Effects of dietary genistein on GH/IGF-I axis of Nile tilapia

Oreochromis niloticus

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Abstract There is considerable concern that isoflavones, such as genistein in fish feed composed of soybean protein, affects somatic growth in fish. Our previous works demonstrated that 30 and 300 μg/g dietary genistein had no significant effect on growth performance in Nile tilapia (Oreochromis niloticus), but the higher level of genistein (3 000 μg/g) significantly depressed growth. This study was conducted to further examine the effects of dietary genistein on the endocrine disruption on growth hormone/insulin-like growth factor-I (GH/IGF-I) axis in Nile tilapia (O. niloticus). Juvenile fish were fed by hand twice daily to satiation with one of four isonitrogenous and isoenergetic diets, each containing either 0, 30, 300 or 3 000 μg/g genistein. Following an 8-week feeding period, plasma GH and IGF-I levels were investigated by radioimmunoassay and gene expression levels of gh, ghrelin, gnrhs, ghr, npy, npyrs, pacap, ghrs, igf-I, igf-Ir, and igfbp3 were examined by real-time PCR. The results show that no significant change in plasma GH and IGF-I levels in fish fed with diets containing 30 μg/g and 300 μg/g genistein. mRNA expression of genes along the GH/IGF-I axis remained unaffected, except for igf-Ir, which was stimulated by the 300 μg/g genistein diet. While in fish fed the 3 000 μg/g genistein diet, the plasma GH and IGF-I levels decreased, and mRNA expression of gh, ghr2, npyr1, igf-I, and igf-Ir were also significantly depressed. In contrast, npy and igfbp3 mRNA expression were enhanced. This study provides convincing evidence for growth impediment by genistein by disturbing the GH/IGF-I axis in Nile tilapia O. niloticus.

Keyword: genistein; hormone/insulin-like growth factor-I (GH/IGF-I) axis; Nile tilapia; growth rate; aquaculture

1 INTRODUCTION

Marine fishes require approximately 30%–50% protein in their feed, most of which is usually provided by fish meal (NRC, 2011). In recent years the price of fish meal has greatly increased because of the limited supplies of fishery resources, thus, soybean meal has gradually become an important alternative ingredient for fish meal because of its favorable amino acid profile, high level of crude protein, and its relatively low price (Refstie et al., 2006; Lilleeng et al., 2007). At present, soybean meal is tentatively substituted for fish meal in some fish feed production. However, as a result of the relatively simple processing technology, soybean meal is rich in isoflavone, also known as phenolic compounds, which may have potential biological effects on fish. In fact, Ishibashi et al. (2002) reported that the total isoflavone content in commercial carp and trout feed was 810 μg/g and 80 μg/g, respectively. The two most abundant and most biologically active isoflavones found in soybeans are genistein and daidzein, whose contents in different soybean origins are approximately 619.1–2 367.9 and 291.5–600.8 μg/g, respectively (Tepavčević et al., 2010). While Mambrini et al. (1999) reported that the maximum content of genistein and daidzein in soybean protein concentrates were ~5 900 and 1 990 μg/g, respectively (Mambrini et al., 1999).

Thus, the effect of isoflavones on fish growth is a concern for the application of the soybean protein in aquatic feed. Moreover, there is little research on the

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effect of single isoflavones on fish growth. Preliminary results have shown that low concentrations of genistein had no significant effect on fish growth, but that high concentrations inhibit growth. Our previous work demonstrated that 30 and 300 μg/g dietary genistein had no significant effect on growth performance in Nile tilapia (O. niloticus), but the higher levels of genistein (3 000 μg/g) significantly reduced the final body weight and specific growth rate (Chen et al., 2015). It has been suggested that genistein may reduce fish growth partly by inhibiting digestion because the activity of major digestive enzymes, including stomach and hepatopancreas protease and amylase in the liver and intestine, were significantly reduced by 3 000 μg/g dietary genistein (Chen et al., 2015). Refstie et al. (2006) also reported that diets in which 24% of total protein came from extracted soybean meal reduced the digestion of both amino acid and lipid metabolism in Atlantic cod (Gadus morhua). However, the endocrine system, especially hormone/insulin-like growth factor-I (GH/IGF-I) axis, plays a crucial role in the regulation of fish growth. It has been demonstrated that dietary daidzein decreases the production of GH in tilapia (Oreochromis aureus) (Yu et al., 2006). At present, data on how isoflavone affects the GH/IGF-I system in fish is scarce. Therefore, this study was conducted to elucidate how genistein affects fish growth based on insights on the GH/IGF-I axis. Juvenile Nile tilapia (O. niloticus) were fed diet containing graded levels of genistein, and then investigated the plasma levels of GH and IGF-I, and determined the mRNA levels of gh, ghr, igf-l, igf-lr, igfbp3, and other regulatory genes along the GH/IGF-I axis.

2 MATERIAL AND METHOD

2.1 Experimental diets

The feed formulation and preparation process was according to that of Chen et al. (2015). In brief, fishmeal and fish oil were used as protein and lipid sources, respectively. Four isonitrogenous and isocaloric diets (diet 1, diet 2, diet 3, and diet 4) were formulated to contain graded levels of genistein (0, 30, 300, and 3 000 μg/g, respectively), which was purchased from Shenzhen Medherb Biotechnology Co. Ltd., Shenzhen, China. The ingredients were ground into a fine powder through a 320-μm mesh, and then thoroughly mixed with fish oil and water to produce stiff dough. The dough was pelletized with an experimental feed mill (F-26 (II), South China University of Technology, China) and dried for 24 h in a ventilated oven at 45°C. After drying, the diets were broken and sieved into proper pellet size (1.5 mm × 3.0 mm), and stored at -20°C.

2.2 Experimental procedures

Juvenile tilapia (O. niloticus) were purchased from the National Tilapia Seed Farm of Qingdao. After acclimating in laboratory rearing conditions and fed with the control diet (diet 1) for 2 weeks, the fish were fasted for 24 h, anesthetized in 75 mg/L MS-222 (Sigma, MO, USA), then weighed. Fish of similar sizes (initial weight 10.47±1.24 g) were then randomly distributed into 12 tanks (50-L capacity filled to 30 L), and each tank was stocked 15 juveniles. Each diet was randomly assigned to triplicate tanks. Fish were hand-fed to apparent satiation twice daily (09:30 and 16:00) for 8 weeks. The consumption of food in each cage was recorded. During the experimental period, rearing water temperature ranged from 26.0 to 30.0°C, dissolved oxygen was maintained at approximately 7 mg/L.

Following feeding for 8 weeks, tilapia were fasted for 24 h and anesthetized in 75 mg/L MS-222 (Sigma), and mean body weight was recorded before harvest. For this study, tissue samples were taken from eight fish that were randomly chosen from the 15 in each of three replicate tanks. The remaining fish were used for experiments reported in another study (Chen et al., 2015). Blood was taken from the caudal vein with chilled heparinized syringes. After centrifugation (1 000×g, 10 min), plasma was frozen in liquid nitrogen for GH and IGF-I measurement by radioimmunoassay. The pituitary, hypothalamus and liver were dissected, then frozen in liquid nitrogen and stored at -80°C for the quantification of gh, ghrelin, gnrhs, npy, npys, pacap, igf-l, ghrs, igf-lr, and igfbp3 mRNA by real-time PCR.

2.3 Radioimmunoassay

GH and IGF-I levels were detected using commercial radioimmunoassay (RIA) kits purchased from the Beijing North Institute of Biological Technology, China. Hormone levels were determined according to the manufacturer’s instructions. The RIA was based upon the competition of certain 125I labeled antigen and unlabeled antigen (standard or unknown) binding to the limited quantity of antibodies. The RIA kits for human GH and IGF-I were validated for use with tilapia samples by demonstrating parallelism between a series of diluted and spiked samples in relation to the standard curve.