Recent literature has demonstrated significant effects of feed restriction and fasting on channel catfish metabolic and endocrine functions (Peterson and Small, 2004; Small and Peterson, 2005). In those studies, conclusions were based on measurements taken at a single point in time. Nothing is known concerning the effects of feeding and fasting on nychthemeral hormone levels in channel catfish. To learn more about the regulation of GH, IGF-I, and cortisol production in channel catfish, we examined nychthemeral hormone profiles in fasted catfish and in catfish fed twice daily to satiety for 21 d.

2. Materials and methods

2.1. Animals

Channel catfish (I. punctatus) fingerlings of the NWAC103 strain were maintained at the USDA-ARS Catfish Genetics Research Unit, Stoneville, MS aquaculture facility following accepted standards of animal care, approved by the Institutional Animal Care and Use Committee (IACUC) according to USDA-ARS policies and procedures. Two weeks prior to starting the experiment, 480 catfish (mean weight=14.8 g) were randomly stocked into forty-eight 76-L aquaria (10 fish/aquarium) and allowed to acclimate. During the 2-week acclimation period, all fish were fed a commercial floating catfish feed (36% crude protein; Land O’Lakes Farmland Feed, Fort Dodge, IA) twice daily (07.00 and 15.00 hours) to satiety. Throughout the acclimation and experimental periods, the fish were reared in 26 °C well-water under a 12L:12D photoperiod, with lights coming on at 06.00 hours daily.

2.2. Experimental design

All the fish were weighed at the start of the experiment and treatments were randomly assigned such that fish in 24 aquaria were fed twice daily to satiety for 21 d and fish in the remaining aquaria were fasted for 21 d. On day 21, fish in the fed treatment were fed once at 0650 hours. Beginning at 0700 hours and continuing every 2 h for 24 h, all the fish from two tanks per treatment were euthanized in a solution of tricaine methanesulphonate (0.3 g/L; Finquil; Argent Chemical Laboratories, Richmond, WA, USA), weighed, and bled from the caudal vasculature into syringes coated with heparin. During the scotophase, fish were rapidly captured by dim flashlight, euthanized in the dark, and then moved to a separate adjoining area where sampling was quickly conducted under dim light. Plasma was separated from whole blood by centrifugation, stored at −80 °C, and later analyzed for GH, IGF-I, and cortisol.

2.3. Immunoassays

2.3.1. Growth hormone ELISA

Plasma GH concentrations were determined using a homologous antigen-capture enzyme-linked immunosorbent assay (ELISA) validated for quantifying circulating levels of channel catfish GH (Drennon et al. 2003). Sensitivity of the assay was 2.0 ng/mL and recovery of channel catfish GH from spiked plasma samples was ≥100%. Intra- and inter-assay coefficients of variation (CV) were 7.7% and 11.2%, respectively. Dose–response inhibition curves using serially diluted pituitary homogenates and plasma samples consistently showed parallelism with the GH standard curve.

2.3.2. IGF-I fluoroimmunoassay

Plasma IGF-I concentrations were determined using a heterologous time-resolved fluoroimmunoassay (TR-FIA) validated for quantifying circulating levels of channel catfish IGF-I (Small and Peterson, 2005). Sensitivity of the assay was 0.20 ng/mL and recovery of IGF-I from spiked plasma samples was ≥95%. Intra- and inter-assay CVs were 5.1% and 9.8%, respectively. Dose–response inhibition curves using serially diluted plasma samples consistently showed parallelism with the IGF-I standard curve.

2.3.3. Cortisol fluoroimmunoassay

Plasma cortisol concentrations were determined using a heterologous TR-FIA kit (R060-101; PerkinElmer Life Sciences, Akron, OH) modified and validated for channel catfish (Small and Davis, 2002). Sensitivity of the assay was 1.2 ng/mL and recovery of cortisol from spiked plasma samples was ≥99%. Intra- and inter-assay CVs were 6.5% and 9.0%, respectively. The displacement curve for serially diluted channel catfish plasma paralleled the cortisol standard curve.
2.4. Statistics

The experimental data for both treatments (fed and fasted) were subjected to two-way analysis of variance (ANOVA) mixed-model procedures using SAS software system version 8.00 (SAS Institute, Cary, NC) with treatment, time, and treatment × time as the fixed effects. When significant differences were found using ANOVA, pairwise contrasts were made using an LSD test to identify significant differences at the 5% level. Results are presented as mean±pooled standard error (S.E.).

3. Results

3.1. Condition of experimental fish

At the end of the 21-d experiment, weight of fed fish increased an average of 66.2% and fasted fish lost 21.7% of body weight on average. Average final weights of fed fish (24.1 g) were significantly greater (P<0.05) than weights of fasted fish (11.9 g; Fig. 1). Survival of experimental fish was 100%.

3.2. Plasma levels of growth hormone

Average nycthemeral plasma GH concentrations were highly variable and not significantly different (P>0.05) between fed (24.7 ng/mL) and fasted (26.8 ng/mL) fish (Fig. 2). Plasma GH concentrations in fasted fish were significantly (P<0.05) higher at 15.00 hours when compared to the lowest concentration at 05.00 hours. In fed fish, no differences (P>0.05) were observed in plasma GH concentrations between times.

3.3. Plasma levels of IGF-I

Average nycthemeral plasma IGF-I concentrations were significantly (P<0.05) higher in fed (23.4 ng/mL) compared to fasted (17.8 ng/mL) catfish (Fig. 3). Plasma IGF-I concentrations in fasted fish increased significantly (P<0.05) at 17.00 hours and were higher than plasma concentrations at all other time-points with the exception of 01.00 hours (P=0.0613). In fed fish, plasma IGF-I concentrations were the highest (P<0.05) at 07.00 hours, the time of morning feeding.

3.4. Plasma levels of cortisol

Average nycthemeral plasma cortisol concentrations were significantly (P<0.05) higher in fed (14.5 ng/mL) versus fasted (11.0 ng/mL) catfish (Fig. 4). Feeding-associated changes in plasma cortisol were observed in fed fish with cortisol levels being highest (P<0.05) immediately after feeding. Significant fluctuations in plasma cortisol levels were observed in fed fish throughout the...
photophase. An increase in circulating cortisol in fed fish during the scotophase between 01.00 and 03.00 hours corresponded to a significant ($P<0.05$) plasma cortisol increase in fasted fish at 03.00 hours.

### 4. Discussion

The effect of fasting on average nychthemeral plasma GH and IGF-I levels in channel catfish is similar to that previously reported by Small and Peterson (2005), in which a single time-point sample after 2 weeks of fasting demonstrated no effect of fasting on plasma GH levels but a decrease in plasma IGF-I levels. After 4 weeks of fasting, however, Small and Peterson (2005) reported both a significant increase in circulating GH levels and a decrease in plasma IGF-I levels. Together with the present study, these results demonstrate that circulating levels of GH respond more slowly to fasting than do plasma IGF-I concentrations in channel catfish and support the conclusion that decreased circulating IGF-I concentrations during fasting are not the result of impaired GH secretion (Thissen et al., 1999). In other teleost species, reduced hepatic binding capacity for GH during fasting appears to be one of the mechanisms responsible for the decline in circulating IGF-I (Pérez-Sánchez et al., 1994, 1995; Gray et al., 1990). Small and Peterson (2005) hypothesized that the observed increase in channel catfish plasma GH after 4 weeks of fasting was due to low circulating IGF-I, resulting in reduced negative feedback on GH synthesis and release. In rainbow trout, IGF-I has been shown to negatively regulate GH release (Pérez-Sánchez et al., 1992).

Plasma IGF-I levels of fasted catfish in the present study increased 2 h after an increase in plasma GH concentration, but a similar pattern was not observed in fed catfish. Since both entrainment and “masking” of hormonal rhythms in fishes by physiological responses to treatments have been documented (see Meier (1993)), it is possible that feeding per se may have masked the temporal increase in IGF-I of fed catfish. Even so, temporal changes in IGF-I and the effects of fasting on nychthemeral concentrations have been largely unexplored. In rats, Donaghyue et al. (1990) observed no evidence of episodic IGF-I release or temporal variations entrained to light or feeding cycles. In fish, studies of diurnal GH and IGF-I release are often disparate or incomplete. Reports of asynchronous release in trout (Le Bail et al., 1991; Gomez et al., 1996) contradict reports of temporal rhythms and episodic release profiles in both trout (Reddy and Leatherland, 1994) and carp (Zhang et al., 1994). Differences in experimental design and physiological status of the fish between studies make direct comparisons and definitive conclusions difficult. This is exemplified by the effect that different feeding protocols have on plasma hormone and metabolite levels (Holloway et al., 1994). When trout are fed at a set time of day versus being fed ad libitum via a demand feeder, the temporal plasma hormone levels change or even disappear (Boujard et al., 1993; Reddy and Leatherland, 1994). Observations such as these led Holloway et al. (1994) to conclude that the time of daily feeding, method of food administration, and presumably the level of feeding were all critical in determining the nature of temporal hormone release.

In fasted trout, plasma cortisol levels have been reported to decrease depending on the degree of food deprivation (Sumpter et al., 1991; Farbridge and Leatherland, 1992; Leatherland and Farbridge, 1992; Holloway et al., 1994). The opposite has been reported for gobies (Kelley et al., 2001) and mixed effects have been previously reported for channel catfish (Peterson and Small, 2004). Peterson and Small (2004) observed an increase in plasma cortisol of catfish fasted for 30 d but observed no effect of fasting on cortisol levels after 14, 45, and 60 d of fasting. Similar to most studies, however, their observations were based on plasma cortisol levels sampled at only one time of the day. When plasma cortisol concentrations were averaged over the nychthemeron in the present study, cortisol levels in fasted catfish decreased after 21 d of fasting and cortisol levels in fed fish exhibited significant temporal fluctuations. Differences in the findings of these two studies might be related to differences in feeding frequency or initial nutritional status of the fish, which might further be associated with nychthemeral fluctuations of plasma cortisol in fed catfish.

Plasma cortisol levels have been extensively studied in fishes and are perhaps the best characterized physiological variable with respect to temporal rhythms (Boujard and Leatherland, 1992). Temporal and feeding-entrained release of cortisol into circulation has been demonstrated in a number of teleost species. Circadian-like rhythms, in which plasma cortisol levels increase during the scotophase, have been reported for killifishes, Fundulus grandis (Garcia and Meier, 1973), goldfish (Peter et al., 1978; Spieler et al., 1991; Farbridge and Leatherland, 1992; Leatherland and Farbridge, 1992; Holloway et al., 1994).

---

**Fig. 4.** The nychthemeral profile of circulating cortisol levels between catfish fed twice daily to satiety and catfish fasted for 21 d. Data plotted are mean±pooled S.E. ($n=20$). Fish in the fed treatment group were fed at 06.50 hours on the day of sampling. The scotophase is indicated by the dark bar along the x-axis. An asterisk indicates significant ($P<0.05$) differences within time between fed and fasted fish.
Noeske, 1984), rainbow trout (Rance et al., 1982), brown trout, *Salmo trutta* (Pickering and Pottinger, 1983), and stickleback, *Gasterosteus aculeatus* (Audet et al., 1986). A similar trend for channel catfish toward increased plasma cortisol levels during the scotophase was observed in the present study. Furthermore, in fed catfish a postprandial cortisol increase followed by temporal fluctuations in plasma levels during the photophase was also observed. Dramatic fluctuations in plasma glucocorticoids over the nychthemeron are common to many vertebrate species (Krieger, 1979; Dauphin-Villemant and Xavier, 1987; Summers and Norman, 1988; Breuner et al., 1999), as is entrainment of glucocorticoid rhythm to the light:dark cycle (Krieger, 1979). In teleost fishes, there is also evidence of temporal cortisol release being entrained to feeding (Bry, 1982; Pickering and Pottinger, 1983; Spieler and Noeske, 1984; Laidley and Leatherland, 1988; Boujard and Leatherland, 1992; Holloway et al., 1994). In the present study, plasma cortisol levels did not increase at 15.00 hours in fed fish, the time at which those fish had been fed throughout the study prior to the day of sampling. This suggests a lack of entrainment of cortisol release to feeding. As such, it can be concluded that the increase in plasma cortisol observed 10 min following the morning feeding might be a physiological result of feeding itself or possibly a result of stress associated with competitive feeding activity, but not a result of anticipated feeding.

Boujard and Leatherland (1992) have suggested that both photoperiod- and feeding-entrained rhythms might be present in fish, one superimposed on the other. This is supported by mammalian literature in which postprandial increases in cortisol are overlaid on a circadian rhythm (Follenius et al., 1982; Shiraishi et al., 1984; Honma et al., 1983, 1984; Saito et al., 1989). Although an increase in plasma cortisol levels during the scotophase, similar to that reported for other teleost fishes, was observed in the present study, definitive conclusions regarding a circadian cortisol rhythm in channel catfish cannot be made since sampling was only conducted every 2 h and during a single nychthemeron.

5. Conclusion

Understanding the regulation of hormone release in fish is important for studies of the endocrine control of physiological traits. In culture, channel catfish are often subjected to periods of restricted feeding and fasting (Hawke et al., 1998; Robinson and Li, 1999) and recent studies have sought to identify the effects of fasting on endocrine, metabolic, and physiological parameters correlated to growth (Peterson and Small, 2004; Small and Peterson, 2005). Studies of the type reported here illustrate the importance of feeding status and sampling time on circulating hormone levels. Temporal differences in circulating hormone levels, whether feeding-entrained or the result of putative circadian rhythms may have major implications on the interpretation of results obtained from single time-point measurements. Further experimental studies with channel catfish should determine whether or not hormone release profiles are altered by the time of daily feeding and the level and frequency of feeding.

Acknowledgments

The authors wish to thank Priscilla Barger and Kira Johnson for their technical assistance. 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


Honma, K.-T., Honma, S., Hiroshige, T., 1983. Critical role of food state, and growth hormone levels in rainbow trout (Oncorhynchus mykiss). Enocrinol. 136, 446–452.

Farbridge, K.J., Leatherland, J.F., 1992. Temporal changes in plasma thyroid hormone, growth hormone and free fatty acid concentrations, and hepatic 5-monodeiodinase activity, lipid and protein content during fasting and re-feeding in rainbow trout (Oncorhynchus mykiss). Fish Physiol. Biochem. 10, 245–258.


