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Quality improvement of fish feed by using insulin-like growth factor 1 as a potential indicator of amino acid deficiency in yellowtail

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ABSTRACT

We developed a new method for improving the quality of fish feed, by using insulin-like growth factor 1 (IGF1) as a potential indicator of dietary amino acid deficiency in yellowtail (*Seriola quinqueradiata*). First, 7 dispensable amino acid solutions (alanine, asparagine, cysteine, glutamine, glycine, serine and proline) and 0.9% NaCl (control) solution were injected into the dorsal muscle of fish using a syringe. Twenty-four hours post-administration, white muscle tissue was collected from the fish opposite to the injection site and assayed for *igf1* mRNA expression. Only fish injected with proline showed a significant increase in *igf1* mRNA expression levels. Subsequently, a feeding trial was conducted with 3 different diets—the control diet; the control diet supplemented with 1% proline; and the control diets supplemented with 3% proline. Juvenile yellowtails were fed exclusively with 1 of the 3 diets until apparent satiation. The body weight and feed efficiency were significantly higher in fish fed with the 3% proline-supplemented diet than fish fed with the control diet. Further, after 3 weeks, the expression levels of *igf1* in white muscle and liver were significantly higher in fish fed with the 3% proline-supplemented diet than in fish fed with the control diet. Our results suggest that *igf1* mRNA expression in the white muscle tissue of yellowtails serves as a reliable indicator of amino acid deficiency. Additionally, the results of our feeding trial suggest that proline is required for juvenile yellowtail growth. Moreover, white muscle *igf1* may represent a valuable tool for improving the quality of fish feed.

1. Introduction

The yellowtail *Seriola quinqueradiata* is an important aquaculture species in Japan, with an annual production of approximately 100,000 tons (Masumoto, 2002). However, sustaining this high level of yellowtail production is difficult, because of the large quantities of fish feed required. In the light of increasing global demand for the wild fish required to produce fishmeal, coupled with a decrease in the potential supply, this issue is of crucial importance (Hardy, 2010). Therefore, it is essential to improve the current aquaculture methods and systems, particularly those relating to fish feed and growth efficiency.

Amino acids are classified as nutritionally essential, i.e., indispensable amino acids (IDAAs), and nutritionally non-essential, i.e., dispensable amino acids (DAAs). Knowledge regarding the nutritional requirement of fish remains limited to a few species, and studies have focused mainly on IDAAs (Lall and Anderson, 2005; National Research Council, 1993; Tibaldi and Kaushik, 2005). The IDAA requirements of yellowtail have been determined for only 4 amino acids, namely, arginine (Ruchimat et al., 1998), histidine (Ruchimat, 1998b), lysine (Ruchimat et al., 1997a), and methionine (Ruchimat et al., 1997b). However, the amino acid requirements of fish – including yellowtail – remain unclear; moreover, there is a high possibility of certain DAAs being re-classified as IDAAs. For example, recent research has identified taurine (2-aminoethane sulfonic acid) – which is synthesized from cysteine – as an IDAA for yellowtail (Takagi et al., 2006, 2008). Similarly, hydroxyproline, which was previously considered to be a DAA, has recently been suggested as an IDAA for fish, with studies recommending that it be included in the feed for Atlantic salmon (*Salmo salar*) (Kousoulaki et al., 2009). Further, comparison of the amino acid composition of various diets used to replace dietary fish meal has identified taurine and hydroxyproline as important for the nutrition of some fish species. Hence, to improve fish growth and growth efficiency, the importance of amino acids and small nitrogen compounds in the fish diet must be elucidated.

Several previous reports have suggested that the growth hormone (GH) — insulin-like growth factor 1 (IGF1) axis, may be used a potential indicator of fish nutritional status. IGF1 plays a pivotal role in fish growth (Beckman, 2011; Picha et al., 2008). Further, nutritional status is an important factor in the regulation of IGF1 synthesis in fish. In barramundi, plasma IGF1 concentrations are closely related to dietary protein levels and growth (Dyer et al., 2004). Gayload et al. (2005) showed that plasma IGF1 concentration and protein retention efficiency in hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) increased in a dose-dependent manner according to dietary tryptophan levels. Banos

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et al. (1999) reported the positive regulation of plasma IGF1 in brown trout (*Salmo trutta*), by intraperitoneally-injected arginine. Chen et al. (2012) showed that the hepatic *igf1* mRNA expression in largemouth bass (*Micropterus salmoides*) increased with increasing dietary arginine levels. Hevrøy et al. (2007) demonstrated that hepatic *igf1* mRNA expression was significantly higher in Atlantic salmon fed with a diet containing high lysine levels than in Atlantic salmon fed with a diet containing low lysine levels.

We previously showed that *igf1* mRNA expression in the white muscle of yellowtail responded differently according to various feeding conditions, and may therefore be used as a reliable indicator of nutritional status (Fukada et al., 2012). White muscle *igf1* mRNA expression was measured in fish following the injection of amino acids. To confirm the igf1 response recorded in our first experiment, we subsequently performed a feeding experiment in which the fish diet was supplemented with an amino acid (proline) found to be lacking in the diet.

2. Materials and methods

2.1. Injection experiments

The injection experiments were performed according to Andoh (2007). Yellowtail (*S. quinqueradiata*) individuals (average weight = 87.1 g), aged <1 year old, were reared at an indoor aquaculture station (Konan, Kochi, Japan). Prior to the experiment, the fish were fed ad libitum with a commercial pellet diet (52.0% crude protein, 10.0% crude fat, Skretting Co. Ltd., Fukuoka, Japan) once per day, 6 days per week for 1 month. For the injection of salmon growth hormone (sGH), fish were transferred into 2 rectangular fiber-reinforced tanks (250-L capacity), with 5 fish per tank. Recombinant sGH (Kyowa Hakko Bio. Co., Tokyo, Japan) was dissolved in distilled water at a concentration of 1 mg/mL. After 24 h of fasting, the fish were injected intramuscularly with 0.9% NaCl solution (control) or 100 μL of sGH solution under anesthesia (0.1 mL/L of 2-phenoxyethanol). Twenty-four hours post injection, the fish were killed with an overdose (0.5 mL/L), and the white muscle was collected.

For the DAA injections, fish were transferred into 8 rectangular fiber-reinforced tanks (250-L capacity) with 7 fish per tank, and starved for 24 h. Aqueous solutions of 7 amino acids – L-alanine (Ala), L-asparagine (Asn), L-cysteine (Cys), L-glutamine (Gln), L-glycine (Gly), L-serine (Ser) and L-proline (Pro) – were obtained from Nacalai Tesque Inc., Kyoto, Japan. Each of the amino acids was dissolved in distilled water at a concentration of 175 mM, and was then injected intramuscularly at a dose of 10 mL/kg body weight (corresponding to 1.75 mmol/kg body weight). As a control, 0.9% NaCl solution was prepared and injected following the same procedure. Injections and sample collection followed the same method as described for the first experiment.

2.2. Real-time quantitative RT-PCR for yellowtail igf1

Yellowtail *igf1* mRNA was quantified according to the method of Fukada et al. (2012). Total RNA was extracted from the fish tissues by using RNAiso Plus (Takara Bio. Inc., Kyoto, Japan). RNA integrity was verified by using an optical density (OD) absorption ratio OD 260 nm/OD 280 nm > 1.8. For the measurement of hepatic *igf1* mRNA expression, 1.2 μg of total RNA was used to synthesize the first strand cDNA. Reverse transcription was performed in a reaction mixture containing 0.5 mM dNTPs, 112.5 ng of random hexamers (Promega, Madison, WI), 50 U of M-MLV reverse transcriptase (Promega), and 1× RT buffer in a final volume of 15 μL. For the measurement of *igf1* mRNA expression in white muscle, total RNA (1.8 μg) was used to synthesize the first strand cDNA. Reverse transcription was performed in a reaction mixture containing 0.5 mM dNTPs, 200 ng of random hexamers (Promega), 120 U of M-MLV reverse transcriptase (Promega), and 1× RT buffer in a final volume of 15 μL. Reverse transcription was performed for 10 min at 25 °C, followed by 60 min at 42 °C, and 5 min at 95 °C. The primers and probe used for the real-time quantitative RT-PCR of yellowtail *igf1* were designed by using the primer express program (Applied Biosystems Inc., Carlsbad, CA) as follows: forward primer, 5′-TTG TGT GTG GAG AGA GGC TTT-3′; reverse primer, 5′-GAA GCA GCA TTC GTC AAC AAT G-3′; and probe, 5′-FAM-ATT TCA GTA AAC CAA CAG GCT ATG GCC CC-TAMRA-3′. The forward primer spanned a predicted intron/exon boundary to avoid genomic DNA amplification.

For the normalization of data, the 18S rRNA gene was used. The 18S primers were derived from a consensus of fish 18S sequences (Tom et al., 2004). The forward primer was 5′-TAC CAC ATC CAA AGA AGG CA-3′ and the reverse primer was 5′-TCG ATC CCG AGA TCC AAC TA-3′. The 18S primer pair used in the present study was previously validated for yellowtail real-time quantitative RT-PCR assay by Murashita et al. (2006). The expression of 18S values (Ct values) did not vary significantly among treatments, in either the injection study or the feeding trial (Pedroso et al., 2009a). The PCR reaction for the *igf1* assay (20 μL) contained 10 μL of TaqMan Universal PCR Master Mix (Applied Biosystems Inc.), 2 μL of first strand cDNA, 1 μM of each of the forward and reverse primers, and 0.2 μM probe. The PCR for the 18S assay (20 μL) contained 10 μL of Power SYBR green PCR master mix (Applied Biosystems Inc.), 1 μM of each of the forward and reverse primers, and 2 μL of the first strand cDNA (1/100 dilution). The amplification and detection of samples were performed using the ABI 7300 system, under the following thermal cycling conditions: 50 °C for 2 min, 95 °C for 15 s, and 60 °C for 1 min (45 cycles). Four serial dilutions of sample cDNA were run to determine the PCR efficiency (E), which was calculated from the regression slope of the assay ($E = 10^{1/\text{slope}}$). The confirmed PCR efficiency (E) was close to the theoretical PCR efficiency (*igf1*, $E = 1.81$, $R^2 = 0.98$; 18S, $E = 1.98$, $R^2 = 0.99$). Steady-state yellowtail *Igf1* mRNA expressions were calculated relative to the 18S rRNA gene, following the method of Pfaffl (2001).

2.3. Experimental diets and feeding trial

Fish meal was used as the major source of dietary protein. Three diets were prepared: the control diet; the control diet supplemented with 1% proline; and the control diet supplemented with 3% proline (Table 1). Feed ingredients were mixed with fresh water (450 mL/kg), and then pelleted (diameter 6 mm), by using a laboratory pellet mill (Hiraga Kosakusho Co. Ltd., Hyogo, Japan). Diets were stored at −20 °C until being fed to the fish.

Yellowtail fish (mean initial weight 111.4 g) were randomly distributed into 9 circular fiber-reinforced tanks (500-L capacity) with 15 fish per tank (i.e., each experiment was repeated in triplicate). Prior to the experiment, the fish were fed ad libitum with a commercial pellet diet (50.0% crude protein, 12.0% crude fat: Skretting Co. Ltd.) once per day, 6 days per week for 2 weeks. Each tank was continuously supplied with filtered and aerated seawater. The experiment was conducted from October 15, 2010 to November 25, 2010. Throughout the trial, the water temperature was maintained at 23.1–27.0 °C, and the fish were hand-fed daily until apparent satiation. After 3 weeks and 6 weeks, all fish were weighed; 5 fish were then randomly selected from each tank, and killed with an overdose of 2-phenoxyethanol. Three fish were used to determine the body composition. All samples were stored at −80 °C until use.

2.4. Growth parameters

The specific growth rate (SGR), feed efficiency, daily feed intake, protein efficiency ratio, and survival rate were calculated by using the following formulas:

SGR (% per day) = (ln [final average body weight] − ln [initial average body weight])/days × 100.