Thesis for the Degree of Master of Science

Effect of dietary inclusion of the ethanol extracts of yacon and ginger on growth, feed utilization, plasma chemistry and body composition of juvenile olive flounder (*Paralichthys olivaceus*) and challenge test against *Vibrio anguillarum* compared to a commercial probiotic (Super lacto®)

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Abstract

The effect of dietary inclusion of the ethanol extracts of yacon (YC) and ginger (GG) on growth, feed utilization, body composition, plasma chemistry of juvenile olive flounder and challenge test against *Vibrio anguillarum* compared to a commercial probiotic (Super lacto®) was determined. Three hundred and sixty juvenile fish were randomly distributed into 12, 50 L flow-through tanks (30 fish per tank). Four experimental diets were prepared in triplicates. The control diet (CON) contained no additive. The 0.5% Super lacto® and 1% ethanol extracts of YC and GG were included into the CON diet, referred to as the

SUP, YCE and GGE diets, respectively. Each diet was hand-fed to satiation twice daily for 8 weeks. After the 8-week feeding trial, fish were artificially infected with *V. anguillarum* and mortality was monitored for the next 7 days after infection. Dietary additives did not affect the weight gain, specific growth rate, feed efficiency, whole body composition and plasma chemistry of fish. However, protein efficiency ratio was significantly higher in fish fed the CON and GGE diets than that of fish fed the SUP and YCE diets. The cumulative mortality of fish fed the YCE and GGE diets was significantly lower than that of fish fed the CON and SUP diets at 72 h after infection until the end of the 7-day post observation. Therefore, the ethanol extracts of YC and GG can be effectively utilized to stimulate the immunity of olive flounder to lower mortality at occurrence *V. anguillarum*.

KEY WORDS: Olive flounder (*Paralichthys olivaceus*); Ginger; Yacon; Challenge test; *Vibrio anguillarum*; Probiotic

배합사료내 야콘과 생강 에탄올 추출물의 첨가가 상업용 프로바이오틱스(Super lacto®)와 비교하여 넙치(*Paralichthys olivaceus*)치어의 성장, 체조성, 혈액성상 및 *Vibrio anguillarum* 면역성에 미치는 영향

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요 약

넙치용 배합사료내 야콘(yacon)과 생강(ginger) 에탄올 추출물의 첨가시 상업용 프로바이오틱스(Super lacto®)와 비교하여 넙치 치어의 성장, 사료이용성, 체조성, 혈액성상 및 *Vibrio anguillarum* 감염에 따른 면역에 미치는 영향을 조사하였다. 총 360 마리의 넙치 치어(시작시 마리당 무게: 7.2 g)를 12 개의 50 L 유수식 수조에 30 마리씩 무작위로 각각 수용하였다. 첨가제가 포함되지 않은 대조구(CON) 사료와 대조구 사료 제조시 물 대신에 0.5%의 상업용 프로바이오틱스(Super lacto®)가 포함된 SUP 사료, 대조구 사료내 물 대신에 1%의 야콘과 생강 에탄올 추출물을 첨가한 YCE 와 GGE 사료를 각각 준비하였다. 총 4 개의 실험사료를 준비하였으며, 각 실험사료는 3 반복구를 두었다. 사육실험 기간은 총 8 주간이며, 8 주간의 사육실험 종료 후 인위적으로 *V. anguillarum* 으로 인위적으로 감염시킨 이후, 총 7 일간의 누적폐사율을 조사하였다. 8 주간의 사육실험 종료시 넙치의 생존율, 증체량, 일일성장률, 체조성, 사료이용성 및 혈액성상학적 차이는 모든 실험구간에 유의적인 차이가 없었다. 그러나 YCE 와 GGE 사료를 공급한 실험구에서의

넙치의 누적폐사율은 세균 감염 이후, 72 시간째부터 관찰 종료시(감염이후 7 일)까지 CON 과 SUP 사료를 공급한 실험구보다 유의적으로 낮은 누적폐사율을 나타냈다. 따라서 넙치용 배합사료내 야콘과 생강의 에탄올 추출물의 첨가는 넙치 치어의 성장과 사료이용성에는 영향을 미치지는 않지만, *V. anguillarum* 에 따른 질병 발생시 넙치의 폐사율을 낮출 수 있는 우수한 면역개선제이었다.

KEY WORDS: 넙치 (*Paralichthys olivaceus*); 생강; 야콘; 면역성 평가; *Vibrio anguillarum*; Probiotic

1. Introduction

Olive flounder (*Paralichthys olivaceus*) has good flavor and excellent growth (Kim et al. 2017b), and is one of the most commercially important marine fish for aquaculture in Eastern Asia including Korea, China and Japan (Cho et al. 2013). An annual aquaculture production of olive flounder reached 41,207 metric tons in Korea in 2017 (KOSIS 2018). However, frequent outbreak of infectious diseases caused by bacteria (*vibriosis*, *edwardsiellosis*, *streptococcosis*, *flexibacteriosis*), viruses (*scuticociellosis*) and parasites (Toranzo et al. 2005; Palaksha 2008; Harikrishnan et al. 2010; Gozlan et al. 2014) affected the survival and growth of fish and caused huge economic losses to fish farmers (Kitamura et al. 2007; Bulfon et al. 2013; Rico et al. 2013). Among the bacterial infections, vibriosis is a major disease in fish caused by several bacterial strains including *V. anguillarumin* Korea (Kim et al. 2014).

Fish farmers generally use various synthetic antibiotics or chemotherapy drugs to treat diseases (Park 2009). Oxytetracycline, norfloxacin, enrofloxacin and different sulphonamids are widely used synthetic antibiotics in aquaculture. (Holmström et al. 2003; Miranda and Zemelman 2003; Akinbowale et al. 2006). However, antibiotics have undesirable effects on environment and non-targeted organisms including human (Abutbul et al. 2005; Le et al. 2005; Reverter et al. 2014; Ameen et al. 2018). In addition, application of these antibiotics may lead to the emergence of antibiotic-resistant bacteria (Smith et al. 1994; Cabello 2006; Park 2009; Bulfon et al. 2013; Rico et al. 2013). The use of synthetic antibiotics in the production of fish is not permitted by the Korean government (Choi et al. 2010) as well as worldwide (Aarestrup et al. 2001; Caspar 2002; Casewell et al. 2003; Demir et al. 2003) for human consumption. Therefore, environment-friendly and cost effective alternative immune modulatory as a substitute for antibiotics in aquatic feed is highly required.

Herb extracts are used to treat certain diseases caused by bacteria, parasites, virus, or fungi (Abutbul et al. 2005; Vaseeharan et al. 2011; Bulfon et al. 2013; Hai 2015; Huang et al. 2015). Yacon (YC) (*Smallanthus sonchifolin*, Poepp. and Endl.), synonym of *Polymnia sonchifolia*, a native fruit of the Andes (Lachman et al. 2003; Genta et al. 2005; Qian et al. 2006; Padla et al. 2012). YC contains phenolic compounds (chlorogenic, caffeic and ferulic acids), which stimulate body immunity, and the antioxidant activity (Yan et al. 1999; Simonovska et al. 2003; Qian et al. 2006; Vos et al. 2006; Fujitani et al. 2007; Ojansivu et al. 2011). In addition, YC contains fructooligosaccharides, which have prebiotic effect and beneficial to lower blood glucose level in rats (Gentaetal. 2005), and YC roots and leaves exhibits excellent antibacterial and antifungal activity (Inoue et al. 1995; Lin et al. 2003; Shin et al. 2017). Low-dose intake of YC has no side reaction or toxic effects on rats (Genta et al. 2005). Improved growth performance and disease resistances of *Streptococcus parauberis*, *Edwardsiella trada* and *S. iniae* in rockfish (*Sebastes schlegeli*) fed diet supplemented with YC have been reported (Kim et al. 2016; Lee et al. 2016; Kim et al. 2017a).

Ginger (GG) (*Zingiber officinale*, Roscoe) belonging to the family Zingiberaceae is a perennial herb (Karuppiah and Rajaram 2012) contains gingerol, gingerdiol and gingerdione, which has antioxidant activity (Akbarian et al. 2011). It is also reported that gingerol can induce interleukin-6 (a protein B cell stimulant) activity, which enhance immunity in human (Benny et al. 2004). The GG also has antibacterial (*Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*) and antifungal (*Rhizoctonia solani*) properties (Mascolo et al. 1989; Agarwal et al. 2001; Ekwenye and Elegalam 2005; Malu et al. 2009) and can be used to prevent or treat drug-resistant microbial infections (Karuppiah and Rajaram 2012). The ethanolic GG extract (5 and 7.5 ppt) effectively treated infections caused by *Gyrodactylus turnbul* in guppies (*Poecilia reticulata*) via bath treatment (Levy et al. 2015). Ameen et al. (2018) also found that the aqueous extracts of GG was most effectively in inhibiting the growth of *Ichthyophonus* sp. in vitro compared to linseed (*Linum usitatissimum*), henna (*Lawsonia inermis*) and white turmeric (*Curcuma zedoaria*) in tilapia (*Oreochromis aureus*)'s feed. The diet supplemented with GG achieved higher weight gain, feed utilization and lower cumulative mortality of juvenile rockfish than the control diet against S. parauberis, E. trada and S. iniae (Kim et al. 2016; Lee et al. 2016; Kim et al. 2017a). Improved growth and immunity were also observed in rainbow trout (*Oncorhynchus mykiss*), Asian sea bass (*Lates calcarifer*) and Niletilapia (*Oreochromis niloticus*) fed the diet containing GG (Nya et al. 2009; Talpur et al. 2013; Hassanin et al. 2014).

Probiotics are live microbial feed additives, which can improve the microbial status of the gastrointestinal tract of host animals (Fuller et al. 1989; Gatesoupe 1999). They can improve the feed digestibility of fish (Verschuere et al. 2000; Merrifield et al. 2010), actively involved in coordinating immune responses (Lazado and Caipang 2014), stimulating non-specific immune system in fish (Verschuere et al. 2000; Balcázar et al. 2006; Merrifield et al. 2010) and preventing outbreak of fish disease (Sugita et al. 1996). Heo et al. (2013) also reported that *Lactobacillus* subsp. *lactis* I2 increased growth and concluded that it has potential as an alternative to antibiotics for controlling . Probiotics (*L. fermentum*) (Super lacto®) is commercially available in Korea as well.

In this study, therefore, we investigated the dietary inclusion of ethanol extracts of YC and GG on the growth, feed utilization and plasma chemistry of the juvenile olive flounder (*Paralichthys olivaceus*) and the challenge test against V. *anguillarum* compared to the commercial probiotic (Super lacto®).

2. Materials and methods

2.1 Fish and the experimental conditions

Juvenile olive flounder were purchased from a private hatchery (Uljin-gun, Gyeongsang Province, 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 flounder feeds (fish feed ingredients) twice a day.

Three hundred and sixty juvenile fish (an initial body weight; 7.2 ± 0.02 g) were randomly distributed into 12,50L flow-through tanks (30 fish per tank). Sand-filtered seawater was supplied throughout the feeding trial at temperature ranging from 10.9 to 23.8 ℃ (mean ± SD: 20.9 ± 1.90 ℃) and flow rate was 1.43-L/min/tank. Proper aeration was supplied to each tank, and the photoperiod followed natural condition. Each diet was randomly assigned to triplicate tanks of fish and hand-fed twice daily (09:00 and 17:00 h) at a satiation level for 8 weeks. Dead fish were removed daily and the bottoms of the containers were cleaned every other day.

2.2 Design of feeding trial and preparation of the experimental diets

Four experimental diets were prepared in triplicates (Table 1). Sixty percent fish meal and 7.5% fermented soybean meals were used as the protein source in the experiment diets. Twenty four percent wheat flour and 4% squid liver and 2% soybean oils were used as the carbohydrate and lipid sources, respectively in the experimental diets. No additives were added into the control (CON) diet. YC and GG were purchased from Tongjong Village (Seoul, Korea) and extracted with absolute ethanol and supplied from Clinical PK & PD LAB, College of Vet medicine, Kyungpook National University (Daegu, Korea). The extracts of YC and GG were filtered through a filter paper (Whatsman filter paper) and concentrated under reduced pressure using a rotary evaporator. A commercial *Lactobacillus*

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

	Experimental diets			
	CON	SUP[1]	YCE[2]	GGE[2]
Ingredient (%)				
Fish meal[3]	60	60	60	60
Fermented soybean meal[4]	7.5	7.5	7.5	7.5
Wheat flour	24	24	24	24
Super lacto® (SUP)	0	0.5	0	0
Ethanol extract of yacon	0	0	1	
Ethanol extract of ginger				1
Squid liver oil	4	4	4	4
Soybean oil	2	2	2	2
Choline	0.5	0.5	0.5	0.5
Vitamin premix[5]	1	1	1	1
Mineral mix[6]	1	1	1	1
Nutrient (%)				
Dry matter	98.5	98.1	98.2	97.8
Crude protein	48.5	49.6	49.3	48.3
Crude lipid	12.8	13.2	12.9	14.7
Ash	13.3	13.5	13.9	13.6

[1]SUP (Super lacto®) was purchased from Chang-Jo Biotec Co Ltd. (Jeju, Korea), which was an aqueous type and included into the experiment diets instead of the same amount of water.

[2]YCE (ethanol extracts of yacon) and [2]GGE (ethanol extracts of ginger) were supplied from Clinical PK & PD LAB, College of Vet medicine, Kyungpook National University (Daegu, Korea) and included into the experimental diets instead of the same amount of water.

[3]Fish meal was purchased from Abank Co Ltd. (Seoul, Korea).

[4]Fermented soybean meal was supplied by CJ CheilJedang Corp. (Seoul, Korea).

[5]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.

[6]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.

(Super lacto®) 0.5% and 1% of the ethanol extracts of YC and GG were included into the experimental diets, referred to as the SUP, YCE and GGE diets, respectively.

The ingredients of the experimental diets were well mixed and pelletized by laboratory pellet extruder (Dongsung mechanics, Busan, Korea). The experiment diets were dried at room temperature overnight and store in -20℃ until used.

2.3 Biological measurements

At the end of the 8-week feeding trial, all surviving fish from each net cage were harvested, collectively weighed and stored for further analysis. Specific growth rate (SGR) (% body weight gain/day) was calculated using the formula given by Britz (1996): SGR = [(ln (Wf) – ln (Wi))/days of feeding] × 100, where ln (Wf) = natural log of the final mean weight of fish and ln (Wi) = natural log of the initial mean weight of fish.

2.4 Analytical procedures of the experimental diets and fish

Three fish from each tank were randomly sampled and frozen at –20 °C for chemical analysis of fish at the end of the 8-week feeding trial. Chemical analysis of the experimental diets and whole body of olive flounder was done according to AOAC (1990). 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), moisture was determined by oven drying at 105°C for 24 h, fiber was determined using an automatic analyser (Fibertec, Tecator, Hoganas, Sweden) and ash was determined using a muffle furnace at 550°C for 4 h.

2.5 Plasma analysis of blood

Olive flounder were starved for 24 h at the end of 8-week feeding trial to analyze the plasma content. Five fish samples randomly chosen from each tank were anesthetized with Ethyl aminobenzoate at a concentration of 50 ppm and blood samples were taken by heparinized syringe from the caudal vein of fish. Plasma was collected after centrifugation (7,000 rpm for 10 min) and stored at –70°C as separate aliquots. Finally, total protein, total cholesterol, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and triglycerides were analyzed by an automatic chemistry system (HITACHI 7180/7600-210, Hitachi, Japan).

2.6 Challenge test

A Healthy and disease-free olive flounders were chosen for challenge test after 8-week feeding trial and then stocked into 12, 50-L static plastic tanks. At the end of the 8-week feeding trial, 15 sampled flounder were injected intraperitoneally with 0.1 mL culture suspension of gram-negative *V. anguillarum* (FP5208) and the concentration was 4.2×10^{6}CFU/L. The cumulative mortality rate was monitored for the following 7 days after pathogen injection. Dead fish were removed every 6 h for the first 4 days and every 12 h for the rest post monitoring period. Fish were starved during the bacterial aggression period.

2.7 Statistical analysis

Significant differences among the means of treatments were determined by one-way ANOVA and Duncan's multi-range test (Duncan 1955) using SPSS program version 19.0 (SPSS Michigan Avenue, Chicago, IL, USA).

3. Results

3.1 Growth performance of olive flounder

Survival (%), weight gain (g/fish) and SGR of olive flounder fed the experimental diets for 8 weeks were shown in Table 2. All the fish remained alive at the end of the 8-week feeding trial. Weight gain ranging from 22.4 to 23.1 g and SGR ranging from 2.52 to 2.56% were not significantly ($P > 0.05$) different among the experimental diets.

3.2 Feed utilization of olive flounder

Feed consumption (g/fish) ranging from 25.3 to 26.4 g, feed efficiency (FE) ranging from 0.88 to 0.89, protein retention (PR) ranging from 37.5 to 38.3 and condition factor (CF) ranging from 0.80 to 0.81 were not significantly ($P > 0.05$) affected by the experimental diets (Table 3).

Significantly ($P > 0.05$) higher protein efficiency ratio (PER) was achieved in fish fed the CON and GGE diets than that of fish fed the SUP and YCE diets. Significantly ($P < 0.05$) lower hepatosomatic index (HSI) was obtained in fish fed the CON diet than that of fish fed all other diets.

3.3 Chemical composition of olive flounder

Moisture ranging from 70.1 to 70.3%, crude protein ranging from 19.5 to 19.6%, crude lipid ranging from 2.6 to 2.7%, and ash content ranging from 4.2 to 4.4% of the whole body of olive flounder were not significantly ($P > 0.05$) affected by the experimental diets (Table 4).

Table 2 Survival (%), weight gain (g/fish) and specific growth rate (SGR) of the olive flounder fed experimental diets containing the ethanol extracts of yacon and ginger and Super lacto® for 8 weeks

Experimental diets	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)	SGR[1] (%/day)
CON	7.2 ± 0.03	29.6 ± 0.38^a	100 ± 0.00^a	22.4 ± 0.36^a	2.52 ± 0.018^a
SUP	7.2 ± 0.01	30.1 ± 0.15^a	100 ± 0.00^a	22.9 ± 0.16^a	2.55 ± 0.011^a
YCE	7.2 ± 0.02	30.1 ± 0.20^a	100 ± 0.00^a	22.9 ± 0.20^a	2.55 ± 0.013^a
GGE	7.2 ± 0.02	30.4 ± 0.08^a	100 ± 0.00^a	23.1 ± 0.06^a	2.56 ± 0.002^a

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

[1]Specific growth rate (SGR) (%/day) = (Ln final weight of fish - Ln initial weight of fish) ×100/days of feeding trial.

Table 3 Feeding consumption (g/fish), feed efficiency (FE), protein efficiency ratio (PER), protein retention (PR), condition factor (CF) and hepatosomatic index (HSI) of olive flounder fed the experimental diets containing the ethanol extracts of yacon and ginger and Super lacto® for 8 weeks

Experimental diets	Feed consumption (g/fish)	FE[1]	PER[2]	PR[3]	CF[4]	HSI[5]
CON	25.3 ± 0.34[a]	0.88 ± 0.003[a]	1.82 ± 0.005[a]	38.3 ± 0.16[a]	0.81 ± 0.003[a]	1.62 ± 0.006[b]
SUP	25.8 ± 0.20[a]	0.89 ± 0.002[a]	1.79 ± 0.004[b]	37.5 ± 0.24[a]	0.81 ± 0.003[a]	1.63 ± 0.003[a]
YCE	26.0 ± 0.33[a]	0.88 ± 0.003[a]	1.78 ± 0.007[b]	37.6 ± 0.12[a]	0.80 ± 0.003[a]	1.65 ± 0.003[a]
GGE	26.4 ± 0.06[a]	0.88 ± 0.001[a]	1.82 ± 0.003[a]	38.1 ± 0.31[a]	0.80 ± 0.000[a]	1.65 ± 0.003[a]

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

[1]Feed efficiency (FE) = 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.

[4]Condition factor (CF) = Fish weight×100/total length.

[5]Hepatosomatic index (HSI) = Liver weight×100/fish weight.

Table 4 Proximate composition of the whole body of olive flounder fed the experimental diets containing the ethanol extracts of yacon and ginger and Super lacto® for 8 weeks

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
CON	70.2 ± 0.09^a	19.6 ± 0.09^a	2.7 ± 0.03^a	4.3 ± 0.08^a
SUP	70.2 ± 0.08^a	19.5 ± 0.07^a	2.6 ± 0.04^a	4.2 ± 0.04^a
YCE	70.1 ± 0.09^a	19.6 ± 0.11^a	2.7 ± 0.05^a	4.4 ± 0.03^a
GGE	70.3 ± 0.10^a	19.6 ± 0.10^a	2.6 ± 0.07^a	4.2 ± 0.10^a

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

3.4 Biological indices of fish

The plasma total protein ranging from 3.24 to 4.49 g/dL, total cholesterol ranging from 173.3 to 208.0 mg/dL, GOT ranging from 42.7 to 68.0 IU/L, GPT ranging from 2.0 to 2.7 IU/L and triglyceride ranging from 174.3 to 338.7 mg/dL were not significantly ($P > 0.05$) affected by the experimental diets (Table 5).

3.5 Cumulative mortality of oliver flounder infected with *Vibrio anguillarum*

The cumulative mortality of fish was not significantly ($P > 0.05$) affected by the experimental diets up to 66 h after *V. anguillarum* infection (Fig. 1). However, significantly ($P < 0.05$) higher cumulative mortality was recorded in olive flounder fed the CON and SUP diets than that of fish fed the YCE and GGE at 76 h until the end of the 7-day post observation after infection of *V. anguillarum*. The cumulative mortality was 97.8% and 95.6% in fish fed the CON and SUP diets respectively, whereas 77.8% cumulative mortality was recorded in fish fed the YCE and GGE diets at the end of 7-day post observation after infection of *V. anguillarum*.

Table 5 Plasma chemistry of olive flounder fed the experimental diets containing the ethanol extracts of yacon and ginger and Super lacto® for 8 weeks

Experimental diets	Total protein (g/dL)	Total cholesterol (mg/dL)	GOT (IU/L)	GPT (IU/L)	Triglyceride (mg/dL)
CON	3.24 ± 0.11[a]	208.0 ± 3.21[a]	42.7 ± 5.81[a]	2.3 ± 0.33[a]	174.3 ± 18.84[a]
SUP	4.49 ± 0.54[a]	173.3 ± 14.50[a]	57.0 ± 8.14[a]	2.7 ± 0.67[a]	338.7 ± 105.53[a]
YCE	3.26 ± 0.10[a]	196.3 ± 3.76[a]	54.3 ± 4.98[a]	2.0 ± 0.58[a]	287.3 ± 90.36[a]
GGE	3.41 ± 0.22[a]	189.3 ± 12.72[a]	68.0 ± 3.51[a]	2.3 ± 0.33[a]	231.7 ± 37.57[a]

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

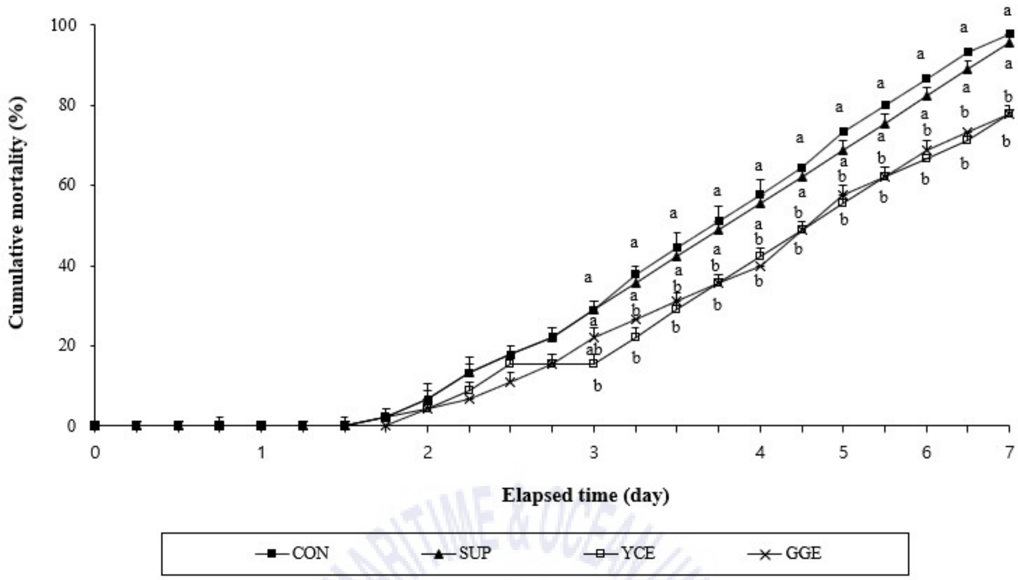

Fig. 1. Cumulative mortality (%) of juvenile olive flounder (*Paralichthys olivaceus*) fed the experimental diets containing the ethanol extracts of yacon and ginger and Super lacto® for 8 weeks, and then infected with *Vibrio anguillarum* for 7 days (mean of triplicates ± SE).

4. Discussion

Frequent outbreak of bacterial, fungal and viral diseases has caused huge economic losses to fish farming in Korea (Shin et al. 2006; Oh et al. 2006; Jung et al. 2008; Palaksha 2008). Overuse of the synthetic antibiotics to reduce microbial infections in aquaculture sectors is a common phenomenon (Cabello 2006; Akinbowale et al. 2006). However, the adverse effect of antibiotics on aquatic environment and human has emerged as major threats for sustainability of aquaculture industry (Jones et al. 2004; Park 2009). Therefore, administration of plant-based remedies is a promising and alternative way to treat fish diseases caused by bacteria, viruses or parasites (Park 2009; Levy et al. 2015). Since YC and GG are rich in some anti-oxidative and antimicrobial compounds (Yan et al. 1999; Nya and Austin 2009; Ghasemzadeh et al. 2010; Talpur et al. 2013; Levy et al. 2015), they are potential substitutes for the synthetic antibiotics in aquafeed to enhance the immunity of fish. Similarly, the use of different feed additives including YC, GG, blueberry, tomato, garlic, *Massa medicata*, *Crataegi fructus*, *Artemisia capillaris* and *Cnidium officinale* have positive effect on growth performance of rockfish (Kim et al. 2016, 2017a), rainbow trout (Nya and Austin, 2009), red sea bream (*Pagrus major*) (Ji et al. 2007a) and Asian sea bass (Talpur et al. 2013) have been reported.

None of survival, weight gain and SGR of olive flounder was affected by the experimental diets in this study. Similarly, Kim et al. (2011) reported that dietary inclusion of the various additives did not affect growth performance of juvenile olive flounder when fish were fed with one of the diets containing different kinds of additives (5% kelp meal, 10% krill meal, 1% garlic powder, 1% citrus meal, 3% onion powder, 1% mugwort powder, 1% licorice powder, 1% wasabi powder and 1% GG) and their mixture (0.2% of each garlic, citrus, onion, GG, mugwort, licorice and wasabi) for 15 weeks. Cho et al. (2013) demonstrated that survival and weight gain of olive flounder were not affected by the dietary inclusion of *Scutellaria baicalensis* extract, but the SGR was higher in fish fed a diet containing 2% *S. baicalensis* extract compared to that of fish fed the diets containing other concentration (0, 0.5, 1, 3 and 5% of *S. baicalensis* extract) for 8 weeks. Conversely, Kim et al. (2017a) showed that rockfish fed the diets containing 1% YC, GG and blueberry exhibited higher weight gain compared to that of fish fed the control diet with 0.01% commercial antioxidant (ethoxyquin) for 8 weeks. Nay and Austin (2009a) presented that an inclusion of GG to the commercial feed improved weight gain and feed efficiency of rainbow trout. The differences in growth performance and feed efficiency of fish could be related with the fish species, dose and type (powder or liquid) of additives, the method of extraction, freshness or the physiological state of fish (Cho 2012; Kim et al. 2013b).

SGR values ranging from 2.52 to 2.56% for the initial weight of 7.2 g olive flounder in this study was relatively higher compared to those ranging from 2.48 to 2.64, from2.27 to 2.51, from 2.17 to 2.30, from 2.2 to 2.5, from 1.85 to 2.03 and from 1.4 to 1.7 % for the same species of fish with initial weights of 1.2, 8.2, 8.5, 12.9, 15.2 and 52.5 g, respectively (Koo et al. 2001; Cho et al. 2007; Ji et al. 2007b; Cho and Kim 2009; Kim et al. 2011; Kim et al. 2013a), indicating that the growth of olive flounder was well achieved in this study.

No difference was found in feed consumption, FE, PR and CF of olive flounder in this study. Similarly, feed efficiency of olive flounder was unaffected by dietary inclusion GG and other additives (garlic, citrus, onion, mugwort, licorice, wasabi, *S. baicalensis* extract) (Cho et al. 2007; Kim et al. 2011). Lee et al. (2016) reported that rockfish fed diet with 1% YC showed the higher FE, PER and PR than those of fish fed the diets with GG and blueberry, whereas feed consumption was the highest in fish fed diet with blueberry. Similarly, higher feed consumption and utilizations (FE, PER and PR) were also reported in rockfish fed the diet containing 1% YC compared to those of fish fed the diets with YC, GG, blueberry, persimmon, tomato, broccoli and cheonggukjang for 7 weeks (Kim et al. 2016).

Lower HSI of fish fed the CON diet compared to all other diets (SUP, YCE and GGE) in this study indicated that dietary inclusion of probiotic (Super lacto®) and the ethanol extracts of YC and GG increased liver weight of olive flounder. Similarly, Kim et al. (2013a) reported that the HSI was higher in olive flounder fed diets containing various chemical additives, such as solid sulfur (0.5 and 1%), fucoidan (0.2 and 0.4%) and glucan (0.1%) compared to that of fish fed the diet with no additive for 6 weeks. In contrast, Cho et al. (2007) showed that greater HSI was found in olive flounder fed the diet with no additive compared to the diets containing various sources of green tea. However, further research will facilitate to find out the exact reason behind the higher HSI in olive flounder fed the phyto-additives.

The chemical composition of the whole body of olive flounder was not affected by the experimental diets in this study, but YC and GG largely changed the chemical composition of rockfish (Lee et al. 2016; Kim et al. 2016). An inclusion of various additives (*Chorella* and different sources of green tea) also changed the chemical composition of the whole body of olive flounder (Koo et al. 2001; Cho et al. 2007).

None of plasma total protein, cholesterol, GOT, GPT and triglyceride level was affected by the experimental diets in this study. Cho et al. (2013) also reported no significant effect on the plasma chemistry of olive flounder fed the diets containing different concentrations of *S. baicalensis* extract. Ji et al. (2007b) showed that administration of various concentrations (0.1, 0.3, 0.5 and 1.0%) of medicinal herb mixture of *M. medicata fermentata*, *C. fructus*, *A. capillaris* and *C. officinale* at the ratio of 2:2:1:1 in a moist diet had no significant influence on the plasma chemistry (glucose, triglyceride, total cholesterol and GPT) of olive flounder, except for GOT. However, dietary inclusion of green tea and *Chlorella* decreased plasma

GPT and low-density lipoprotein cholesterol, and GOT and GPT of olive flounder, respectively (Koo et al. 2001; Cho et al., 2007). Talpur et al. (2013) reported that the blood glucose, lipid, triglyceride and cholesterol levels decreased in Asian sea bass fed the diet containing GG compared to those of fish fed the diet with no additive when fish were fed with one of diets containing different concentrations of GG (0.1, 0.2, 0.3, 0.5 and 1%) for 15 days. The mechanisms behind the result had not been explained yet, but the additive sources may affect the plasma chemistry of fish.

Lower cumulative mortality of fish fed the YCE and GGE diets at 72 h after artificial infection of *V. anguillarum* until the end of 7-day post observation compared to that of fish fed the CON and SUP diets indicated that administration of the ethanol extracts of YC and GG were more resistant against *V. anguillarum* than the commercial probiotic (Super lacto®). Kim et al. (2013b) also reported that dietary inclusion of various concentration of flowering plant, *S. baicalensis* extract (a commercially available extract) did not affect growth performance of Eastern catfish (*Silurus asotus*), but improved survival of fish infected with *V. anguillarum* and *S. iniae* compared to that of fish fed the control diet after 8-week feeding trial. Similarly, dietary inclusion of 1% YC, GG and blueberry reduced cumulative mortality of rockfish infected with *S. parauberis* compared to all other diets (control and diets containing cheonggukjang, persimmon, tomato and broccoli) (Kim et al. 2016). YC and GG were also effective against artificial infection of *E. tarda* (Kim et al. 2017a) and *S. iniae* in rockfish (Lee et al. 2016). Sebiomo et al. (2010) reported that both aqueous and ethanol extracts of GG showed the highest antibacterial activity compared to the commercial antibiotics (chloramphenicol, ampicillin and tetracycline) against *S. aureus* and *S. pyogenes* in vivo. Talpur et al. (2013) explained that oral administration of various concentrations (0.1, 0.2, 0.3, 0.5 and 1%) of GG increased survival of Asian sea bass infected with *V. harveyi* and modulated plasma lysozyme activity, phagocytic activity of the head kidney macrophages and serum anti-protease activity. Harikrishnan et al. (2011) mentioned that herbal treatments are cheaper and more accurate method than the chemical drugs and have potential to eradicate the disease problem in aquaculture. Therefore, the high survival of olive flounder fed the YCE and GGE diets in this study may be related with the presence of different biologically active compounds, supported by several studies showing that YC and GG improve the immunity of fish against *E. tarda*, *S. parauberis* or *V. harveyi* (Kim et al. 2007; Talpur et al. 2013; Kim et al. 2016). Further research is needed to determine the effect of different doses of the ethanol extracts of YC and GG on growth performance and immune response of olive flounder against other pathogens.

5. Conclusion

Although the growth, feed utilization (FE and PR), chemical composition and plasma chemistry of olive flounder were not affected by dietary inclusion of the ethanol extracts of YC and GG in the 8-week feeding trial in this study, they effectively lowered the cumulative mortality of olive flounder infected with *V. anguillarum* compared to the control diet or the diet containing commercial probiotic (Super lacto®). Therefore, the ethanol extracts of YC and GG seems to play as natural immunostimulant against *V. anguillarum* rather than growth promoter in olive flounder.

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