Chapter 15
Functional Feed Additives to the Diet of Golden Pompano *Trachinotus ovatus* Juveniles

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Abstract Golden pompano *Trachinotus ovatus* is an economically important warm-water farmed marine fish species. For the past few years, as *T. ovatus*-intensive aquaculture expanded and culture density raised, diseases have happened more repeatedly, causing many economic losses. In order to improve survival, several chemotherapeutic agents, vaccines, and antibiotics as well as some immunostimulants have been used to prevent bacterial, viral, fungal, and parasitic diseases at many hatcheries and fish farms. Therefore, the optimum type and supplementation level of functional additives are essential to the growth performance of *T. ovatus* juveniles in aquaculture. In this chapter, we review the functional feed additives (soybean isoflavones and marine red yeast *Rhodotorula mucilaginosa*) and their effects on the growth performance, nonspecific immune response, and disease resistance of juvenile *T. ovatus*.

Keywords Juvenile *Trachinotus ovatus* · Functional additives · Feed · Marine red yeast · Soybean isoflavones

15.1 Introduction

Golden pompano *Trachinotus ovatus* is an economically important warm-water marine fish species farmed in south coast of China and Southeast Asia (Sun et al. 2014; Zheng et al. 2014). As intensive aquaculture expanded and culture density

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increased, diseases happened more repeatedly, especially from May to October, causing many economic losses (Xia et al. 2012; Zhang et al. 2014a, b). Fish under intensive culture conditions are more susceptible to pathogen infection than wild fish. The environmental stress caused by discharge of farms into estuaries and bays and the stress caused by high rearing density are factors that make animals more sensitive to pathogens (Bilen et al. 2011). For reduce mortality, several fish farms and hatcheries use many chemotherapeutic agents, vaccines, and antibiotics, as well as some immunostimulants, to prevent bacterial, viral, fungal, and parasitic diseases (Dügenci et al. 2003). However, the drug applications listed above are quite expensive in intensive culture. In addition, they may cause adverse effects like pollution, bioaccumulation, and antibiotic resistance, which can be transferred to wild and human pathogenic microorganisms, thereby posing a threat to human health and sociopolitical and environmental issues (Harikrishnan et al. 2011).

Immunostimulants increase resistance to infectious disease by enhancing nonspecific defense mechanisms. Consequently, the response duration is very short because there is no memory component (Sakai 1999). Since immunostimulants are considered safe and effective against various pathogens, the use of immunostimulants in fish culture to enhance immunity and disease resistance has received considerable attention (Sakai 1999; Harikrishnan et al. 2011). Immunostimulants can be applied via injection, oral administration, or bathing (Sakai 1999; Yin et al. 2006; Jeney and Anderson 1993a, b). Although intraperitoneal injection has been verified to be the most speedy and effective way of administration, incorporation in the diet is considered as most suitable for fish culture, as this method is non-stressful (Siwicki et al. 1994; Esteban et al. 2001). Several immunostimulants, such as levamisole (Siwicki et al. 1990; Jeney and Anderson 1993a, b), chitosan (Siwicki et al. 1994), yeast (Siwicki et al. 1994), glucan (Jorgensen and Robertsen 1995), lipopolysaccharide (Solem et al. 1995), growth hormone (Sakai et al. 1995, 1996), glucan plus vitamin C (Verlhac et al. 1996), yeast RNA (Sakai et al. 2001), and zeranol (Keles et al. 2002), have been administered as feed additives to modulate nonspecific immunity of fish, such as *Ictalurus punctatus* Rafinesque, *Cyprinus carpio* L., *Salmo salar* L., and *Oncorhynchus mykiss*. In this chapter, we aim to evaluate the role of dietary additives of soybean isoflavones and marine red yeast *Rhodotorula mucilaginosa* as an immunostimulant to promote growth performance and enhance the nonspecific immune ability and production of golden pompano.

15.2 The Effects of Dietary Soybean Isoflavones (SI) in the Feed of Juvenile Golden Pompano

Isoflavones are a class of molecules called flavonoids, whose basic structural unit is composed of two benzene rings connected by a heterocyclic pyran ring and belongs to a large family of polyphenols (Barnes et al. 2011; Chen et al. 2011). Because isoflavones are structurally similar to natural estrogen, they can exert a variety of estrogen-like biological effects in animals (Ng et al. 2006). In mammals, isoflavones have a wide range of biological activities including antioxidant effects (Jiang et al. 2007), antiestrogenic effects (Cassidy et al. 1995), anticancer effects (Dijsselbloem et al. 2004), anti-inflammatory effects (Verdrengh et al. 2003; Hämäläinen et al. 2007), enzyme-inhibitory effects (Yamashita et al. 1990), cardioprotective effects (Anthony et al. 1996), and antifungal effect (Naim et al. 1974).

15.2.1 Growth

Soybean isoflavones (SI), which are a plant chemical with estrogenic activity, can weakly bind to estrogen receptors, causing competition between natural estrogens and isoflavones (Turan 2006; Kelly et al. 1993). However, Martin et al. (1978) suggested that isoflavones may act as anti-estrogens in the presence of high levels of endogenous estrogens (Martin et al. 1978). Because isoflavones may have hormone-like functions, they may affect animal growth. The effects of soybean isoflavones on growth performance are somewhat variable. For example, supplemental SI can increase the growth rate in barrows (Cook 1998), African catfish *Clarias gariepinus* (Burchell 1822) (Turan and Akyurt 2005), tilapia *Oreochromis aureus* (Yu et al. 2006) and tilapia *Oreochromis aureus* (Turan 2006). Zhou et al. (2015) found that with the increase of dietary SI, the growth performance of *T. ovatus* was significantly improved when dietary SI level is up to 40 mg kg^{-1}, indicating that dietary SI might promote the growth of fish at a suitable dose.

15.2.2 Nonspecific Immune Response

Proteins are the most important compound in the serum, with albumin and globulin being the major serum proteins (Kumar et al. 2005; Jha et al. 2007). The hemolymph protein content is used as an immune parameter to indicate whether or not a fish is healthy (De Smet and Blust 2001). The complement system is the major humoral component of the innate immune responses and plays an essential role in alerting the host immune system of the presence of potential pathogens as well as their clearance (Muller-Eberhard 1988). The complement system is initiated by one or a combination of three pathways, namely, the classical, lectin, and alternative. All three pathways merge at a common amplification step involving C3 and proceed through a terminal pathway that leads to the formation of a membrane attack complex, which can directly lyse pathogenic cells (Boshra et al. 2006). The results in the previous study on *T. ovatus* showed that the total plasma protein and C3 content were significantly increased by feeding the dose of SI at 40 mg kg^{-1} feed (Zhou et al. 2015). However, these immune parameters were not further increased by feeding the fish with the diet supplemented with SI at the level of 60 and 80 mg kg^{-1} feed, suggesting that high supplementation, especially the dose of 40 mg kg^{-1} feed is optimal for the increase of plasma total protein and C3 content. To date, there is no exact explanation on how SI works to increase plasma total protein and C3 content in fish. Further studies are necessary to explore the mechanism of the effects of SI on plasma total protein and C3 content of fish.

The innate immune system of fish is considered to be the first line of defense against a broad spectrum of pathogens and is more important for fish as compared with mammals. Lysozyme level or activity is an essential index of innate immunity of fish and is ubiquitous in its distribution among living organisms (Saurabh and Sahoo 2008). The plasma LYZ activity of *T. ovatus* increased with dietary SI level, increasing from 0 to 40 mg kg^{-1} SI, with no significant differences among the treatments with over 40 mg kg^{-1} SI (Zhou et al. 2015). Similarly, a large number of immunostimulants have been reported to increase serum lysozyme levels in fish that may be due to either an increase in the number of phagocytes secreting lysozyme or due to an increase in the amount of lysozyme synthesized per cell (Kumari and Sahoo 2006).

Respiratory bursts are produced by phagocytes in order to attack invasive pathogens during phagocytosis and have been widely used to evaluate the defense ability against pathogens. However, the excessive accumulation of reactive oxygen intermediates (ROIs) is extremely toxic to host cells (Dalmo et al. 1997). It is clear that the SI has an enhancing effect on respiratory burst activity of golden pompano (Zhou et al. 2015). Data from this study showed that compared with the control, the 40 mg kg^{-1} SO group significantly increased respiratory burst activity, and the other groups supplemented with 20, 60, and 80 mg kg^{-1} SI had a tendency of an increase in respiratory burst activity. A previous study has demonstrated that dietary administration of immunostimulant (such as emodin) significantly affected respiratory burst activity of Wuchang bream (Zhang et al. 2014a, b). These findings suggest that long-term feeding of the proper immunostimulant (SI) supplementation can maintain the activation of phagocytic cells throughout the experimental period and is fundamental in achieving disease resistance.

15.2.3 Hepatic Antioxidant Status and the Expression of Hepatic Gene HSP70

The nonspecific defense mechanisms of fish include neutrophil activation, production of peroxidase and oxidative free radicals, and initiation of other inflammatory factors (Ainsworth et al. 1991). The stress response might also impact factors such as total antioxidation capacity and levels of glutathione, catalase, SOD, and various peroxidases (Liu et al. 2010; Itou et al. 1996). The SI has exhibited antioxidant effects both in vitro and in vivo (Chen et al. 2011). Isoflavone-supplemented diets can reduce lipid peroxidation and F2-isoprostane levels, a biomarker of lipid peroxidation, in humans (Wiseman et al. 2000). Disilvestro et al. (2005) reported that the

SI in capsules could elevate erythrocyte superoxide dismutase in human (Disilvestro et al. 2005). Previous studies showed that SI supplemented at the dose of 20 mg kg^{-1} feed significantly increased the SOD activity in *L. vannamei* when compared with other treatments (Chen et al. 2011), and the hepatic antioxidative capacity was strongly increased by dietary SI in tilapia (*Oreochromis aureus* Steindachner) (Yu et al. 2006). Cai and Wei (1996) suggested that dietary genistein enhances the activities of antioxidant enzymes (SOD, CAT, glutathione reductase, and glutathione peroxidase) in various organs in mice, which may be a mechanism of genistein's chemopreventive action (Cai and Wei 1996). Consistent with these studies, Zhou et al. observed that compared to the control the treatments supplemented with 40 mg kg^{-1} SI increased activities of hepatic antioxidant enzymes (SOD, T-AOC, CAT) whereas malondialdehyde content was reduced (Zhou et al. 2015). The present results imply that SI can improve antioxidative status, inhibit free radical formation, and reduce the harm of lipidic superoxide in juvenile golden pompano.

Heat shock proteins (HSPs), also known as stress proteins and extrinsic chaperones, are a suite of highly conserved proteins of varying molecular weight (*c.* 16–100 kDa) produced in all cellular organisms when they are exposed to stress (Roberts et al. 2010). HSP70 is mainly involved in stress protection, improving cell survival and raising tolerance to environmental stressors or harm (Basu et al. 2002). Thus, HSP70 has been widely used as a bioindicator of stress. HSP70 is induced by heat and chemical shocks in fish, like in mammals (Gornati et al. 2004). A number of studies have shown that Chinese herbs enhance the expression of HSP70 in Wuchang bream (Liu et al. 2012) and white shrimp (Lei and Zeng 2008), and higher dietary carbohydrate increases the expression level of HSP70 (Zhou et al. 2013). Moreover, HSP70 mRNA of broilers was also changed positively by the dietary genistein (Kamboh et al. 2013). Similarly, the relative level of hepatic HSP70 mRNA of *T. ovatus* increased with increasing dietary SI levels up to 40 mg kg^{-1} and thereafter levelled off, indicating that dietary SI could enhance the expression of HSP70 (Zhou et al. 2015).

15.2.4 Challenge Test

Currently, because the methodology to comprehensively investigate immunity and disease resistance of fish is still limited, a useful biomarker for disease resistance of fish is difficult to identify. Therefore, bacterial challenge tests have often been used as a final indicator of fish health status after nutrition trials (Lin et al. 2012; Jin et al. 2013). Vibriosis is caused by *V. harveyi*, a halophilic gram-negative bacterium that is known to cause disease to fish, shrimp, and shellfish either in the culture system or in the wild aquatic environment (Sharma et al. 2012; Austin and Zhang 2006). A previous study showed that dietary SI showed an improved survival rate of *L. vannamei* against an intramuscular challenge with *V. alginolyticus* (Chen et al. 2011). This agrees well with the results of other workers (Huang et al. 2005), who found SI-supplemented diets improved survival rate against an intramuscular injecting with *V. parahaemolyticus*. Zhou et al. (2015) found that dietary SI showed an increased survival rate of *T. ovatus* against challenge with *Vibrio harveyi* (Zhou et al. 2015). Therefore, SI showed positive effects on preventing golden pompano against *Vibrio harveyi* infection.

15.3 Effects of Dietary Additive of Marine Red Yeast *Rhodotorula mucilaginosa* on Golden Pompano

Yeast can affect nonspecific immunity and protection against furunculosis in rainbow trout (Siwicki et al. 1994). Yeast may improve fish health as antagonists to pathogens and by immunostimulation (Andlid et al. 1995). Rorstad et al. (1993) also reported that yeast glucan showed an adjuvant effect when included in vaccines against furunculosis in Atlantic salmon (*Salmo salar* L.). Nakano et al. (1999) observed that red yeast had a reducing effect on oxidized oil-induced oxidative stress in rainbow trout (*Oncorhynchus mykiss*). Xia et al. (2013) observed that marine red yeast *Rhodotorula mucilaginosa* could promote the growth and immunity of *Litopenaeus vannamei*.

15.3.1 Growth

The marine red yeast has been widely used for its potential beneficial effect in aquaculture (Yang et al. 2010; Zhang et al. 2013; Sun et al. 2015). Zhou et al. (2016) found that the WG and SGR of *T. ovatus* fed with 1‰ *R. mucilaginosa* diet were higher than those of control group. Previous studies also showed that compared to the control group, weight gain (WG) and specific growth rate (SGR) of *Litopenaeus vannamei* with fed *R. paludigenum* supplementation increased significantly (Yang et al. 2010; Scholz et al. 1999). Zhang observed that addition of 1 g kg^{-1} *Rhodotorula benthica* into brown fish meal can significantly improve feeding rate, protein efficiency rate, and growth performance of turbot, a similar growth level to white fish meal (Zhang et al. 2013). Tovar-Ramírez et al. (2004) found that final mean weight of sea bass larvae in the group fed with 1.1% of marine yeast *D. hansenii* CBS8339 was twice as that of the other groups. These results suggested that the marine red yeast produces many bioactive substances, such as protein, amino acids, fatty acid, polysaccharide, and carotenoids, which could promote the growth of aquatic animals.

15.3.2 Serum Biochemical and Immune Parameters

LYZ is a bactericidal peptide, which is an important component of the immune defense of marine fish species (Liu et al. 2012). It is responsible for breaking down the polysaccharide walls of many kinds of bacteria and thus provides some protection against pathologic infection (Lie et al. 1989; Hauge et al. 2002). The AKP is an important component of lysosomal enzymes that originate from hemocytes to destroy extracellular binvadersQ (Cheng and Rodirick 1975). Therefore, phagocytic competence and AKP activity are related to the quantity and quality of hemocytes. The activities of the immunity active factors (serum LYZ and AKP) in the serum of the lady crab *Charybdis japonica* have different degrees of the enhancement in 48 h after infection with the polysaccharide of oceanic red yeast (Sun et al. 2015; Wang et al. 2011). Similarly, compared to the control, the *R. mucilaginosa* diets significantly increased serum LYZ and AKP activities of juvenile *T. ovatus* (Zhou et al. 2016). Nitric oxide produced by NOS is associated with diverse actions in neurotransmission, vascular systems, and immunity, including antimicrobial and antiviral activities by inhibiting DNA as well as protein and lipid synthesis (Bredt and Snyder 1994; Karupiah et al. 1993; Howe et al. 2002; Lepoivre et al. 1990). Compared to other treatments, the group supplemented with 2‰ *R. mucilaginosa* significantly increased serum NOS activity (Zhou et al. 2016). This agrees well with the finding of Zhang et al. (2011), who found that the shrimp (*Penaeus japonicus*) fed the diet with both *Bacillus* probiotics and IMO (T3) produced significantly higher immune parameters (LYZ activity and NOS activities) than the control group. These results suggested that oceanic red yeast has immune stimulation to some extent.

15.3.3 Hepatic Antioxidative Status

The increase in free radical content may lead to an increase in lipid peroxidation content and lipid peroxidation injury in fish (Liu et al. 2012). The breakdown of hepatic lipid peroxide yields large amounts of aldehydes, alcohols, and hydrocarbons such as MDA, a strongly toxic chemical. The antioxidant enzyme system plays a prominent role in resisting lipid oxide damage (Holmblad and Soderhall 1999; Lopes et al. 2001). Dietary supplementation with marine red yeast can significantly enhance antioxidant activity in aquatic animals (Bon et al. 1997; Li and zhang 2004). A previous study showed the SOD activity of hepatopancreases from *L. vannamei* in groups fed with the live yeast diet and the dry yeast diet was significantly higher than that in the control group, whereas no statistical difference was found in MDA content of hepatopancreases (Yang et al. 2010). Zhou et al. (2016) found that compared to the control, the groups supplemented with 2–4‰ *R. mucilaginosa* increased hepatic SOD activity, whereas the 2–5‰ *R. mucilaginosa* treatment groups decreased hepatic MDA content, especially in the 4‰ *R. mucilaginosa* group. Taken together, our results suggest that the supplementation with *R. mucilaginosa* reduces the potential for oxidative damage in *T. ovatus*.

15.3.4 *Effect of* R. mucilaginosa *on Survival in* T. ovatus

Currently, bacterial challenge test has often been used as a final indicator of fish health after nutrition trials (Zhou et al. 2015). Vibriosis is caused by *V. harveyi*, a halophilic gram-negative bacterium causing disease in fish, shrimp, and shellfish (Sharma et al. 2012; Austin and Zhang 2006). A previous study showed that supplementation of red yeast could make the red yeast colonize in the intestine of fish larvae, which could affect the growth of the larvae and accelerate the maturity of the digestive system to improve survival rates (Gatesoupe 2007). The survival rate of the mice in the group fed with the diet supplemented with the astaxanthin produced by red yeast was higher than that of the control group (Bennedsen et al. 1990). Similarly, dietary *R. mucilaginosa* showed an increased survival rate of *T. ovatus* against challenge with *V. harveyi* (Zhou et al. 2016).

15.3.5 *The Effect of* R. mucilaginosa *on Hemolymph Complement 3 and Complement 4 of* T. ovatus *After* V. harveyi *Infection*

The complement system is the major humoral component of the innate immune responses and thus plays an essential role in alerting the host immune system of the presence of potential pathogens as well as their clearance, which is initiated by one or a combination of three pathways, namely, the classical, alternative, and lectin (Holland and Lambris 2002; Zhou et al. 2014). The complement C3 is the central component of the complement system, being activated into its respective cleavage products C3a and C3b through one of the three pathways (Boshra et al. 2006). The complement C4 plays an integral role in the activation of the classical and lectin pathways (Boshra et al. 2006). A previous study showed that after challenge by *Aeromonas veronii*, there was no significant difference in complement 3 among all groups (Yu et al. 2014). However, compared to the control group prior to infection, the serum C3 level significantly increased in the group supplemented with *R. mucilaginosa*; compared to the control after infection, the 0.1–0.3‰ *R. mucilaginosa* groups significantly increased serum C4 levels (Zhou et al. 2016).

15.4 Conclusion

In conclusion, dietary soybean isoflavones and *R. mucilaginosa* supplementation, as an immunostimulant, could promote growth performance and enhance the nonspecific immune ability and production of golden pompano in aquaculture.

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