Lee et al. Fisheries and Aquatic Sciences (2020) 23:7
https://doi.org/10.1186/s41240-020-00157-8

Fisheries and Aquatic Sciences

RESEARCH ARTICLE

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Dietary inclusion effect of yacon, ginger, and blueberry on growth, body composition, and disease resistance of juvenile black rockfish (*Sebastes schlegeli*) against *Vibrio anguillarum*

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Abstract

Background: To minimize the use of antibiotics and to obtain a more sustainable fish culture and aquaculture industry, development of alternative natural source of immunostimulant to replace antibiotic in aquafeed is highly needed.

Objective: Dietary inclusion effect of yacon (YC), ginger (GG), and blueberry (BB) on growth, body composition, and disease resistance of black rockfish against *Vibrio anguillarum* was compared to ethoxyquin (EQ).

Methods: Four hundred eighty juvenile (an initial weight of 4.2 g) fish were randomly distributed into 12 of 50 L flow-through tanks (forty fish per tank). Four experimental diets were prepared; the control (Con) diet with 0.01% EQ inclusion, and YC, GG, and BB diets at 1% each additive inclusion. Each additive was included into the experimental diets at the expense of wheat flour. Each diet was assigned to triplicate tanks of fish and hand-fed to satiation twice daily for 8 weeks. At the end of 8-week feeding trial, 20 fish from each tank fish were artificially infected by intraperitoneal injection with 0.1 mL of culture suspension of pathogenic *V. anguillarum* containing 3.3×10^6 cfu/mL respectively. Fish were monitored for the following 8 days after *V. anguillarum* infection and dead fish were removed every 6 h for the first 4 days and 12 h for the rest of the study.

Results: Weight gain, specific growth rate (SGR), and feed efficiency ratio (FER) of fish fed the YC diet was higher than those of fish fed all other diets. However, feed consumption, protein efficiency ratio, and protein retention was not affected by dietary additive. Moisture, crude protein, and crude lipid content of the whole body of fish were affected by dietary additive. Analysis of the Kaplan-Meier survival curves showed that survival of fish fed the YC, BB, and GG diets was higher than the Con diet.

Conclusion: Oral administration of YC can improve not only weight gain, SGR, and FER of black rockfish, but lower mortality of rockfish at occurrence of *V. anguillarum*.

Keywords: Black rockfish (*Sebastes schlegeli*), Yacon, Ginger, Blueberry, *Vibrio anguillarum*

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Background

Black rockfish (*Sebastes schlegeli* Hilgendorf 1880) has been one of the most commercially important marine aquaculture fish species in Korea, Japan, and China since over two decades ago due to its fast growth and high resistance against disease. Therefore, many studies to elucidate dietary nutrient requirements (Lee 2001; Lee et al. 2002; Yan et al. 2007), optimum dietary protein to lipid ratio (Cho et al. 2015), alternative animal and/or plant protein sources for fish meal in the diet (Lee et al. 1996; Lim et al. 2004; Jeon et al. 2014), optimum dietary carbohydrate to lipid ratio (Lee and Kim 2009), effect of dietary lipid sources (Aminikhoei et al. 2013), optimum feeding frequency and rate (Lee et al. 2000; Mizanur et al. 2014), and effect of dietary additive to improve lysozyme activity and stress recovery (Hwang et al. 2013), and immune response against *Vibrio alginolyticus* (Kim et al. 1999) and *Vibrio ordalii* (Lim et al. 2009) infection for black rockfish have been reported.

Application of antibiotics has been widespread to reduce mortality during year-round fish culture. As oral administration of synthetic antibiotic in aquafeed, however, is prohibited in some countries (Tang et al. 2001) due to high possibility of contamination of environment and culturing fish used for human consumption (Alderman and Hastings 1998), development of the new natural source of dietary additive that has no side effect on environment and culturing fish to replace synthetic antibiotics keeps being needed.

Vibriosis is a major disease occurring in marine and brackish water fish and characterized by hemorrhagic septicemia (Demircan and Candan 2006). *Vibrio* spp. has been one of main pathogens causing high mortality occurred in marine fish including olive flounder *Paralichthys olivaceu*s, black rockfish, and red sea bream *Pagrus major* and shrimp farm during summer season year after year in Korea (Choi et al. 2010a; Jung et al. 2012). Therefore, development of new natural source of dietary additive to lower mortality of fish at occurrence of *V. anguillarum* is highly desired.

Yacon *Polymnia sonchifolia* Poeppig and Endlicher (YC) containing the polyphenols had an antioxidant activity (Park et al. 2012). Its leaves showed the antibacterial activity against *Staphylococcus aureus* (Choi et al. 2010b). In our early study, YC was the most effective additive to improve weight gain and feed utilization of black rockfish among the several feed additives tested (Kim et al. 2016).

Ginger *Zingiber officinale* Roscoe (GG) containing gingerols and shogaols is known to have antibacterial properties (Ekwenye and Elegalam 2005; Sebiomo et al. 2011) and antioxidant activity (Balestra et al. 2011). Akintobi et al. (2013) reported that water and ethanol extracts of GG showed the highest inhibition against *Salmonella typhi* and *Proteus mirabilis*, respectively and postulated that the extracts of GG possessed antimicrobial compounds, which could be used as substitutes for the antibiotics. In addition, oral administration GG effectively improved weight gain and disease resistance of several fish (Nya and Austin 2009; Talpur et al. 2013; Hassanin et al. 2014).

Blueberry *Vaccinium ashei* Reade (BB) containing anthocyanin had an antioxidant activity (Vizzotto et al. 2013; Deng et al. 2014) and showed antioxidant effect on animals (Molan et al. 2008; Papandreou et al. 2009).

Ethoxyquin (EQ) is the most widely used as a synthetic antioxidant in fish feed to prevent rancidity (Weil et al. 1968; FAO 1970; Thorisson et al. 1992; Drewhurst 1998). The optimal EQ concentrations of 13.8 and 50 mg/kg diet were reported for Japanese sea bass *Lateolabrax japonicus* and large yellow croaker *Pseudosciaena crocea*, respectively (Wang et al. 2010, 2015). The upper limit for EQ in fish feed established in the EU is 150 mg/kg diet (Council Directive 2003). In addition, Bohne et al. (2007) proposed that EQ-derived residues in salmon might have high toxicological effects for human consumers.

The widespread use of antibiotics should be minimized because it causes antibiotic contamination of the environment and fish used for human consumption (Alderman and Hastings 1998) as well as leading to the development of antibiotic resistance. For instance, Colquhoun et al. (2007) observed oxolinic acid resistance among *V. anguillarum* strains isolated from diseased Atlantic cod *Gadus morhua*. In spite of these drawbacks, antimicrobial therapy is still required for containment of vibriosis outbreaks. To minimize the use of antibiotics and to obtain a more sustainable fish culture and aquaculture industry, development of alternative natural source of immunostimulant to replace antibiotic in aquafeed is highly needed.

Those natural sources (YC, GG, and BB) of plant-originated ingredients have high potential as an alternative antibiotic in rockfish feed when fish were fed with one of the diets containing various sources of additives for 7 weeks, and then infected with *Strepotococcus parauberis* (Kim et al. 2016). The other plant-originated additives were also effective to improve not only weight gain and feed utilization, but also disease resistance in some marine fish (Ji et al. 2007a; Punitha et al. 2008; Talpur and Ikhwanuddin 2012; Talpur et al. 2013; Talpur 2014).

Therefore, the effect of dietary inclusion of YC, GG, and BB on growth, body composition, and disease resistance of juvenile black rockfish against *V. anguillarum* was compared to EQ in this study.

Methods

Fish and the experimental conditions

Juvenile black rockfish were purchased from a private hatchery (Tongyeong City, Gyeongsangnam-do, Korea) and acclimated to the experimental conditions for 2 weeks before an initiation of the feeding trial. During the acclimation period, fish were hand-fed a commercial extruded pellet (Suhyup Feed Co. Ltd., Gyeongsangnmado, Korea) twice a day at a ratio of 2–3% body weight of fish. Four hundred eighty juvenile fish (an initial body weight of 4.2 g) were randomly chosen and distributed into 12 of 50 L flow-through tanks (water volume: 40 L) (40 fish per tank). The flow rate of water into each tank was 1.41 L/min/tank. The water source was sand-filtered natural seawater and aeration was supplied into each tank. Water temperature monitored daily from 17.4 to 23.9 °C (mean ± SD: 20.1 ± 3.23 °C) and photoperiod followed natural conditions.

Preparation of the experimental diets

Four experimental diets were prepared; the control (Con) diet with 0.01% EQ inclusion, and YC, GG, and BB diets at 1% each additive inclusion (Table 1). Each additive, which is a commercially available product (Tojongherb Co Ltd., Seoul, Korea), was included into the experimental diets at the expense of wheat flour. The Con diet was prepared to satisfy dietary nutrient requirements for black rockfish (Kim et al. 2001; Kim et al. 2004). Fish meal and fermented soybean meal were used as the protein source in the experimental diets. Wheat flour, and squid liver and soybean oils were used as the carbohydrate and lipid sources, respectively in the experimental diets.

The ingredients of the experimental diets were well mixed with water at a ratio of 3:1 and pelletized by lab pellet-extruder (Dongsung mechanics, Busan, Korea). The experimental diets were dried at room temperature overnight and stored in − 20 °C until used. Each diet was randomly assigned to triplicate tanks of fish and hand-fed to satiation twice daily (07:00 and 17:00 h) for 7 day a week for 8 weeks. Tanks were siphon-cleaned daily.

Analytical procedures of the experimental diets and fish

Ten fish at the start and seven fish from each tank at the termination of the feeding trial were sampled and sacrificed for proximate analysis. Crude protein was determined by the Kjeldahl method (Kjeltec 2100 Distillation Unit, Foss Tecator, Hoganas, Sweden), crude lipid was determined using an ether-extraction method (Soxtec TM 2043 Fat Extraction System, Foss Tecator, Sweden), moisture was determined by oven drying at 105 °C for 24 h, fiber was determined using an automatic analyzer (Fibertec, Tecator, Sweden), and ash was determined using a muffle furnace at 550 °C for 4 h.

Serum chemistry of fish

Blood samples were obtained from the caudal vein of five randomly chosen fish from each tank by using syringes after they were starved for 24 h. Serum was collected after centrifugation (900 g for 10 min); stored in the freezer at − 70 °C as separate aliquots for analysis of total protein, total cholesterol, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and triglyceride; and analyzed by using automatic chemistry system (HITACHI 7180/7600-210, Hitachi, Japan).

Table 1 Ingredients of the experimental diets containing the various sources of additives (DM basis, %)

	Experimental diets			
	Con	YC[a]	GG[a]	BB[a]
Ingredients (%)				
Fish meal[b]	63.5	63.5	63.5	63.5
Fermented soybean meal[c]	7.0	7.0	7.0	7.0
Wheat flour	22.99	22.0	22.0	22.0
Ethoxyquin[d]	0.01			
Additives		1	1	1
Squid liver oil	2.0	2.0	2.0	2.0
Soybean oil	2.0	2.0	2.0	2.0
Vitamin premix[e]	1.0	1.0	1.0	1.0
Mineral premix[f]	1.0	1.0	1.0	1.0
Choline	0.5	0.5	0.5	0.5
Nutrients (%)				
Dry matter	90.7	90.5	91.1	91.0
Crude protein	50.4	50.8	50.2	50.1
Crude lipid	10.8	10.9	11.2	11.0
Ash	10.1	10.2	10.2	10.5

[a]YC (yacon), GG (ginger), and BB (blueberry) powder were purchased from Tojongherb Co Ltd. (Dongdaemun-gu, Seoul, Korea)
[b]Fish meal and was purchased from Abank Co Ltd. (Seocho-gu, Seoul, Korea)
[c]Fermented soybean meal was supplied by CJ CheilJedang Corp. (Jung-gu, Seoul, Korea)
[d]Ethoxyquin was supplied from Chunhajeil feed Co Ltd. (Daedeok-gu, Daejeon, Korea)
[e]Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbic acid, 121.2; DL-α-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003
[f]Mineral premix contained the following ingredients (g/kg mix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0.

Challenge test

Twenty externally normal fish shown to be free from bacterial infection were selected from each tank and stocked into 12, static 50 L tanks at the end of the 8-week feeding trial. Fish was used for the *V. anguillarum* challenge and water was not exchanged. The bacteria used for the challenge were reference pathogenic strain of gram-negative *V. anguillarum* (FP2247) isolated from black rockfish.

The culture suspension of *V. anguillarum* were grown on agar for 24 h, collected, washed, and suspended in sterile 0.85% saline solution and counted. Then, fish were artificially infected by intraperitoneal injection with 0.1 mL of culture suspension of pathogenic *V. anguillarum* containing 3.3×10^6 cfu/mL respectively. Fish were monitored for the following 8 days after *V. anguillarum* infection and dead fish were removed every 6 h for the first 4 days and 12 h for the rest of the study. Fish was starved throughout the 8-day challenge test.

Calculations and statistical analysis

The following variables were calculated: specific growth rate (SGR, %/day) = (Ln final weight of fish − Ln initial weight of fish) × 100/days of feeding trial, feed efficiency ratio (FER) = weight gain/feed consumed, protein efficiency ratio (PER) = weight gain of fish/protein consumed and protein retention (PR) = protein gain × 100/protein consumed.

SAS version 9.3 (SAS Institute, Cary, NC, USA) was used to conduct a one-way ANOVA. Tukey's honestly significant difference (HSD) test was used to determine the significance ($P < 0.05$) of the differences among the means responses to dietary treatments. Percentage data was arcsine-transformed prior to statistical analysis. The survival patterns were analyzed using Kaplan–Meier survival curves, Log-rank and Wilcoxon tests.

Results

Growth performance of black rockfish

No significant difference in survival of black rockfish was observed at the end of the 8-week feeding trial (Table 2). However, weight and SGR of fish fed the YC diet were significantly higher than those of fish fed all other (GG, BB, and Con) diets. Weight gain and SGR of fish fed the GG and BB diets were also significantly ($P < 0.05$) higher than those of fish fed the Con diet.

Feed utilization

Feed consumption (g/fish) of fish was not significantly affected by dietary additive (Table 3). FER of fish fed the YC diet was significantly higher than that of fish fed the all other diets. No significant difference in FER was observed among fish fed the Con, GG, and BB diets. PER of fish was not significantly affected by dietary additive. PR of fish fed the YC and GG diets was significantly higher than that of fish fed the Con and BB diets.

Chemical composition of the whole body of fish

Moisture content of the whole body of fish fed the BB diet was significantly higher than that of fish fed the all other diets (Table 4). Moisture content of the whole body of fish fed the Con diet was also significantly higher than of fish fed the YC and GG diets. Crude protein content of the whole body of fish fed the GG diet was significantly higher than that of fish fed the all other diets, followed by the YC, Con, and BB diets. Crude lipid content of the whole body of fish fed the YC and GG diets was significantly higher than that of fish fed the Con and BB diets. Ash content of the whole body of fish was not significantly affected by dietary additive.

Plasma chemistry of fish

Plasma chemistry (total protein, total cholesterol, GOT, GPT, and triglycerides) of fish was not significantly affected by dietary additive (Table 5).

Challenge test

Survival of fish fed the experimental diets containing various sources of additive for 8 weeks, and then infected with *V. anguillarum* for 8-day post observation is depicted in Fig. 1. Analysis of the Kaplan-Meier survival curves showed that the survival of black rockfish fed the YC (51.7 ± 0.02%; means of triplicate ± SE), BB (46.7 ± 0.02%), and GG (26.7 ± 0.02%) diets was significantly higher than that of fish fed the Con (1.7 ± 0.02%) diet. In addition, the survival of fish fed the YC and BB diets was also significantly higher than that of fish fed the GG diet.

Discussion

Oral administration of feed additives (YC, GG, and BB) used in this study achieved improved weight gain of black rockfish compared to the Con diet. The greatest improvement in weight gain, SGR and FER was obtained in fish fed the YC diet agreed with Kim et al. (2016)'s study showing that YC was the most effective additive to improve growth performance and FER of black rockfish. Improved weight gain, SGR and PR of fish fed the GG

Table 2 Survival (%), weight gain (g/fish) and specific growth rate (SGR, %/day) of black rockfish *Sebastes schlegeli* fed the experimental diets containing the various sources of additives for 8 weeks

Experimental diets	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)	SGR[1] (%/day)
Con	4.3 ± 0.00	16.4 ± 0.03	100.0 ± 0.00[a]	12.1 ± 0.03[c]	2.41 ± 0.002[c]
YC	4.2 ± 0.01	16.7 ± 0.01	100.0 ± 0.00[a]	12.5 ± 0.01[a]	2.45 ± 0.003[a]
GG	4.2 ± 0.01	16.5 ± 0.06	100.0 ± 0.00[a]	12.3 ± 0.06[b]	2.43 ± 0.006[b]
BB	4.3 ± 0.00	16.6 ± 0.03	100.0 ± 0.00[a]	12.3 ± 0.03[b]	2.43 ± 0.002[b]

Values (means of triplicates ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$)

[1]SGR (%/day) = (Ln final weight of fish − Ln initial weight of fish)×100/days of feeding trial

Table 3 Feed consumption (g/fish), feed efficiency ratio (FER), protein efficiency ratio (PER), and protein retention (PR) of black rockfish (*Sebastes schlegeli*) fed the experimental diets containing the various sources of additives for 8 weeks

Experimental diets	Feed consumption (g/fish)	FER[1]	PER[2]	PR[3]
Con	11.5 ± 0.03^a	1.06 ± 0.003^b	2.10 ± 0.006^a	36.3 ± 0.19^b
YC	11.5 ± 0.03^a	1.08 ± 0.003^a	2.13 ± 0.006^a	37.7 ± 0.10^a
GG	11.5 ± 0.05^a	1.07 ± 0.008^b	2.12 ± 0.017^a	38.2 ± 0.35^a
BB	11.6 ± 0.07^a	1.06 ± 0.004^b	2.12 ± 0.009^a	35.7 ± 0.24^b

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$)
[1]Feed efficiency ratio (FER) = Weight gain of fish/feed consumed
[2]Protein efficiency ratio (PER) = Weight gain of fish/protein consumed
[3]Protein retention (PR) = Protein gain × 100/protein consumed

diet compared to the Con diet in this study partially coincided with other studies (Nya and Austin 2009; Talpur et al. 2013; Hassanin et al. 2014) showing that weight gain and feed utilization (FER or PER) of rainbow trout *Oncorhynchus mykiss*, Asian sea bass *Lates calcarifer*, and Nile tilapia *Oreochromis niloticus* improved effectively when 0.1–1% GG was included in diets. Similarly, dietary inclusion of 0.5% *Chlorella* powder and *Lycium chinense* improved weight gain and FER of black rockfish (Bai et al. 2001; Lim et al. 2009). Gabor et al. (2010) reviewed the use of phytoadditives, such as garlic *Allium sativum*, onion *Allium cepa*, oregano *Origanum vulgare*, GG Echinacea *Echinadea purpurea*, cinnamon *Cinnamomum verum*, or nettle *Urtica dioica* in diets for growth performance of several fish and emphasized the major advantages in the use of phytoadditives is the fact that they are natural substances and do not pose any threat to fish, man, or environment. Therefore, those phytoadditives can be considered as alternative antibiotics in fish feed without serious concern of either fish-consumer or undesirable effect on environment.

The chemical composition of the whole body black rockfish, except for ash content, was affected by dietary additives in this study. A similar result showing that dietary additive affected the whole body composition of black rockfish was reported (Bai et al. 2001; Yun et al. 2016). Moisture content seemed to be reversely related to crude protein and lipid contents of the whole body of rockfish in this study. Unlike this study, however, the chemical composition of the whole body, muscle, viscera, and liver of rockfish was not affected by dietary inclusion of herb medicine mixture of *Artemisia asiatica* and *Epimedium koreanum* (Seo et al. 2009).

Plasma criteria (total protein, total cholesterol, GOT, GPT, and triglycerides) of fish measured in this study was not affected by the experimental diets due to wide variation in plasma values within the same treatment.

The dead black rockfish in 8-day post observation after *V. anguillarum* infection in this study exhibited the typical symptoms of diseased fish infected with *V. anguillarum*, such as exophthalmos, externally hemorrhages at the base of pectoral fins, on lateral line, around eyes and around the anal region, darkening of the skin, and swelling of abdomen with accumulation of ascites reported by Kent and Poppe (2002), Li et al. (2005), and Demircan and Candan (2006)'s studies.

Lower mortality (48.3, 53.3, and 73.3%) of black rockfish fed the YC, BB, and GG diets compared to the Con diet (98.3%) at the end of 8-day post observation indicated that YC, BB, and GG were all effective, especially YC was the most effective feed additive to lower mortality of rockfish at occurrence of *V. anguillarum*. Outstanding improvement in disease resistance against *V. anguillarum* of black rockfish fed the YC diet in this study indicated that YC was the most effective immunostimulant for rockfish. Comparison of administration of YC, GG, and BB of olive flounder with commercial immunostimulant (probiotics) or antibiotics against disease resistance is needed in the future.

Similarly, oral administration of YC, BB, and GG for 7 weeks lowered mortality of black rockfish when fish were infected with *Streptococcus parauberis* (Kim et al. 2016). Dietary inclusion of GG effectively improved disease resistance of rainbow trout against *Aeromonas hydrophila*, Asian seabass against *V. harveyi*, and Nile tilapia against *A. hydrophila* infections, respectively (Nya and Austin 2009; Talpur et al.

Table 4 Proximate composition (%) of the whole body of juvenile black rockfish *Sebastes schlegeli* fed the experimental diets containing the various sources of additives

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
Con	69.4 ± 0.23^b	17.3 ± 0.03^c	8.0 ± 0.06^b	5.1 ± 0.03^a
YC	68.2 ± 0.03^c	17.6 ± 0.00^b	8.2 ± 0.03^a	5.0 ± 0.06^a
GG	68.2 ± 0.03^c	17.8 ± 0.03^a	8.2 ± 0.03^a	5.1 ± 0.03^a
BB	70.0 ± 0.03^a	16.9 ± 0.21^d	7.8 ± 0.03^c	5.1 ± 0.00^a

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$)

Table 5 Plasma chemistry of black rockfish *Sebastes schlegeli* at the end of the 8 weeks feeding trial

Experimental diets	Total protein (g/dL)	Total cholesterol (mg/dL)	GOT (IU/L)	GPT (IU/L)	Triglyceride (mg/dL)
Con	4.2 ± 0.02[a]	203.0 ± 5.86[a]	64.0 ± 5.51[a]	1.0 ± 0.00[a]	269.0 ± 8.33[a]
YC	4.3 ± 0.34[a]	217.7 ± 18.81[a]	57.7 ± 1.76[a]	1.7 ± 0.33[a]	306.0 ± 14.36[a]
GG	4.4 ± 0.08[a]	217.0 ± 6.24[a]	57.0 ± 9.24[a]	2.7 ± 0.67[a]	287.3 ± 20.12[a]
BB	4.4 ± 0.18[a]	218.0 ± 12.17[a]	61.7 ± 6.12[a]	2.3 ± 0.33[a]	293.0 ± 16.09[a]

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$)

2013; Hassanin et al. 2014). Akintobi et al. (2013) showed that high antimicrobial activity of GG extract against six pathogenic microorganisms, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus subtilis*, and *Salmonella typhi in vitro* test and explained that GG can be used as substitute for antibiotics. Lower mortality (48.3 and 53.3%) of black rockfish fed the YC and BB diets compared to the GG diet (73.3%) at the end of 8-day post observation also indicated that YC and BB were also more effective than GG to lower mortality of rockfish at occurrence of *V. anguillarum*. In considering Kim et al. (2016)'s study showing that YC, GG, and BB were effective to lower mortality of black rockfish at occurrence *S. parauberis* and this study, they are all effective as an immunostimulant for both bacterial gram-negative and gram-positive pathogens. Vallejos-Vidal et al. (2016) reviewed several plants, herbs, algae extracts, and pathogen associated molecular patterns as immunostimulants in aquafeeds.

The plant-originated additives were effective to improve disease resistance in some marine fish (Sivaram et al. 2004; Ji et al. 2007a; Punitha et al. 2008; Kim et al. 2012; Talpur and Ikhwanuddin 2012; Talpur et al. 2013; Talpur 2014). Ji et al. (2007b) also reported that dietary inclusion of mixture herb of *Massa medicate fermentata*, *Crataegi fructus*, *Artemisia capillaries*, and *Cnidium officinale* at a ratio of 2:2:1:1 at the concentration 0.3, 0.5, and 1% improved not only weight gain and FER including fatty acid utilization, but also stress recovery of olive flounder against air exposure and anesthetizing test compared to the control diet without any additive or 0.1% mixture inclusion diet.

Conclusions

Oral administration of YC can improve not only weight gain, SGR, and FER of black rockfish, but lower mortality of rockfish at occurrence of *V. anguillarum*.

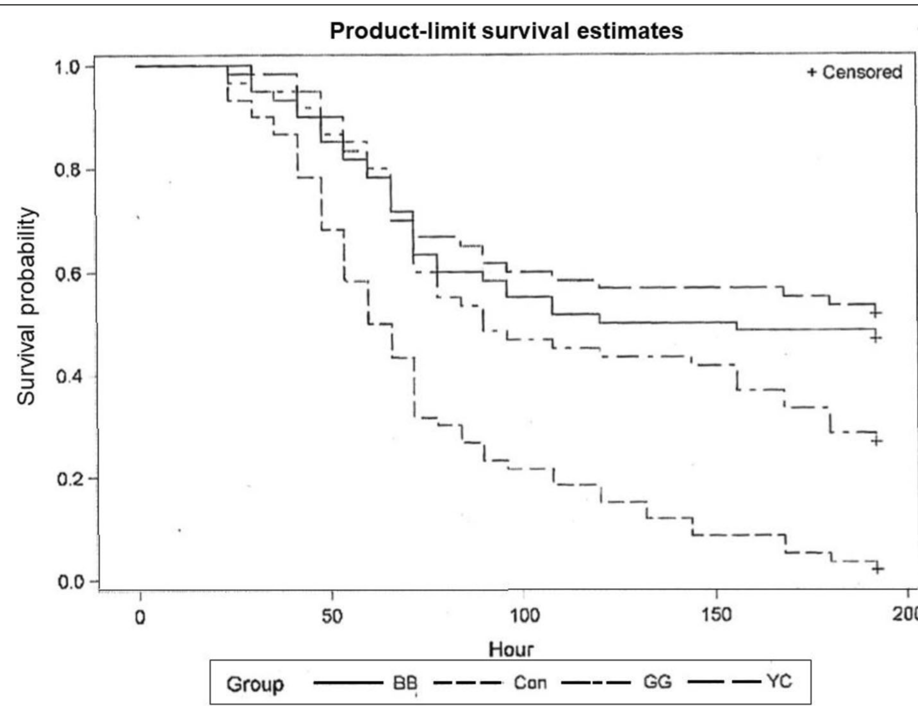

Fig. 1 Kaplan–Meier survival curves of juvenile black rockfish *Sebastes schlegeli* fed the experimental diets containing various sources of additives for 8 weeks, and then infected with *Vibrio anguillarum* ($P < 0.0001$, Log-rank and Wilcoxon tests)

Abbreviations
YC: Yacon; GG: Ginger; BB: Blueberry; EQ: Ethoxyquin; SGR: Specific growth rate; FER: Feed efficiency ratio; PER: Protein efficiency ratio; PR: Protein retention; GOT: Glutamic oxaloacetic transaminase; GPT: Glutamic pyruvic transaminase

Acknowledgments
This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2017R1A2B4009773).

Authors' contributions
SHC designed the study and prepared the draft. KWL and HSJ ran the feeding trial and analyzed the chemical composition of the diets and fish. All authors read and approved the final manuscript.

Funding
The design of the study, collection, analysis, and interpretation of the data and writing of the manuscript were supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2017R1A2B4009773).

Availability of data and materials
All datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval
All experimental protocols followed the guidelines of the Institutional Animal Care and Use by Committee of the Korea Maritime and Ocean University.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 27 November 2019 Accepted: 10 March 2020
Published online: 14 April 2020

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