



## Effect of Various Sources of Dietary Additives on Growth, Body Composition, and 1 Challenge Test Survival of Juvenile Rockfish *Sebastes schlegeli*

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### Abstract

Whether the inclusion of the various sources of commercially available dietary additives [ginger (GG), cheonggukjang (CJ), blueberry (BB), persimmon (PM), tomato (TT), broccoli (BC) and yacon (YC)] has an effect on growth, body composition, and challenge test survival of juvenile rockfish *Sebastes schlegeli* was investigated. Twenty-four groups of 70 fish (1680 in total) were each fed one of eight experimental diets: a control diet (Con) without additives, and diets containing GG, CJ, BB, PM, TT, BC or YC. Fish were hand-fed to satiation twice daily for 7 weeks. At the end of this period, 20 fish from each tank were infected with *Streptococcus parauberis* and monitored for 10 days. Fish fed the YC diet had greater weight gain compared with fish fed the other diets. Feed efficiency and protein efficiency ratio of fish fed the TT and YC diets were higher than of fish fed other diets. Cumulative mortality of fish fed the GG, BB, and YC diets was lower than of fish fed the other diets on day 5 post infection. We conclude that yacon was the best dietary additive for improving rockfish weight gain, while ginger, blueberry, and yacon effectively lowered mortality of rockfish infected with *S. parauberis*.

**Keywords:** Rockfish (*Sebastes schlegeli*), additive, challenge test, *Streptococcus parauberis*.

### Introduction

In annual aquaculture production volume, the rockfish *Sebastes schlegli* has been highly ranked in Korea for the last few decades, second only to the olive flounder *Paralichthys olivaceus* (MFAFF, 2014). A variety of feeding trials has been performed with the species to determine dietary nutrient requirements (Kim *et al.*, 2001; Lee *et al.*, 2002; Yan *et al.*, 2007), the digestibility of various feed ingredients (Lee, 2002), alternative animal and/or plant protein sources for fish meal in the diet (Lee *et al.*, 1996), optimum feeding rate depending on temperature (Mizanur *et al.*, 2014), optimum feeding frequency (Lee *et al.*, 2000), dietary additives to improve lysozyme activity and stress recovery (Hwang *et al.*, 2013), and immune response (Kim *et al.*, 1999).

High fish mortality rates commonly occur in year-round cultures at fish farms due to annual recurring disease outbreaks. As the dietary administration of certain synthetic chemicals such as antibiotics to fish intended for human consumption is prohibited in some countries (Tang *et al.*, 2001), the development of alternative natural sources of dietary antibiotic additives that improve growth performance

and/or immune response and lowers fish mortality in the event of disease outbreak remains essential (Defoirdt *et al.*, 2011; Talpur, 2014). An oral administration of 0.5% aloe lowered cumulative mortality of rockfish infected by *Vibrio alginolyticus* (Kim *et al.*, 1999). Nya and Austin (2009a) showed that dietary inclusion of 0.5% and 1% garlic effectively lowered mortality of rainbow trout infected with *Aeromonas hydrophila*. A combined vaccine containing formalin-inactivated *Edwardsiella tarda*, *Streptococcus iniae*, and *S. parauberis* successfully induced humoral and protective immunity in olive flounder (Han *et al.*, 2011).

Ginger *Zingiber officinale* Roscoe (GG), containing gingerols and shogaols, is well known to display antioxidant properties (Balestra *et al.*, 2011) and is widely used for the treatment of several diseases (Ali *et al.*, 2008). Orally administrated GG has been shown to be effective as an antioxidant in animals (Kota *et al.*, 2008; Lee *et al.*, 2013). Dietary inclusion of GG effectively improved not only weight gain, but also disease resistance of rainbow trout *Oncorhynchus mykiss* and Asian seabass *Lates calcarifer* (Nya and Austin, 2009a; Talpur *et al.*, 2013). The traditional fermented soyfood cheonggukjang (CJ) contains isoflavones and

anthocyanin, which have antioxidant and free radical-scavenging properties (Shon *et al.*, 2007; Kim *et al.*, 2009). Antioxidant properties have also been demonstrated for Southern black blueberry *Vaccinium ashei* Reade (BB) (anthocyanin; Jeong *et al.*, 2012), persimmon *Diospyros kaki* L. (PM) (polyphenols; Hwang *et al.*, 2011; Kim *et al.*, 2011; Zhang *et al.*, 2011), tomato *Solanum lycopersicum* L. (TT) (lycopene; Kim and Chin, 2011; Goyal *et al.*, 2013), broccoli *Brassica oleracea* L. (BC) (glucosinolates; Banerjee *et al.*, 2012) and yacon *Polymnia sonchifolia* Poeppig and Endlicher (YC) (polyphenols; Kim *et al.*, 2010a; Park *et al.*, 2012). The effectiveness of oral application in animals was shown for all of these species (BB: Dunlap *et al.*, 2006; Molan *et al.*, 2008; Papandreou *et al.*, 2009; PM: Kim *et al.*, 2003; Chen *et al.*, 2012; TT: Bobek, 1999; Moreira *et al.*, 2005; BC: Muller *et al.*, 2012; Tomofuji *et al.*, 2012; YC: Kim, 2013).

In this study, we investigated the effect of dietary inclusion of these additives on growth, body composition, and challenge test survival of juvenile rockfish.

## Materials and Methods

### Fish and Experimental Conditions

Juvenile rockfish were purchased from a private hatchery and acclimated to the experimental conditions for 2 weeks. During the acclimation

period, fish were hand-fed a commercial extruded pellet (Suhyup Feed, Gyeongsangnam-do, Korea) twice a day at a ratio of 2-3% of fish body weight. A total of 1680 fish averaging 3.0 g were randomly distributed into 24 fiber-reinforced plastic flow-through tanks with a volume of 200 L, yielding 70 fish per tank. The water source was sand-filtered natural seawater. Constant aeration was supplied to each tank. The flow rate of water into each tank was 4.6 L/min. Water temperature ranged from 15.8 to 23.1°C (mean±SD: 20.5±2.64°C) and the photoperiod was left to natural conditions.

### Preparation of the Experimental Diets

Eight experimental diets were prepared; a control diet (Con) with no additive, and GG, CJ, BB, PM, TT, BC and YC diets (Table 1). One percent (dry matter) of each additive, which is commercially available in Korea, was included into the treatment diets at the expense of wheat flour. The Con diet was prepared to satisfy dietary nutrient requirements for rockfish (Kim *et al.*, 2001; Kim *et al.*, 2004). Fish meal, dehulled soybean meal, and casein were used as protein sources in the experimental diets, wheat flour as carbohydrate source, and squid liver and soybean oils as lipid sources.

The ingredients of the experimental diets were well mixed with water at a ratio of 3:1 and pelletized by a lab pellet-extruder (Dongsung Mechanics, Busan, Korea). Diets were dried at room temperature

**Table 1.** Ingredients of the experimental diets (% DM)

Ingredients	Experimental diets							
	Con	GG <sup>1</sup>	CJ <sup>1</sup>	BB <sup>1</sup>	PM <sup>2</sup>	TT <sup>2</sup>	BC <sup>2</sup>	YC <sup>2</sup>
Fish meal <sup>3</sup>	58	58	58	58	58	58	58	58
Soybean meal <sup>4</sup>	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Casein	4	4	4	4	4	4	4	4
Wheat flour	21	20	20	20	20	20	20	20
Additives		1	1	1	1	1	1	1
Squid liver oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Soybean oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Carboxymethyl cellulose (CMC)	1	1	1	1	1	1	1	1
Vitamin premix <sup>5</sup>	1	1	1	1	1	1	1	1
Mineral premix <sup>6</sup>	1	1	1	1	1	1	1	1
Choline	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
<b>Nutrients (% DM)</b>								
Dry matter	92.1	92.5	92.0	92.0	91.8	91.8	92.3	92.2
Crude protein	50.2	50.3	50.6	50.8	50.4	50.6	50.2	50.7
Crude lipid	11.6	11.2	11.4	11.4	11.6	11.3	11.4	11.3
Ash	9.4	9.7	9.6	9.5	9.3	9.5	9.4	9.3
Carbohydrate	28.8	28.8	28.4	28.3	28.7	28.6	29.0	28.7

<sup>1</sup>GG (ginger), <sup>1</sup>CJ (cheonggukjang) and <sup>1</sup>BB (blueberry), and <sup>2</sup>PM (persimmon), <sup>2</sup>TT (tomato), <sup>2</sup>BC (broccoli) and <sup>2</sup>YC (yacon) were commercially available products and purchased from Tojongherb Co Ltd. (Dongdaemun-gu, Seoul, Korea) and Yundoo Co Ltd. (Uiijeongbu-si, Gyeonggi-do, Korea), respectively

<sup>3</sup>Fish meal and <sup>4</sup>Soybean meal were purchased from Abank Co Ltd. (Seocho-gu, Seoul, Korea).

<sup>5</sup>Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbic acid, 121.2; DL- $\alpha$ -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.

<sup>6</sup>Mineral premix contained the following ingredients (g/kg mix): MgSO<sub>4</sub>·7H<sub>2</sub>O, 80.0; NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, 370.0; KCl, 130.0; ferric citrate, 40.0; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 20.0; Ca-lactate, 356.5; CuCl<sub>2</sub>, 0.2; AlCl<sub>3</sub>·6H<sub>2</sub>O, 0.15; KI, 0.15; Na<sub>2</sub>Se<sub>2</sub>O<sub>3</sub>, 0.01; MnSO<sub>4</sub>·H<sub>2</sub>O, 2.0; CoCl<sub>2</sub>·6H<sub>2</sub>O, 1.0.

overnight and stored at -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 hours) for 7 days a week over 7 weeks.

### Analytical Procedures of the Experimental Diets and Fish

Ten fish at the start and seven fish at the termination of the feeding trial were sampled from each tank and sacrificed for proximate analysis conducted according to standard AOAC (1990) protocols. Crude protein content was determined by the Kjeldahl method using an Auto Kjeldahl System (B-324/435/412: Buchi, Flwail, Switzerland), and crude lipid content was measured using the ether-extraction method using a Soxtec TM 2043 Fat Extraction System (Tecator, Hoganas, Sweden). Moisture content was determined by oven drying at 105°C for 24 h, fiber content was determined using an automatic analyzer (Fibertec: Tecator, Sweden), and ash content was determined using a muffle furnace at 550°C for 4 h.

### Challenge Test

At the end of 7-week feeding trial, 20 fish of externally normal appearance shown to be free from bacterial infection were selected from each tank and stocked into 24 static 200 L tanks. The fish were used for the *Streptococcus parauberis* challenge and water was not exchanged. The pathogenic gram-positive *S. parauberis* (KCTC11980BP) reference strain isolated from rockfish was used for the challenge.

The culture suspension of *S. parauberis* was grown on agar for 24 h, collected, washed and suspended in sterile 0.85% saline solution, and then counted. Fish were then artificially infected by intraperitoneal injection with 0.1 mL of pathogenic *S. parauberis* culture suspension containing  $1.2 \times 10^5$  cells/ml. The fish were monitored for ten days post-infection; dead fish were removed every 6 h for the first four days and every 12 h for the remainder of the study. Cumulative mortality of fish was calculated as follow: cumulative mortality (%) = cumulative

number of dead fish at each elapsed time  $\times$  100/total number of infected fish. Fish were starved throughout the 10-day challenge test.

### Statistical Analysis

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 statistical significance (at a level of  $P < 0.05$ ) of the differences among mean response to the dietary treatments. Percentage data was arcsine-transformed prior to statistical analysis.

### Results and Discussions

Weight gain and survival of fish supplied with the different experimental diets are shown in Table 2. The survival of rockfish for the 7-week feeding trial was over 98% and was not significantly affected by the various sources of dietary additives. Weight gain (g/fish) for fish fed the YC diet was significantly greater than that for fish fed the other diets. This is in contrast to Kim *et al.* (2010b) who reported that dietary supplementation of 5 and 10% YC effectively lowered the weight gain of rats fed a high fat-high cholesterol diet. The GG, BB and Con diets also increased weight gain but to a lesser degree. The GG result is consistent with previous reports showing that the weight gain of rainbow trout and Asian sea bass improved proportionally when GG was included in the diet (Nya and Austin, 2009b; Talpur *et al.*, 2013).

In this study, the weight gain of fish fed the CJ, PM and BC diets was significantly lower compared to fish fed the Con diet. The poorest weight gain was observed in fish fed the BC diet. It was previously shown that dietary inclusion of BC extract did not affect weight gain of broilers chickens compared to a control diet (Mueller *et al.*, 2012). There was also no effect on weight gain in rats fed a high cholesterol diet with an oral supplementation of BC (Tomofuji *et al.*, 2012). The effect of CJ and PM on weight gain in the present study is consistent with previous studies, as the oral administration of 19.7% CJ lowered weight gain of rats fed high cholesterol diet (Kim *et al.*,

**Table 2.** Survival (%) and weight gain (g/fish) of juvenile rockfish *Sebastes schlegeli* fed experimental diets containing the various sources of additives for 7 weeks

Experimental diets	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)
Con	3.0±0.00	16.2±0.15 <sup>bc</sup>	100.0±0.00	13.2±0.15 <sup>bc</sup>
GG	3.0±0.00	16.5±0.07 <sup>b</sup>	99.0±0.48	13.5±0.07 <sup>b</sup>
CJ	3.0±0.00	15.2±0.09 <sup>d</sup>	100.0±0.00	12.2±0.09 <sup>d</sup>
BB	3.0±0.00	16.3±0.09 <sup>bc</sup>	99.5±0.48	13.3±0.09 <sup>bc</sup>
PM	3.0±0.01	15.1±0.12 <sup>d</sup>	100.0±0.00	12.1±0.12 <sup>d</sup>
TT	3.0±0.00	16.0±0.09 <sup>c</sup>	100.0±0.00	13.0±0.10 <sup>c</sup>
BC	3.0±0.01	13.0±0.17 <sup>e</sup>	99.0±0.95	10.0±0.18 <sup>e</sup>
YC	3.0±0.01	17.4±0.27 <sup>a</sup>	98.6±1.43	14.4±0.28 <sup>a</sup>

Values (means of triplicates±SE) in the same column sharing the same superscript letter are not significantly different ( $P > 0.05$ ). GG, CJ, BB, PM, TT, BC, and YC: refer to the footnotes of Table 1.

2009). The addition of 0.2% PM leaf extract to this type of rat diet did not result in any change in weight gain although it has been suggested that supplementation of PM leaf extract promoted the excretion of fecal sterols and led to decreased absorption of dietary cholesterol (Kim *et al.*, 2003). Hypo- and hyper-energetic diets supplemented with 0.5% TT did not affect weight gain in rats (Moreira *et al.*, 2005). Additional dietary additives, such as garlic (*Allium sativum*), neem (*Azadirachta indica*) and peppermint (*Mentha piperita*) have been reported to effectively improve weight gain in fish (Talpur and Ikhwanuddin, 2012, 2013; Talpur, 2014).

Feed consumption, feed and protein efficiency ratios and protein retention of fish supplied with the different experimental diets are shown in Table 3. Feed consumption of the YC diet was significantly higher compared to all other diets except for the BB diet. Feed consumption of the Con, GG and BB diets was significantly higher than that of the CJ, PM, TT and BC diets. The lowest feed consumption was observed with the BC diet. Similarly, dietary inclusion of CJ was reported to lower feed consumption by rats (Kim *et al.* 2009). The oral administration of TT lowered food intake of rats of both hypo- and hyper-energetic diets (Moreira *et al.*, 2005). Supplementation of YC into the high fat-high cholesterol diet lowered rat feed consumption (Kim *et al.*, 2010b). However, dietary inclusion of BC did not affect feed intake of broiler chickens and rats (Muller *et al.*, 2012; Tomofuji *et al.*, 2012).

Feed efficiency (FE) and protein efficiency ratio (PER) of fish fed the TT and YC diets were significantly higher compared to the other diets based on lower feed consumption but relative higher weight gain (Table 3). FE and PER of fish fed the Con and GG diets were significantly higher than those of fish fed the CJ, BB, PM and BC diets. Previous studies similarly found that dietary inclusion of GG effectively improved FE and PER of fish (Nya and Austin, 2009b; Talpur *et al.*, 2013). The poorest FE and PER were observed in fish fed the BC diet. Protein retention (PR) of fish fed the GG, BB and YC

diets was significantly higher than of fish fed the other diets. PR of fish fed the TT diet was significantly higher than of fish fed the CJ, PM and BC diets, but not of fish fed the Con diet. Again, the poorest PR was obtained in fish fed the BC diet. Oral administration of fermented soybean did not affect growth and feed utilization of olive flounder, but increased antioxidant activity in diet and nonspecific immune response of fish (Kim *et al.*, 2010c).

The increased feed consumption and improved FE and PER of fish fed the YC diet suggested that YC could have potential as a growth-promoting agent for rockfish. However, the weight gain of rats fed a high fat-high cholesterol diet supplemented with 10% YC was lower than of rats consuming an equal amount of control diet (Kim *et al.*, 2010b) and there was no effect on weight gain, feed intake and FE of broiler chickens fed with the dietary inclusion of 0.5, 1 and 2% YC byproduct in a 5-week feeding trial (Kim, 2013). However, in the latter study the level of thiobarbituric acid reactive substance (TBARS) in chicken thigh meat decreased, and DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical-scavenging activity increased in a dose-dependent manner. In rockfish, dietary inclusion of green tea extract lowered weight gain and FE, but improved lipid utilization, lysozyme activity and stress recovery (Hwang *et al.*, 2013).

Moisture, crude protein and lipid content and ash content of fish supplied with different experimental diets are shown in Table 4. Moisture content of fish fed the CJ diet was significantly higher than of fish fed the Con, BB, PM, TT and BC diets, but not significantly different to fish fed the GG and YC diets. The lowest moisture content was obtained from fish fed the TT diet. Crude protein content of fish fed the GG, BB and YC diets were significantly higher compared to fish fed the Con, CJ, PM, TT and BC diets. Crude lipid content of fish fed the YC diet was significantly higher than of fish fed the Con, GG, BB, PM, TT and BC diets, but not significant to fish fed the CJ diet. In contrast, Kim *et al.* (2010b) reported that the dietary inclusion of YC could potentially

**Table 3.** Feed consumption (g/fish), feed efficiency (FE), protein efficiency ratio (PER) and protein retention (PR) of juvenile rockfish *Sebastes schlegeli* fed experimental diets containing the various sources of additives for 7 weeks

Experimental diets	Feed consumption (g/fish) <sup>1</sup>	FE <sup>2</sup>	PER <sup>3</sup>	PR <sup>4</sup>
Con	12.4±0.16 <sup>c</sup>	1.06±0.002 <sup>b</sup>	2.11±0.005 <sup>b</sup>	33.3±0.47 <sup>bc</sup>
GG	12.7±0.07 <sup>bc</sup>	1.07±0.006 <sup>b</sup>	2.11±0.010 <sup>b</sup>	39.1±0.84 <sup>a</sup>
CJ	11.7±0.06 <sup>d</sup>	1.04±0.003 <sup>c</sup>	2.04±0.006 <sup>c</sup>	31.9±0.52 <sup>c</sup>
BB	12.8±0.05 <sup>ab</sup>	1.04±0.009 <sup>c</sup>	2.05±0.016 <sup>c</sup>	37.6±0.81 <sup>a</sup>
PM	11.7±0.06 <sup>d</sup>	1.03±0.005 <sup>c</sup>	2.04±0.009 <sup>c</sup>	32.7±0.31 <sup>c</sup>
TT	11.9±0.04 <sup>d</sup>	1.09±0.005 <sup>a</sup>	2.16±0.010 <sup>a</sup>	34.9±0.29 <sup>b</sup>
BC	10.5±0.16 <sup>e</sup>	0.95±0.004 <sup>d</sup>	1.90±0.005 <sup>d</sup>	29.5±0.57 <sup>d</sup>
YC	13.2±0.27 <sup>a</sup>	1.09±0.005 <sup>a</sup>	2.15±0.011 <sup>a</sup>	38.8±0.34 <sup>a</sup>

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

1 Feed consumption = Dry total feed consumption / number of fish.

2 Feed efficiency (FE) = Weight gain of fish / feed consumed.

3 Protein efficiency ratio (PER) = Weight gain of fish / protein consumed.

4 Protein retention (PR) = Protein gain×100 / protein consumed.

GG, CJ, BB, PM, TT, BC, and YC: refer to the footnotes of Table 1.

reduce lipid storage of rats fed a high cholesterol diet as the YC supplement might improve serum, liver and adipose tissue lipid metabolism. Administration of green tea extract has been shown to lower total lipid content in rockfish (Hwang *et al.*, 2013). The ash content of fish fed the Con diet was significantly higher than of fish fed the other diets, followed by the BC and YC diets. The ash content of fish fed the BB diet was significantly higher than of fish fed the GG and TT diets, but not significantly different to fish fed the PM diet. The lowest ash content was obtained from fish fed the CJ diet.

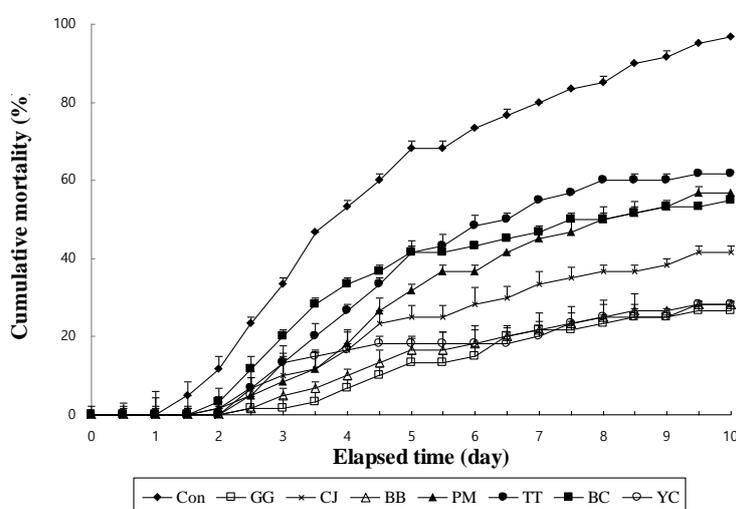
Cumulative mortality of fish supplied with different experimental diets is shown in Figure 1. Cumulative mortality of fish fed the Con diet was significantly higher than of other fish at 36 h post infection. This indicates that all dietary additives used in this study were effective in lowering the mortality of rockfish infected with *S. parauberis*. The dead fish exhibited typical symptoms similar to those of olive flounder infected with *S. parauberis*, such as exophthalmic eyes, hemorrhages on the operculum and gills, darkening of the skin and distended abdomen (Baek *et al.*, 2006). Cumulative mortality of fish fed the GG and BB diets was significantly lower

than of fish fed the BC diet at 60 h post infection, but not significantly different to fish fed the CJ, PM, TT and YC diets. Cumulative mortality of fish fed the GG, BB and YC diets was significantly lower compared to fish fed the other diets at day 5 post infection, but did not differ significantly from each other. At day 10 the lowest cumulative mortality was observed in fish fed the GG diet, followed by the BB, YC, CJ, BC, PM, TT and Con diets. This lower mortality indicates that including GG, BB and YC diets as a feed additive could have high potential to lower *S. parauberis*-related mortality in rockfish. A previous study reported similar results, finding 64% mortality in control rainbow trout infected with *A. hydrophila* compared with 16%, 4% and 0% mortality in fish receiving 1, 0.05 and 0.5% GG, respectively, when fed supplemented commercial diet for two weeks prior to infection (Nya and Austin, 2009b). Ohara *et al.* (2008) reported that the ginger family *Zingiberaceae* contains the most abundant and stable antibacterial components against *S. mutans* among 81 tested edible plants. Cumulative mortality of fish fed the CJ diet was also significantly lower than of fish fed the Con, PM, TT and BC diets 5 days post infection. The dietary additives garlic, neem and

**Table 4.** Proximate analysis for composition (%) of the whole body of juvenile rockfish *Sebastes schlegeli* fed the experimental diets containing the various sources of additives for 7 weeks

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
Con	69.7±0.67 <sup>cd</sup>	15.6±0.15 <sup>b</sup>	7.8±0.03 <sup>bc</sup>	4.5±0.07 <sup>a</sup>
GG	71.8±0.09 <sup>ab</sup>	17.8±0.26 <sup>a</sup>	7.2±0.09 <sup>d</sup>	3.8±0.03 <sup>de</sup>
CJ	72.3±0.52 <sup>a</sup>	15.4±0.22 <sup>b</sup>	7.9±0.03 <sup>ab</sup>	3.8±0.06 <sup>e</sup>
BB	70.4±0.66 <sup>bcd</sup>	17.7±0.21 <sup>a</sup>	7.2±0.03 <sup>d</sup>	4.0±0.06 <sup>c</sup>
PM	70.0±0.23 <sup>cd</sup>	15.8±0.07 <sup>b</sup>	7.7±0.12 <sup>c</sup>	4.0±0.03 <sup>cd</sup>
TT	68.9±0.32 <sup>d</sup>	15.9±0.12 <sup>b</sup>	7.7±0.03 <sup>c</sup>	3.8±0.03 <sup>de</sup>
BC	69.9±0.66 <sup>cd</sup>	15.3±0.26 <sup>b</sup>	7.6±0.06 <sup>c</sup>	4.3±0.06 <sup>b</sup>
YC	70.9±0.27 <sup>abc</sup>	17.5±0.20 <sup>a</sup>	8.1±0.09 <sup>a</sup>	4.2±0.03 <sup>b</sup>

Values (means of triplicates±SE) in the same column sharing the same superscript letter are not significantly different ( $P>0.05$ ). GG, CJ, BB, PM, TT, BC, and YC: refer to the footnotes of Table 1.



**Figure 1.** Cumulative mortality (%) of juvenile rockfish *Sebastes schlegeli* fed the experimental diets containing the various sources of additives for 7 weeks, and then infected by *Streptococcus parauberis* for 10 days (means of triplicates±SE). GG, CJ, BB, PM, TT, BC, and YC are explained in the footnotes of Table 1.

peppermint have been reported successfully replace antibiotics in aquafeed and to lower the mortality of Asian seabass infected with *Vibrio harveyi* (Talpur and Ikhwanuddin, 2012, 2013; Talpur, 2014).

The dietary inclusion of 1 and 2% *Broussonetia kazinoki* was shown to effectively improve immune responses against *S. parauberis* infection in olive flounder (Kim et al., 2012). Dietary supplementation of 0.5-2% YC byproduct lowered mortality of chickens in a 5-week feeding trial and effectively decreased the level of thiobarbituric acid reactive substances, but increased the total phenol content and DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity in the thigh meat (Kim, 2013). Dogs fed a diet containing 2% BB in the form of lowbush blueberry (*V. angustifolium*) may be better protected against exercise-induced oxidative damage (Dunlap et al., 2006). Molan et al. (2008) reported that water-soluble BB extract may be a good satiety inducer and weight management modulator, and that a reduction in food intake and a decrease in body weight in rats may therefore not have been merely a consequence of antioxidant mechanisms.

In conclusion, of the tested dietary supplements, yacon showed the best results in improving weight gain in rockfish, and yacon and tomato were effective in improving feed efficiency and protein efficiency ratio. Ginger, blueberry and yacon were effective in lowering the cumulative mortality of rockfish infected with *S. parauberis*.

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