Fish & Shellfish Immunology 57 (2016) 362–370

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

Role of dietary ginger *Zingiber officinale* in improving growth performances and immune functions of *Labeo rohita* fingerlings

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ARTICLE INFO

Article history:
Received 29 April 2016
Received in revised form
23 August 2016
Accepted 25 August 2016
Available online 26 August 2016

Keywords:
Ginger
Skin mucus
Growth performance
Cytokine genes
Aeromonas hydrophila

ABSTRACT

This study evaluated the effects of ginger (*Zingiber officinale*) as a feeding supplement on the growth, skin mucus immune parameters, and cytokine-related gene expression of *Labeo rohita,* and its susceptibility to *Aeromonas hydrophila* infection. Diets containing six different concentrations of dried ginger (0% [basal diet], 0.2% [G2], 0.4% [G4], 0.6% [G6], 0.8% [G8], and 1.0% [G10] were fed to fish (average weight: 12.3 g) for 60 days. Growth parameters were examined at 30 and 60 days post-feeding. Skin mucosal immune responses and gene expression were examined 60 days post-feeding. Results showed that growth parameters such as final weight gain (93.47 ± 1.73 g) and specific growth rate (3.41 ± 0.14) were significantly higher in G8 than in the control. Among the skin mucosal immune parameters examined, lysozyme (46.5 ± 3.8 U mg^{-1}), immunoglobulin level (8.9 ± 0.4 unit-mg mL^{-1}), protein level (44.3 ± 2.2 mg mL^{-1}) were significantly higher in G8. However, alkaline phosphatase activity (171.6 ± 10.2 IU L^{-1}) was high ($P < 0.05$) in the G10 group. Skin mucus of G8 exhibited significantly higher inhibition zones when tested against pathogenic bacterial strains. For cytokine-related genes, antioxidant genes (zinc/copper superoxide dismutase [SOD1], glutathione peroxidase [GPx], anti-inflammatory cytokines (interleukin-10 [IL-10], transforming growth factor-beta [TGF-β]), signalling molecules nuclear factor erythroid 2-related factor 2 [Nrf2], and Inhibitor protein κBα [IκB-α]) were all up-regulated in the head kidney, intestine, and hepatopancreas of fish that were fed experimental diets. In addition, expression abundance was significantly higher in most tissues in G2 and/or G10, than in the control. Conversely, expression of genes encoding pro-inflammatory cytokines (IL-1β, tumour necrosis factor-alpha [TNF-α]), signalling molecules Kelch-like-ECH-associated protein 1 (Keap1), and nuclear factor kappa B p65 (NF-κBp65) were down-regulated in treatment groups. Moreover, fish fed a 0.8% [G8] ginger supplemented diet exhibited significantly higher relative post-challenge survival (65.52%) against *Aeromonas hydrophila* infection. Collectively, these results suggest that dietary supplements of ginger (at 0.8%) can promote growth performance, skin mucus immune parameters, and strengthen immunity of *L. rohita*. Therefore, ginger represents a promising food additive for carps in aquaculture.

1. Introduction

Global aquaculture production is increasing and playing a vital role in boosting human nutrition, since it provides almost half of all fish consumed by the human population [1]. Besides its contribution to food security, the aquaculture industry is a source of income and employment in many countries [2]. Around 70% of Indian aquaculture is comprised of three major carp species *Catla catla*, *Cirrhinus mrigala*, and *Labeo rohita* [3]. Intensive aquaculture causes environmental stress to fish, which can increase susceptibility to various pathogens, including viruses, bacteria, fungi, and parasites [4]. Disease outbreaks cause either huge economical loss through mortality or lower profit margins [5]. Antibiotics, disinfectants, and therapeutants are commonly used to control diseases in aquaculture [6]. However, limitations to such products have arisen from public health concerns over evidence of food contamination, bacterial drug resistance, and negative effects on the environment [2,7]. Therefore, natural or eco-friendly therapeutic approaches need to be developed for the sustainability of aquaculture.

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http://dx.doi.org/10.1016/j.fsi.2016.08.056
1050-4648/

'An immunostimulant is a naturally occurring compound that modulates the immune system by increasing the host's resistance against diseases that in most circumstances are caused by pathogens' [8]. The immunostimulant plants or their by-products contain several phenolic, polyphenolic, alkaloid, quinone, terpenoid, lectin, and polypeptide compounds, many of which are effective alternatives to antibiotics, chemicals, vaccines, and other synthetic compounds. They also facilitate growth, anti-stress, environmental friendly and antimicrobial properties [9]. A wide range of medicinal plants show potential effects on growth and survival properties of aquatic organisms. Whole plant or parts (leaf, root or seed) or extract compounds have been used as feed additives in aquaculture [10]. The growing interest of using herbal immunostimulants in aquaculture has increased world-wide because they are easy to prepare, cheap, and they contain natural organic compounds that do not cause any threat to fish heath or to human health [5,10]. Recently, we have demonstrated that dietary administration of guava leaves at 0.5% for 60 days [4], *Chlorophytum borivilianum* polysaccharide at 0.4% for 28 days [11], or banana peels at 5% for 60 days [12] could improve the cytokine responses and disease resistance of *L. rohita*. However, the effects of a wide range of medicinal plants or their by-products on aquatic species remain unknown.

Zingiber officinale Roscoe (Zingiberaceae), commonly known as ginger, is indigenous to tropical Asia, mainly to southern China and India. The rhizome of the plant has a wide range of prophylactic and therapeutic properties [13]. Ginger is reported to have anti-inflammatory [14] and anti-diabetic [15] properties. Pharmacological studies also reveal that ginger has anti-cancer, chemopreventive, and chemotherapeutic effects on a variety of tumour cell lines and on animal models [16]. Ginger rhizome extracts contain specific phenolic compounds, gingerols, and their derivatives have various biological actions [13]. The component 6-GN has been reported to inhibit NO production and reduce iNOS in LPS-stimulated J774.1 macrophages [17]. Lee et al. [18] reported that 6-GN displayed anti-inflammatory properties by decreasing inducible NO synthase and TNF-α expression through the suppression of IκB-α phosphorylation, NF-κB nuclear activation, and PKC-α translocation. However, very few studies have demonstrated the effect of ginger on growth and immune systems of aquatic animals. For example, ginger increased immune responses and resistance against pathogens in tilapia (*Oreochromis mossambicus*) [19], and Asian sea bass (*Lates calcarifer*) [5]. Furthermore, bathing *Gyrodactylus turnbulli*-infected fish (*Poecilia reticulata*) in ethanolic ginger extract significantly reduced infection prevalence and intensity when compared to water extract and ethanol controls [20].

Skin mucus is a key component of fish immunity, and contains a number of innate immune components, such as lysozymes, proteases, immunoglobulins, lectins, and proteolytic enzymes [21]. Fish primarily depend on this innate immune system [22]. Earlier studies reported that dietary supplements of garlic [23] and extract of date palm fruits [24] increase skin mucosal immune responses of Caspian roach and common carp, respectively. In addition, analysis of the expression of cytokine genes is commonly regarded as an effective method of measuring the immune response in various fish species [2,4,24]. Therefore, boosting fish mucosal immune responses, as well as cytokine responses, using herbal-based immunostimulants may be the most promising approach for disease control [25]. To date, no study has examined the effect of ginger on cytokine-related gene expression in fish. Thus, the present study aimed to investigate the effects of dietary supplements of ginger on the growth, skin mucosal immune parameters, and cytokine gene expression of *L. rohita*, and disease resistance against pathogen infection. We also aimed to explore the potential of ginger as a feed additive in aquaculture.

2. Material and methods

2.1. Feed preparation

Ginger (*Zingiber officinale* Roscoe) rhizomes were purchased from a local market in Thanjavur, Tamil Nadu. Ginger was washed with tap water, peeled, sliced, and shade-dried at room temperature, and then oven-dried at 50 °C, before being powdered mechanically using a grinder, passed through an 80-mesh sieve, and stored at room temperature. Moisture content of dried ginger was 8.6%.

The composition of the basal diet is shown in Table 1. Proximate analysis of basal diet, performed according to the method outlined by the Association of Official Analytical Chemists (AOAC) [26], revealed a composition of 28.4% protein, 6.7% lipid, and 13.6% ash. The basal diet was used as the control diet. Basal diets were supplemented with ginger powder at five concentrations (g Kg^{-1}): 2.0 g (G1), 4.0 g (G4), 6.0 g (G6), 8.0 (G8), and 10.0 g (G10). All ingredients were blended thoroughly into a mixture and then pelletized, air-dried, ground, and sieved into appropriate pellet size. The pellets were then stored at −20 °C until further use.

2.2. Experimental design

L. rohita fingerlings (mean bodyweight 12.3 ± 0.11 g) were acclimatised to laboratory conditions in 500 L flow-through tanks at 24 ± 2 °C for 2 weeks and fed a basal diet. Approximately 20% of the water in all tanks was exchanged daily and 100% of the water was exchanged weekly. Basic physicochemical parameters of the water were analysed weekly [27]. Oxygen and ammonia concentrations were 6.1–7.3 mg L^{-1} and 0.03–0.06 mg L^{-1}, respectively, and pH ranged from 7.0 to 8.0 throughout the study period.

Fish were randomly assigned and divided into 200 L flow-through tanks where six experimental groups (three replicates in each group) were established. Each group consists of 75 fish (25 fish × 3 tanks). Fish were fed one of the six diets (basal diet, G2, G4, G6, G8, or G10) for 60 days, at 3–5% of body weight for three times (08:00 h, 13:00 h, and 18:00 h) daily. The amount of feed consumed was determined by daily recovery of excess feed, which was then adjusted every 15 days by batch weighing after 24 h of starvation.

2.3. Growth performance

Ten fish were randomly selected from each tank (i.e. 10 × 3 = 30 fish per group) at the end of 30 and 60 days of experimental

Table 1
Formulation and chemical composition of basal diet (g kg^{-1} dry matter).

Ingredients	Concentrations (g kg^{-1})
Ground nut oil cake	400
Rice bran	350
Wheat floor	150
Fish meal	75
Vegetable oil	20
Mineral and vitamin mixture[a]	5
Proximate analysis (g kg^{-1})	
Crude protein	284
Crude lipid	67
Ash	136

[a] Every 250 g of mineral-vitamin mixture (Supplevite-M, Sarabhai Zydus Animal Health Ltd., India) provides vitamin A, 500000 IU; vitamin D3, 100000 IU; vitamin B2, 0.2 g; vitamin E, 75 units; vitamin K, 0.1 g; calcium pantothenate, 0.25 g; nicotinamide, 0.1 g; vitamin B12, 0.6 mg; choline chloride, 15 g; calcium, 75 g; manganese, 2.75 g; iodine, 0.1 g; iron, 0.75 g; zinc, 1.5 g; copper, 0.2 g and cobalt, 0.045 g.