Comparative Biochemistry and Physiology, Part A 166 (2013) 215–221

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Effects of dietary arginine on endocrine growth factors of channel catfish, *Ictalurus punctatus*

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ARTICLE INFO

Article history:
Received 9 April 2013
Received in revised form 12 June 2013
Accepted 17 June 2013
Available online 21 June 2013

Keywords:
Arginine
Channel catfish
Growth hormone
IGF-I
Thyroxine
Triiodothyronine

ABSTRACT

Thyroid (TH) and growth (GH) hormones, and insulin-like growth factor I (IGF-I) are anabolic regulators in fish and responsive to nutrient intake. A study was conducted to determine if previously reported growth effects of dietary arginine (ARG) in channel catfish were related to the activation of endocrine axes. In a first experiment, catfish were fed incremental levels of ARG (0.5 – 4% of diet) for 6 weeks and sampled at 2-week intervals. In a second experiment, fasted (48 h) fish were fed a single ration of ARG (0.5 or 4% of diet) and sampled at various intervals (0 to 72 h postprandial, PP). Experiment 1 did not reveal any influence of ARG on circulating TH, GH, or IGF-I despite the significantly increased growth of fish fed ARG-enriched diets. In experiment 2, feeding the 4% ARG diet significantly increased the amplitude of pulsatile plasma GH levels and also significantly increased IGF-I mRNA in liver and muscle, (at 2 h PP) and plasma IGF-I levels (at 6 h PP). Although relatively infrequent sampling failed to reveal alterations in TH or GH levels in response to ARG-induced growth activation, PP high frequency sampling unveiled high amplitude pulsatile GH secretions and may be important in activating IGF production in target tissues. Additionally, expressed and secreted IGF-I exhibited discernible patterns which closely correlate with ARG-induced growth effects in catfish.

1. Introduction

Promoting growth rates and improving efficiency of feed utilization are the two most economically important priorities in aquaculture. Hence, biotechnological approaches that help grow fish more efficiently can reduce production costs and improve the economic potential of aquaculture (Naylor et al., 2009). Arginine (ARG) is an essential amino acid for young animals experiencing rapid growth (Wu et al., 2009). It is the most abundant nitrogen carrier in tissue proteins and is used in multiple synthetic pathways, involving key enzymes such as arginase, nitric oxide synthase, arginyl-tRNA synthetase, among others (Morris, 2006; Wu et al., 2009). As such, ARG serves as a precursor for the synthesis of creatine, ornithine, proline, glutamate, polyamines, and nitric oxide and displays remarkable metabolic and regulatory versatility in cells (Wu et al., 2004; Morris, 2006; Yao et al., 2008). In addition to the critical roles these molecules play in reproduction and development, the growth-promoting effects of ARG supplementation can in part be ascribed to its ability to activate production of hormones that promote an efficient nutrient utilization (Kim et al., 2004; Collier et al., 2005; Yao et al., 2008; Wu, 2009; Wu et al., 2009).

Pituitary (growth hormone, GH), thyroid (triiodothyronine, T_3 and tetraiodothyronine, T_4) and pancreatic hormones (insulin and glucagon) all have been implicated in the normal growth of fish (Reindl and Sheridan, 2012) and in an increased uptake of amino acids into muscle protein (Matty, 1986). The regulation of GH, T_3 and T_4 axes in fish involves multifactorial controls (MacKenzie et al., 1993; Riley et al., 1996; Gaylord et al., 2001; Peterson and Small, 2005; Peterson et al., 2009). Food intake and quality of feed, including amino acid profile, have a direct influence on growth-related hormone dynamics in several fish species (Matty, 1986) including red drum, *Sciaenops ocellatus* (MacKenzie et al., 1993), gilthead sea bream, *Sparus aurata* (Gómez-Requeni et al., 2003), rainbow trout, *Oncorhynchus mykiss* (Riley et al., 1996), chinook salmon, *Oncorhynchus tshawytscha* (Pierce et al., 2005), tilapia, *Oreochromis mossambicus* (Fox et al., 2010) and channel catfish, *Ictalurus punctatus* (Gaylord et al., 2001; Peterson et al., 2009). Some studies have reported a direct secretagogue effect of ARG on fish hormones, but these have mainly focused on its insulinotropic potential. For instance, after intramuscular injection of ARG, barfin flounder (*Verasper moseri*) produced higher levels of plasma insulin than after glucose injection (Andoh, 2007). Similar results were found in rainbow trout, where intraperitoneally (IP) injected ARG increased plasma levels of glucagon, glucagon-like

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http://dx.doi.org/10.1016/j.cbpa.2013.06.016

peptide-1 and insulin (Mommsen et al., 2001). In addition, increased circulating insulin levels have been observed in coho salmon, *Oncorhynchus kisutch* (Plisetskaya et al., 1991), brown trout, *Salmo trutta*, common carp, *Cyprinus carpio* (Baños et al., 1997), and largemouth bass, *Micropetrus salmoides* (Sink and Lochmann, 2007), upon IP- or intragastric-ARG administration. Moreover, in coho and chinook salmon as well as in rainbow trout, ARG supplementation in the diet (2–3%) caused increased plasma insulin levels, although a stimulatory effect on growth was observed only in rainbow trout (Plisetskaya et al., 1991). Finally, in the latter fish, diets deficient in ARG depressed thyroidal status; whereas, ARG supplementation slightly increased T_3 and T_4 plasma levels (Riley et al., 1996).

Previous studies in our laboratory have indicated that dietary ARG supplementation can enhance growth of channel catfish – the most intensively cultured teleost species in North America (Buentello and Gatlin, 2000). In our most recent experiment, fish fed two to four times the previously established dietary ARG requirement of 1% (Robinson et al., 1981) had remarkable increases in weight gain (30–50%) and 11% more protein deposition in carcass as compared to fish fed the ARG requirement (Pohlenz et al., 2012). To determine whether the growth-promoting effects of ARG supplementation were in part due to activation of the GH-insulin-like growth factor (IGF) and/or thyroid axes, we undertook a series of studies to examine the effects of acute and prolonged dietary ARG supplementation on circulating hormone levels and tissue IGF-I gene expression in channel catfish.

2. Materials and methods

2.1. Experimental diets

Experimental diets were prepared as previously described (Table 1, Pohlenz et al., 2012). All diets were formulated to contain 26% crude protein from casein, gelatin and crystalline L-amino acids premix, and 8% lipid from both corn and menhaden oil. These diets provided 0.5, 1, 2 or 4% of ARG (dry weight), by supplementing ARG (USB, Cleveland, OH, USA). Based on the previously established minimum dietary requirement for channel catfish (Robinson et al., 1981) the control diet provided 1% ARG. Diets were maintained isonitrogenous by adjusting the levels of a 50:50 glycine–aspartate mix. Composition of diets was confirmed for proximate analysis (AOAC, 2005) and ARG levels (HPLC, Buentello and Gatlin, 2000).

Table 1
Formulation and analyzed proximal composition of experimental diets (g/100 g dry mass).

Ingredient	Dietary ARG(%)			
	0.5	1.0 control	2.0	4.0
Casein[a]	10.4	10.4	10.4	10.4
Gelatin[a]	2.7	2.7	2.7	2.7
AA mix[a, b]	7.1	7.10	7.1	7.1
Dextrin[a]	25.4	25.4	25.4	25.4
Celufil[a]	28.0	28.6	29.8	32.3
Corn oil[c]	4.0	4.0	4.0	4.0
Menhaden oil[c]	4.0	4.0	4.0	4.0
Vitamin Premix[d]	3.0	3.0	3.0	3.0
Mineral premix[e]	4.0	4.0	4.0	4.0
$Ca(PO_4)$[f]	1.0	1.0	1.0	1.0
Carboxymethyl cellulose[a]	2.2	2.2	2.2	2.2
Asp/Gly mix[a, b]	8.2	7.1	4.9	0.2
L-ARG-HCl[a]	0.0	0.5	1.5	3.6
ARG in diet[g] (%)	0.5	1.0	2.0	4.0
Dry Matter (%)	92.9	93.8	92.2	93.4
Crude Protein (%)	24.7	25.0	25.0	25.1
Lipid (%)	8.3	8.3	8.5	8.4
Ash (%)	3.4	3.4	3.4	3.4

[a] USB, Cleveland, OH, USA.
[b] As Buentello and Gatlin (2000).
[c] Omega Protein, Reedville,VA, USA.
[d] As Moon and Gatlin (1991).
[e] As Moon and Gatlin (1991), prepared by MP Biomedicals, Solon, OH, USA.
[f] Fisher Scientific, Waltham, MA, USA.
[g] Analyzed by HPLC (Buentello and Gatlin, 2000).

2.2. Experimental fish and trial conditions

Juvenile catfish were held in 110-L glass aquaria connected to a common recirculating system equipped with biological and particulate filtration. A constant flow of 1 L/min was maintained in all tanks. Oxygen saturation and water temperature were maintained above 90% and at 27 ± 1 °C, respectively. A 12:12 h light:dark cycle was provided via fluorescent lamps regulated with a timer. Water quality was monitored every other day for pH, hardness, alkalinity, nitrite, ammonia, temperature and dissolved oxygen and kept within optimal levels for catfish culture. Feeding rate was set at a level approaching satiation (~4% of biomass) provided in two daily feedings (morning and evening). Fish were weighed once a week and the ration was adjusted accordingly. Procedures used in this study were approved by the Texas A&M University System Animal Care and Use Committee (AUP # 2007-188).

2.3. Experiment 1. Six-week feeding trial – effects of graded levels of arginine on growth performance and endocrine factors

One hundred and eighty juvenile catfish, with an average initial weight of 22.9 ± 0.5 g were placed into 12, 110-L aquaria, at a density of 15 fish per aquarium. Acclimation took place over a 2-week period, during which fish were fed the control diet. After the acclimation period, each diet was randomly assigned to triplicate aquaria. At 2, 4 and 6 weeks after feeding the experimental diets, three fish per tank were randomly selected for blood sample collection. Blood samples (~1 mL) were obtained from the caudal vasculature with heparinized needles 16 h post-feeding. Blood plasma was separated by centrifugation at 6000 *g* for 12 min. Tissue samples of liver (2 g) and muscle (2 g left side, dorsal) also were collected on week 6. Plasma and tissue samples were quickly frozen in liquid nitrogen and kept at −80 °C until analysis. In addition, whole-body crude protein concentration was evaluated on week 0 and 6 samples by determining total nitrogen by the Dumas method (AOAC, 2005). Standard performance indicators including absolute weight gain, protein retention and survival were computed on week 6 for fish fed each experimental diet. Prior to all sample collections, fish were euthanized with a tricainemethane sulphonate (Tricaine-S, Western Chemical Inc., Ferndale, WA, USA) overdose (300 mg/L).

2.4. Experiment 2. Effects of bolus dose of dietary arginine on endocrine factors

Two hundred and eight juvenile channel catfish with an average mass of 55 ± 2 g/fish were randomly assigned to 26, 40-L tanks (eight fish/aquarium) and were maintained as described in Section 2.2. Fish were acclimated for 2 weeks to the experimental conditions and fed a commercial extruded diet (Rangen Inc., Angelton, TX, USA; crude protein 40%, lipid 10%, ash 13.2% and dry matter 90%). After the conditioning period, fish in all tanks were fed the control diet from experiment 1 for an additional week, at which time plasma ARG levels were at a homogenous low level of ~80 nmol/mL. This level was within the range of previously reported plasma ARG levels for channel catfish fed a similar diet (Buentello and Gatlin, 2000). Then all fish were fasted for 48 h and a single ration of two experimental diets was provided at 4% of the fishes' body weight. For this experiment, only the low (0.5% ARG) and high (4% ARG) diets were provided. Each of the two diets was fed to fish held in thirteen individual aquaria resulting in eight replicate fish per sampling period (0, 1, 2, 4, 6, 8, 12, 16, 24, 36, 48, 60 and 72 h postprandial, PP). Samples of blood, liver and muscle from euthanized fish were

collected for analysis of plasma ARG and hormonal protein and tissue mRNA concentrations as described in Section 2.5.

2.5. Blood and gene expression analysis

Plasma ARG was analyzed via HPLC following a fluorometric technique (Buentello and Gatlin, 2000) that incorporates pre-column derivatization with o-phthaldialdehyde (Sigma, St. Louis, MO, USA). Total T_3 and T_4, were analyzed by a radioimmunoassay technique as previously described (Loter et al., 2007) with modifications described by Cohn et al. (2010). The assay's sensitivity was 0.1 ng/mL. Intra-assay coefficient of variation (CV) was <7% for both hormones; whereas, the inter-assay CV was <12% and 19% for T_3 and T_4, respectively. Thyroid hormone samples were run in duplicate.

Growth hormone in plasma was assessed using an enzyme-linked immunosorbent assay (ELISA) as described by Drennon et al. (2003). Briefly, immunoplates (Costar, Acton, MA, USA) were coated with ligand in carbonate buffer and incubated overnight (4 °C). After addition of normal goat serum (NGS, Sigma) plates were further incubated overnight. Plasma samples were diluted 1:4 with assay buffer and mixed with NGS and primary antibody (rabbit anti-catfish GH). The assay's sensitivity under these conditions was 0.2 ng/mL and the intra- and inter-assay CVs were <8% and <11%, respectively. Growth hormone samples were run in triplicate.

Plasma IGF-I was analyzed using a competitive time-resolved fluoroimmunoassay validated for channel catfish (Small and Peterson, 2005). The assay was conducted in Reacti-Bind™ goat anti-rabbit coated clear 96-well plates (Pierce, Rockford, IL, USA). Plasma samples were acid-ethanol-extracted prior to analysis. The IGF-I assay sensitivity was 0.2 ng/mL and intra- and inter-assay CVs were <7 and <12%, respectively. Serial dilutions of plasma were parallel to the standard curve and recovery of IGF-I from spiked plasma samples was >90%. Plasma IGF-I sample analyses were run in triplicate.

IGF-I mRNA in skeletal muscle and liver tissues were measured with the technique described by Peterson et al. (2009). Quantitative real-time polymerase chain reaction (qPCR) was performed using the iCycler iQ RT-PCR detection system (BioRad, Hercules, CA, USA) to quantify IGF-I and ribosomal 18S subunit mRNA. All amplifications were performed in triplicate, and specific quantities of IGF-I mRNA were normalized against the amount of 18S amplified.

2.6. Statistical analysis

Data from all experiments were evaluated for normality using Shapiro–Wilk test. Results from experiment 1 were analyzed via one-way analysis of variance using Duncan's multiple-range test for mean separation. All data from experiment 2, with the exception of the pulsatile data for GH, were subjected to Student's t-test. The pulsatile data for GH was analyzed using the Kruskal–Wallis test to determine if there were significant changes within treatment groups over time. If significance was found, the Mann–Whitney U-test was then utilized as a post-hoc evaluation to determine which time points had significant changes in GH levels. Analyses were conducted using the Statistical Analysis System (SAS 9.2, 2012, SAS Institute Inc., Cary, NC, USA) or the Statistical Package for the Social Sciences (SPSS 20, 2011, IBM Corporation, Armonk, NY, USA) software. A level of $P \leq 0.05$ was taken to indicate statistical significance.

3. Results

3.1. Experiment 1. Six-week feeding trial – effects of graded levels of arginine on growth performance and endocrine factors

Increasing levels of ARG in the diet resulted in significant effects on weight gain and protein retention (Fig. 1) after 6 weeks of feeding. Fish fed 2 and 4% ARG diet had significantly ($P < 0.0001$) higher

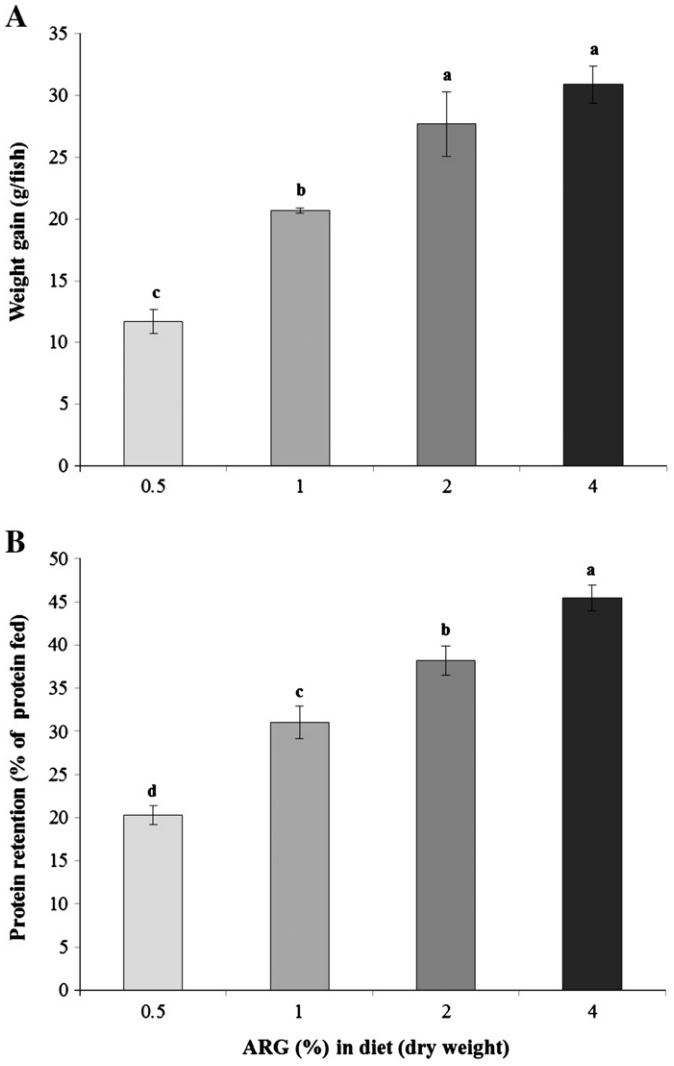

Fig. 1. Specific performance indicators of juvenile channel catfish fed graded amounts of ARG for 6 weeks (from Pohlenz et al., 2012). A, absolute weight gain (final mean body weight – initial mean body weight); B, protein retention ([final whole-body protein – initial whole-body protein]/dry protein fed × 100). Bars represent means ± standard error (n = 3). Letters above bars indicate significant differences ($P < 0.05$) among treatments as evaluated by Duncan's multiple range test.

weight gain than fish fed the 1% ARG diet (previously determined requirement, Robinson et al., 1981) and deficient diet (0.5% ARG). Also, protein retention was significantly ($P < 0.0001$) higher – 23 and 46% for fish fed the 2 and 4% ARG diets, respectively – as compared with fish fed the control diet. No statistical differences were found in fish survival among treatments (data not shown).

At the second week of feeding, plasma ARG levels were significantly lower ($P < 0.005$) in fish fed the ARG-deficient diet as compared to fish fed the other three inclusion levels (Table 2). Interestingly, at week 4 and 6 fish fed the deficient diet had plasma ARG levels that were not different from those of fish fed the control diet. No significant ($P > 0.05$) differences were found at any sampling time for plasma GH, IGF-I, T_3, or T_4 levels among fish fed the various diets (Table 2). In addition, IGF-I mRNA levels in liver tissue were not significantly ($P > 0.05$) different in catfish fed the control, 2 and 4% ARG diets. However, fish fed the ARG-deficient diet had a significant (2.5-fold) increase from the control fish (Fig. 2). No differences were found in muscle IGF-1 mRNA levels among treatments (data not shown).

Table 2
Long-term plasma ARG and hormone levels of juvenile channel catfish fed diets with four different levels of ARG for 6 weeks.

	Sample time-point	Arginine level (% of diet)				*Pr* > F	Pooled std. error
		0.5	1.0	2.0	4.0		
ARG (nmol/mL)	2-week	75.7[a]	131.3[b]	164.5[b]	184.5[b]	0.005	16.75
	4-week	85.5[a]	139.3[ab]	172.3[b]	206.8[b]	0.019	16.17
	6-week	91.8[a]	124.6[ab]	156.1[b]	164.3[b]	0.015	10.21
GH (ng/mL)	2-week	17.1	19.2	28.3	20.0	0.21	3.56
	4-week	24.9	27.7	29.7	27.4	0.35	1.76
	6-week	26.3	19.3	22.2	22.2	0.23	2.15
T_3 (ng/mL)	2-week	2.2	1.8	2.9	1.7	0.62	0.82
	4-week	3.3	3.2	2.1	2.8	0.64	0.70
	6-week	1.1	1.0	0.7	1.2	0.66	0.35
T_4 (ng/mL)	2-week	1.0	1.4	0.9	1.2	0.67	0.28
	4-week	3.2	9.8	7.2	9.0	0.14	1.89
	6-week	1.6	1.8	1.7	1.8	0.20	0.24
IGF-I (ng/mL)	6-week	9.0	10.9	7.8	11.7	0.39	1.65

Values represent means of three replicate tanks. Different superscript letters within a row indicate differences ($P < 0.05$) as evaluated by Duncan's multiple range test.

3.2. Experiment 2. Effects of bolus dose of dietary arginine on endocrine factors

Plasma ARG levels of fasted catfish experienced a striking increase upon feeding a single ration of the 4% ARG diet as early as 1 h PP (Fig. 3-A). This level was 4-fold higher than that of fish fed 0.5% ARG (dashed line), but the difference increased to 18-fold at peak plasma ARG levels (4 h PP) when comparing fish fed the 0.5 and 4% ARG diets. The plasma ARG concentration in fish fed a single ration of the 4% ARG diet progressively decreased until reaching basal levels at 72 h PP, maintaining, however, a significantly higher concentration than those fed the 0.5% ARG up to 48 h PP (Fig. 3-A). ELISA analyses (Fig. 3-B) did not detect significant differences in circulating GH over 72 h when the meal contained 0.5% ARG. In contrast, significant, high-amplitude oscillations in GH were observed over the first 36 h following a single meal containing 4% ARG. Fish fed this diet exhibited pulsating peaks in GH at 4, 8, 16, and 36 h PP, in each case significantly exceeding the GH level in the immediately previous sampling time. Interestingly, plasma IGF-I in fish fed the ARG 4% diet was significantly ($P < 0.05$) higher at 6 and 72 h PP than circulating IGF-I levels in fish fed the 0.5% ARG (Fig. 3-C). For fish fed the ARG 4% diet the 6 h plasma IGF-I peak was preceded by a significantly increased IGF-I expression in liver (Fig. 3-D) and muscle tissue (Fig. 3-E) at 2 h PP; 8- and 7-fold, respectively, from IGF-I expression in fish fed the 0.5% ARG diet. Finally, T_4 and T_3 plasma levels displayed significant differences at only one sampling interval each, when comparing fish fed the 0.5% or 4% ARG diets: at 1 h PP for T_4 and 24 h PP for T_3; both thyroid hormones being higher in fish fed the 4% ARG diet (data not shown).

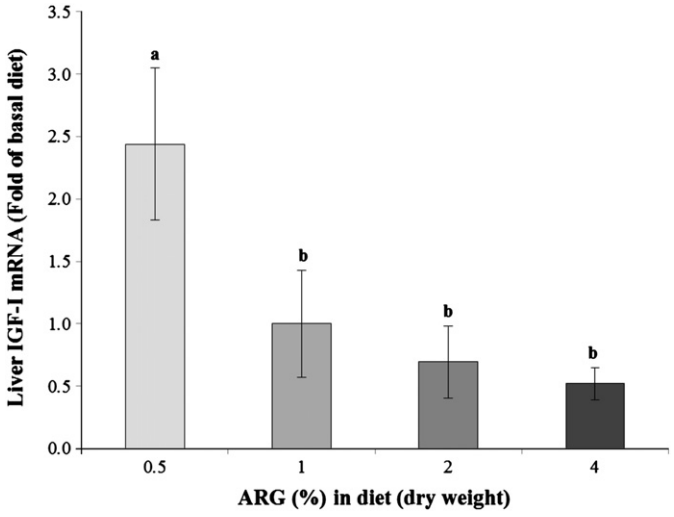

Fig. 2. Liver IGF-I mRNA levels at week 6 in juvenile channel catfish fed graded levels of ARG and sampled 16 h PP. Results are expressed as fold inductions compared to the control group (1% ARG). Bars represent means ± standard error (n = 6). Letters above bars indicate differences ($P < 0.05$) among treatments as evaluated by Duncan's multiple range test.

4. Discussion

This study provided a good opportunity to test the hypothesis that dietary ARG activates somatotropic axes in fish. It also highlighted the potential of a series of metabolic assays for evaluating somatotropic effects brought about by specific nutrients. In addition, this study helped clarify some of the mechanisms by which amino acids may potentially modulate the endocrine function in fish. Originally, we had hypothesized that ARG's remarkable effects on fish growth and productive parameters (reported in Pohlenz et al., 2012) were due in part to its possible actions as a secretagogue, up-regulating the biosynthesis and/or secretion of pituitary GH or thyroid hormones. This up-regulation could then activate specific peripheral responses that promote nutrient uptake and utilization, including thyroid hormones and/or IGF production.

Results of this study indicate that supplementation with dietary ARG – beyond the established minimum requirement – increases whole-body protein retention and improves weight gain in catfish, further highlighting the importance of this amino acid in growth regulation. These results are in agreement with previous reports for other vertebrates including swine (Kim et al., 2004; Yao et al., 2008) and rats (Cui et al., 1999), as well as with reports for fish species involving rainbow trout (Plisetskaya et al., 1991; Riley et al., 1996; Fournier et al., 2002), pacu, *Piaractus mesopotamicus* (Tesser et al., 2005), turbot, *Psetta maxima* (Fournier et al., 2002), gilthead sea bream (Fournier et al., 2002) and European seabass, *Dicentrarchus labrax* (Fournier et al., 2002; Tulli et al., 2007), but not for coho salmon (Plisetskaya et al., 1991). A more in-depth discussion on growth-related parameters and ARG supplementation in channel catfish is reviewed in Pohlenz et al. (2012).

To further elucidate ARG's growth-promoting action, the potential effects of this amino acid as activator of somatotropic and thyroid axes were evaluated. Whereas some evidence exists linking thyroid hormones levels to growth rates in fish, including catfish (Gaylord et al., 2001; Loter et al., 2007), the biological significance of ARG's effects on the thyroid axis found in the present experiments seems to be marginal, which is in line with findings in rainbow trout where no consistent increase on these hormones were found with increasing dietary ARG, despite obvious growth enhancement (Riley et al., 1996). Likewise, despite having documented significant increases in weight gain with increasing levels of dietary ARG in catfish (Experiment 1), we observed no significant differences in circulating GH or IGF-1. Similarly, no effects were recorded for muscle IGF-1 expression, not even when contrasting the fastest and slowest growing fish at 2, 4, and 6 weeks. Significantly, the IGF-1 mRNA levels found in liver tissue were highest for fish fed the ARG-deficient diet. Although these results disagree with those found for higher vertebrates (Collier et al., 2005; Wu et al., 2009), fish studies have similarly failed to detect clear correlations between growth rate and GH (reviewed in Canosa et al., 2007). Given the established somatotropic action of exogenous GH in fish, including catfish (Silverstein et al., 2000; Peterson et al., 2007), it is surprising that there is little experimental evidence to support increased circulating GH in rapidly-growing fish (Canosa et al., 2007). However, studies in mammalian species have shown that GH can be released from the pituitary in discrete pulses, and evidence exists for similar episodic releases in fish (Zhang et al., 1994; Benedet et al., 2010).

In mammals, the amplitude of circulating GH pulses, but not their inter-peak intervals, best reflects the rate of somatic growth and increased circulating IGF-I (Butler and Le Roith, 2001). In spite of this

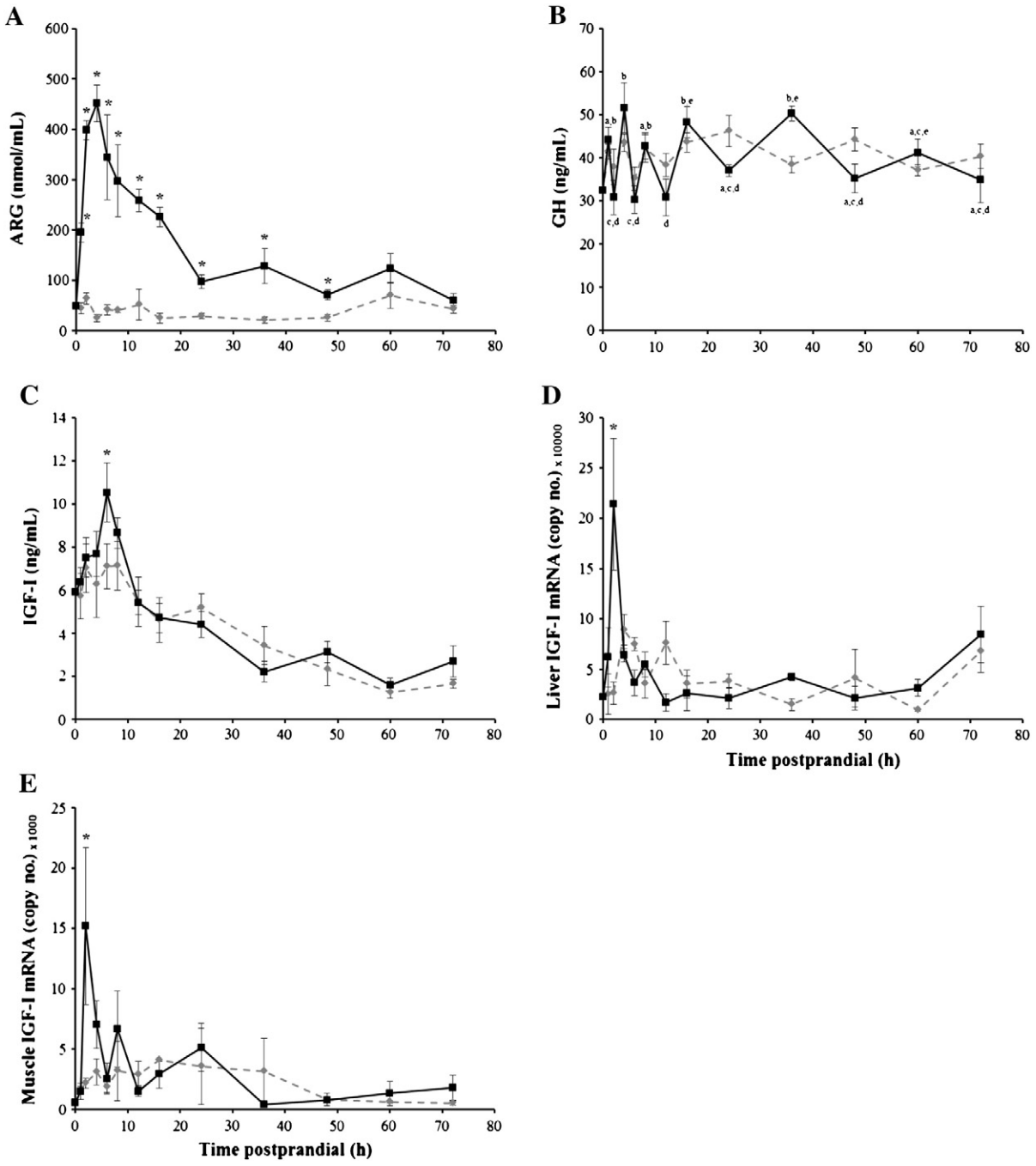

Fig. 3. Postprandial plasma levels in juvenile channel catfish throughout a 72 h period (x axis) after feeding a single ration (4% of biomass) of either ARG-deficient (0.5%, dashed line) or ARG-supplemented (4%, continuous line) diets. A, plasma ARG concentrations (nmol/mL); B, plasma GH concentrations (ng/mL), C, plasma IGF-I concentrations (ng/mL); D, liver IGF-I mRNA concentrations (copy number); E, muscle IGF-I mRNA concentrations (copy number). Markers represent means ± standard error (n = 8). Asterisk above markers (A, C, D, E) indicate significant ($P < 0.05$) differences between treatments at that time point as evaluated by Student's *t*-test. Letters above markers (B) indicate significant ($P < 0.05$) differences within time points of fish fed 4% ARG as evaluated by Mann–Whitney U-test.

observation, no previous studies have examined the impact of diet composition on the amplitude of GH pulses in fish. Periodical plasma sampling conducted for an extended period of time has revealed diurnal patterns of GH release for salmonids (Gomez et al., 1996; Björnsson et al., 2002), goldfish (Marchant and Peter, 1986) and catfish (Small and Peterson, 2005) but these GH releases were not established as pulsatile. Asynchronous, episodic levels of circulating GH have been found in cannulated grass carp, *Ctenopharyngodon idellus* (Zhang et al., 1994) and rainbow trout with accompanying levels of IGF delayed by 1.5 h in trout (Niu et al., 1993). However, pulsatile release within individual fish could be masked by asynchronous release of GH among individuals averaged together in a treatment group.

To our knowledge, this is the first study with teleost fish to demonstrate that the amplitude of GH pulses may correlate with specific nutrient intake or IGF production. This gap in information is likely due to the difficulty in cannulating fish (Canosa et al., 2007). In channel catfish, a two fold increase in both GH and IGF-I may occur within a 2 h period and the magnitude of this increase can be amplified by alterations in diet, such as feed restriction (Small and Peterson, 2005).

Under these circumstances, measurement of circulating hormone levels at a single time point after 6 weeks may not accurately reflect the temporal changes induced in episodic GH secretion by dietary manipulation. Therefore, we also tested a different strategy in which fish were fasted for 48 h, fed a single meal of either high or low ARG content, and then sampled with high frequency over a 72 h period following feeding. Interestingly, this high-resolution sampling was able to distinguish a clear episodic GH pattern in the blood, reflecting a pulsatile GH secretion, with inter-peak times of about 4 h, with high plasma ARG levels observed in fish fed the 4% ARG diet. There were no significant changes of GH within the low-ARG fed group at the times sampled, suggesting that dietary ARG supplementation (hence high peaks of plasma ARG) can impact GH secretion by increasing the amplitude of the pulse generator in briefly-fasted animals. Most interestingly, there was a significant increase in the tissue IGF-I mRNA and in the circulating IGF-I observed only in the animals exhibiting the high magnitude GH pulses, suggesting IGF-I may be dependent on the GH pulse amplitude, as opposed to dependency on a single high peak. Previous fish studies of GH or IGF-I response to feeding and specific dietary constituents, including our first experiment, may have missed such results because of relatively infrequent sampling protocols (normally just once after a prolonged treatment, Gómez-Requeni et al., 2004; Pierce et al., 2001).

Results on ARG clearance from fish plasma deserve special consideration. A 50% decrease from maximum values of plasma ARG – which occurred 4 h PP – was observed in ARG-fed fish at 8 h PP. In contrast with higher vertebrates, ARG appears to slowly surge in fish and also takes longer to be cleared from circulation (Wu et al., 2007). A relatively shorter half-life of ARG has been established between 1 and 2 h after an oral dose for several mammalian species (Böger and Bode-Böger, 2001; Wu et al., 2007). Although for catfish ARG residence time in circulation proved longer than for mammalian species, similarly to these higher vertebrates, circulating ARG eventually declined (Fig. 3-A) if fish were not continually fed. Therefore, it is plausible that ARG, through secondary metabolism, may also affect non-endocrine pathways upon supplementation and thus influence fish growth. In this sense, the reported occurrence of high arginase activity in several fish tissues (Felskie et al., 1998; Wright et al., 2004) including catfish, may also funnel the conversion of ARG into anabolically-important metabolites (Wilson and Poe, 1974; Mommsen et al., 2001), such as polyamines, and thus elicit more potent growth responses (Li et al., 2009; Wu, 2010). Previous research in our laboratory has reported higher plasma concentrations of ornithine – a key precursor for polyamine synthesis – in fish fed supplemented levels of ARG (Buentello and Gatlin, 2000; Pohlenz et al., 2012). In addition, Arndt et al. (1994), found a strong correlation between ornithine decarboxylase – a key enzyme in polyamine synthesis from ARG-derived ornithine – and specific growth rate in Atlantic salmon, *Salmo salar*, further supporting the growth findings in the present study after a 6-week feeding trial. However, further research is necessary to more fully evaluate this notion.

In conclusion, despite significant increases in growth rates for fish fed ARG-supplemented diets in a 6-week feeding trial, no clear effects were identified on circulating GH or thyroid axes. However, after a single bolus feeding of ARG, GH and IGF-I responses were discernible with high frequency sampling. Thus, it appears that single measurements of blood concentrations of these hormones may not be ideally suited to describe the relationship between dietary ARG and growth in catfish. Interestingly, results from these studies do indicate that IGF-I, measured as circulating levels or mRNA expression, may be more useful indicators of an ARG-induced acute activation of the somatotropic axis in fish. This effect may be localized at the IGF-I level, and not provide activation to the entire axis; although the observed changes in GH pulsatility may suggest otherwise. Finally, it is also possible that the important role of ARG in energy compartmentalization and nitrogen metabolism may affect other metabolic pathways (e.g., up-regulation of polyamine biosynthesis) upon ARG supplementation and thus influence fish growth through non-endocrine mechanisms. However, more research is necessary to elucidate the full extent of ARG's influence in the somatic growth of channel catfish.

Acknowledgements

This study was supported in part by the TAMU-CONACYT Collaborative Research Grant Program (Buentello, MacKenzie and Tovar, Co-PIs). Funding for Camilo Pohlenz was provided in part by Consejo Nacional de Ciencia y Tecnología (CONACyT-México), Secretaría de Educación Pública (SEP, Mexican Government) and the National Research Council of Norway through the Aquaculture Protein Centre, CoE, Norway.

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