[UCI]I804:21028-200000009246

Thesis for the Degree of Doctor of Philosophy

Administration of feed ingredient to improve attractiveness, growth and disease resistance of juvenile rockfish *Sebastes schlegeli* Hilgendorf 1880

Hee Sung Kim

Department of Convergence Study on the Ocean Science and Technology

The Graduate School

Korea Maritime and Ocean University

February 2018

Administration of feed ingredient to improve attractiveness, growth and disease resistance of juvenile rockfish *Sebastes schlegeli* Hilgendorf 1880

Advisor: Prof. Sung Hwoan Cho

by

Hee Sung Kim

A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In the Department of Convergence Study on the Ocean Science and Technology, the Graduate School of Korea Maritime and Ocean University

February 2018

Administration of feed ingredient to improve attractiveness, growth and disease resistance of juvenile rockfish *Sebastes schlegeli* Hilgendorf 1880

A dissertation

by

Hee Sung Kim

Approved as to style and content by:

Sang-Min Lee, Ph.D.
Chairman

Jin-Hyung Yoo, Ph.D.
Committee

Bo-Young Jee, Ph.D.
Committee

Mun-Gyeong Kwon, Ph.D
Committee

Sung Hwoan Cho, Ph.D.
Committee

December 2017

CONTENTS

Chapter 3. Effects of dietary inclusion of yacon, ginger and blueberry on growth, body composition and challenge test of juvenile rockfish *Sebastes schlegeli* against *Edwardsiella tarda*

Chapter 4. Attractiveness/palatability of various protein sources and their function as feeding attractant to promote feed consumption, growth performance, feed utilization and body composition in juvenile rockfish *Sebastes schlegeli* through dietary supplementation

List of Tables

List of Figures

배합사료 원료에 따른 조피볼락의 먹이유인도, 성장 및 질병 방어능

김 희 성

한국해양대학교 해양과학기술전문대학원 해양과학기술융합학과

요 약

조피볼락은 빠른 성장과 질병에 대한 강한 저항성을 가지고 있어 지난 30년간 한국, 중국과 일본 등의 동아시아에서 상업적으로 중요한 해산 양식 대상 어종이다. 국내 조피볼락의 연중 양식으로 인하여 빈번하게 발생하는 질병 저감을 위하여 주로 합성항생제(항산화제)를 사료내 혼합하여 사용하고 있는 실정이다. 그러나 합성항생제의 사용은 환경오염뿐만 아니라 양식어류를 섭취하는 인간에게까지 부정적인 영향을 미칠 수 있으므로 한국, 유럽 등 많은 나라에서 사용을 금지하고 실정이다. 또한 국내 조피볼락 양식 산업 현장에서는 배합사료보다 생사료 공급을 더 선호하고 있으며, 이는 배합사료보다 생사료에 대한 조피볼락의 선호도가 더 높아서 결국 사료섭취량이 높아지는 결과를 야기한 것으로 나타난다. 생사료의 사료섭취량이 높은 것은 생사료내 함유되어 있는 먹이유인물질이나 먹이섭취촉진물질이 배합사료에 비하여 생사료에 다량 함유되어 있는 원인에 기인한 것으로 사료된다. 과거

어종별에 따른 사료섭취량 향상을 위한 먹이유인물질 또는 먹이섭취촉진물질 규명에 대한 연구가 수행된 바 있으나, 이들의 연구는 주로 먹이 추출물에 함유된 특정 합성유인물질에 대한 실험대상 어종의 생리영양학적 반응이나 전기신경생리학적 반응을 모니터링 한 결과로서 양식 산업이나 양어용 배합사료 제조시 아주 제한적으로 사용되고 있으며, 양어용 배합사료 제조시 바로 적용시킬 수 있는 먹이유인물질 또는 먹이섭취촉진물질을 다량으로 함유한 사료원료를 개발하여 적용시킬 수 있는 실용적인 기술 개발이 필요하다. 따라서 본 연구에서는 조피볼락의 먹이유인도, 성장 및 질병 방어능을 향상시키기 위하여 배합사료내 합성항생제(항산화제)를 대체할 수 있는 천연소재의 사료첨가제 사용에 따른 조피볼락의 성장, 체조성과 그람 양·음성 세균의 인위 감염에 미치는 효과 및 조피볼락에 의한 다양한 단백질원의 먹이유인도를 평가하고, 이들 중 우수한 먹이유인도를 보인 단백질원의 배합사료내 첨가에 따른 조피볼락의 성장, 사료 이용성 및 체조성에 미치는 효과를 평가하였다.

1. 배합사료내 다양한 천연소재 사료첨가제가 조피볼락의 성장, 체조성 및 인위 감염에 미치는 효과

본 연구는 조피볼락용 배합사료내 천연소재 사료첨가제인 생강[ginger (GG), *Zingiber officinale* Roscoe), 청국장(CJ), 블루베리[blueberry (BB), *Vaccinium ashei* Reade], 감[persimmon (PM), *Diospyros kaki* L.], 토마토[tomato (TT), *Solanum lycopersicum* L.], 브로콜리[broccoli (BC), *Brassica oleracea* L.] 및 야콘[yacon (YC), *Polymnia sonchifolia* Poeppig and Endlicher]의 첨가가 조피볼락 치어의 성장, 체조성 및 인위 감염에 미치는 영향을 평가하였다. 총 1,680마리의 조피볼락 치어를 무작위로 선별하여 24개의 200 L 유수식 수조에 수용하였다. 총 8종류의 실험사료를 준비하였으며, 천연소재의 사료첨가제를 첨가하지 않은 대조구(Con) 사료와 GG, CJ, BB, PM, TT, BC 및 YC를 각각 1%씩 첨가한 실험사료를 제조하였다. 실험사료는 1일 2회 만복시까지 공급하였으며, 총 사육기간은 7주이었다. 사육실험 종료시 생존한 조피볼락 20마리를 각 수조에서 무작위로 추출하여 *Streptococcus parauberis* (KCTC11980BP)로 인위적인 감염 후 10일간의 누적폐사율을 관찰하였다. YC 사료를 공급한 어류의 성장[어체중 증가(weight gain)와 일일성장률(specific growth rate, SGR)]이 다른 모든 실험사료를 공급한 어류보다 우수하게 나타났다. 사료효율(feed efficiency, FE)과 단백질전환효율(protein efficiency ratio, PER)은 TT와 YC 사료 공급구가 나머지 다른 사료 공급구보다 높게 나타났다. *S. parauberis* 감염 후 5일째에는 GG, BB와 YC 사료를 공급한 어류의 누적폐사율이 다른 실험사료를 공급한 어류보다 낮게 나타났다. 이상의 결과를 고려할 때 조피볼락용 배합사료내 YC의 첨가는 조피볼락의 증체량을 향상시키며, GG, BB와 YC의 첨가는 *S. parauberis*의 질병 발생시 조피볼락의 폐사율 저감에 효과적인 것으로 판단된다.

2. 배합사료내 야콘, 생강과 블루베리 첨가에 따른 조피볼락 치어의 성장, 체조성 및 *Edwardsiella tarda* 인위 감염에 미치는 영향

본 연구는 배합사료내 야콘(YC), 생강(GG)과 블루베리(BB)의 첨가에 따른 조피볼락의 성장, 체조성 및 *Edwardsiella tarda* (ET-1)의 인위감염에 미치는 영향을 상업용 항산화제인 ethoxiquin (EQ) 첨가 사료와 비교하였다. 마리당 평균 4.3 g의 조피볼락 치어를 12개의 유수식 수조에 각각 40마리씩 수용하였다. 총 4종류의 실험사료를 준비하였으며, 0.01% 소맥분 대신에 상업적으로 시판중인 0.01% EQ를 첨가한 사료를 대조구(Con)로 이용하였다. 또한 1% 소맥분 대신에 상업적으로 이용가능한 천연소재의 사료첨가제 YC, GG와 BB를 첨가한 실험사료를 준비하였으며, 실험구는 3반복구를 두었다. 모든 실험어는 8주간 매일 1일 2회 손으로 만복시까지 사료를 공급하여 주었다. 사육실험 4주와 8주째 각각의 사육수조에서 생존한 조피볼락을 무작위로 각각 10마리와 15마리씩 추출하여 *E. tarda*로 인위적으로 감염시켰다. 8주간의 사육실험 종료시 YC 사료를 공급한 어류의 어체중 증가와 일일성장률은 다른 모든 실험사료 공급구보다 우수하게 나타났다. 사료효율, 단백질전환효율과 단백질축적율은 YC 사료 공급구가 나머지 다른 실험사료 공급구보다 높게 나타났다. 4주째와 8주째 인위적인 세균 감염실험 결과, Con 사료 공급구의 누적폐사율이 다른 실험사료보다 높게 나타났다. 이상의 결과를 고려할 때, 조피볼락 치어용 배합사료내 YC, GG와 BB의 첨가는 조피볼락의 어체중 증가와 일일성장률 향상에 효과적이었다. 또한 YC, GG와 BB를 사료내 첨가하여 4주와 8주간 공급시 모두 *E. tarda* 질병 발생에 따른 조피볼락의 폐사율을 감소시키는데 효과적이었다. 특히 YC, GG와 BB를 첨가한 사료를 4주 동안만 공급하여도 *E. tarda* 질병 발생시 조피볼락의 폐사율을 감소시키는데 효과적이었다.

3. 조피볼락에 있어서 다양한 단백질원의 먹이유인도 평가 및 이들의 배합사료내 첨가에 따른 사료섭취량, 성장, 사료이용성 및 체조성 향상에 미치는 먹이유인물질로서의 기능 평가

본 연구는 다양한 단백질원에 대한 조피볼락 치어의 먹이유인도를 평가하며, 우수한 먹이유인도를 보인 단백질원의 배합사료내 첨가에 따른 조피볼락의 성장, 사료이용성 및 체조성에 미치는 효과를 평가하였다. 예비실험에서는 반복구 없이 총 16 종류[6 종 어분(멸치분, 청어분, 전갱이분, 가수분해어분, 명태어분, 정어리분), 4 종 갑각류분(게분, 크릴분, 새우머리분, 새우분), 4 종 연체동물분(홍합분, 굴분, 오징어간분, 오징어분)과 2 종 식물성단백질분(콘글루텐분, 탈지대두박분)]의 단백질원료를 이용하여 특수 제조된 먹이유인도 평가 실험 장치에 동일한 크기의 치어 30 마리씩을 수용하여 토너먼트 방식으로 유인도를 평가하였다. 그리고 이들 중에서 높은 먹이유인도를 보인 5 종의 사료 원료를 선정하여, 특수 제조된 먹이유인도 평가 실험 장치에서 동일한 크기의 치어 30 마리씩을 수용하였다. 먹이유인도는 토너먼트 방식으로 3 반복구를 두어서 평가하였다. 최종적으로 높은 먹이유인도를 보인 5 종의 사료 원료와 양호한 먹이유인도를 보인 멸치분을 대조구로 이용한 사료를 준비한 총 6 종류의 실험사료를 준비하여 3 반복구를 두어서 8 주간의 사육실험을 실시하였다. 8 주간의 사육실험에는 360 마리의 조피볼락 치어를 18 개의 유수식 수조에 분산 수용하였다. 대조구(Con) 사료에는 55%의 멸치분을 함유하였으며, Con 사료에 첨가한 멸치분의 5% 대신에 우수한 유인도를 보인 5 종류의 단백질원을 각각 5%씩 첨가한 사료를 준비하였다. 모든 실험어는 1 일

2회 만복시까지 손으로 공급하였다. 예비실험 결과, 조피볼락의 먹이유인도는 전갱이분(jack mackerel meal, JM), 새우분(shrimp meal, SHM), 오징어분(squid meal, SQM), 정어리분(sardine meal, SM), 명태분(pollack meal, PM)에서 높게 나타나는 경향을 보였다. 3반복구를 두어서 먹이유인도를 평가한 결과, 조피볼락의 먹이유인도는 JM, SM, SQM, PM과 SHM의 순서로 높게 나타났다. 8주간의 사육실험에 있어서는 체중증가와 일일성장률은 JM 사료 공급구가 다른 모든 실험사료 공급구보다 높게 나타났으며, 다음으로 SM, PM, SQM, SHM과 Con 사료 공급구 순으로 높게 나타났다. 사료섭취량은 JM 사료 공급구가 다른 모든 실험사료 공급구보다 높게 나타났으며, 다음으로 SM, PM, SQM, SHM과 Con 사료 공급구 순으로 나타났다. 비만도(condition factor, CF)는 JM 사료를 공급한 실험어가 가장 높게 나타났으며, 다음으로 SM, PM, SQM, SHM과 Con 사료 공급구 순으로 나타났다. 이상의 결과 조피볼락용 배합사료내 5%의 JM 첨가는 사료섭취량, 어체중 증가, 일일성장률 및 비만도를 가장 효율적으로 향상시켰으며, 그 다음으로 SM, PM, SQM, SHM과 Con사료의 순이었다. 이러한 결과를 고려할 때 먹이유인도를 고려한 사료원료의 선택과 배합사료내 첨가로 조피볼락의 사료섭취량 향상, 어체중 증가, 일일성장률 향상 및 비만도를 효율적으로 증가시킬 수 있을 것으로 판단된다.

Administration of feed ingredient to improve attractiveness, growth and disease resistance of juvenile rockfish *Sebastes schlegeli* Hilgendorf 1880

Hee Sung Kim

Department of Convergence Study on the Ocean Science and Technology,
Korea Maritime and Ocean University

Abstract

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. 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 due to high possibility of contamination of environment and culturing fish used for human consumption. Most rockfish aquaculture production in Korea has occurred by feeding moist pellet (MP). Formulated feed (FF) is still difficult rockfish farmers to adopt due to their biased perception of the superior production and feed consumption of fish fed MP over FF. A lower feed

consumption of fish fed on FF compared to MP could result from a deficiency or lower amount of feeding attractants and/or stimulants in the former. Determining the attractability of aquatic animals based on either omission tests or monitoring the physiological and electro(neuro)physiological responses of the components in the synthetic extracts of prey is a very complicate process. No study has examined whether the addition of a selected feed ingredient affects fish performance based on its attractability. This application is simple, highly desirable and recommendable in formulating practical diets to improve the production of fish. In this study, therefore, we investigated the effect of dietary inclusion of natural sources of feed additives on growth, body composition and challenge test of juvenile rockfish against gram-positive and -negative pathogenic. Also, the attractability of the selected feed ingredient among the various aquatic animal- and terrestrial plant-originating sources was evaluated in juvenile rockfish, and their dietary supplementation effects on the growth performance, feed utilization and body composition of fish were determined and compared.

1. Effect of various sources of dietary additive on growth, body composition and challenge test of juvenile rockfish *Sebastes schlegeli*

We investigated 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*. Twenty-four groups of 70 fish (1,680 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 rock fish weight gain, while ginger, blueberry, and yacon effectively lowered mortality of rockfish infected with *S. parauberis*.

2. Effects of dietary inclusion of yacon, ginger and blueberry on growth, body composition and challenge test of juvenile rockfish *Sebastes schlegeli* against *Edwardsiella tarda*

Effects of dietary inclusion of yacon (YC), ginger (GG) and blueberry (BB) on growth, body composition and challenge test of rockfish against *Edwardsiella tarda* compared to ethoxyquin were investigated. Three hundred sixty fish were randomly distributed into 12 flow-through tanks. Four experimental diets were prepared; the control diet (Con) with 0.01%

ethoxyquin, and YC, GG and BB diets. Each diet was assigned to triplicate tanks of fish and hand-fed for 8 weeks. Externally normal fish after 4^{th} and 8^{th} week feeding trial were infected with *Edwardsiella tarda* for challenge test. Weight gain and specific growth rate (SGR) of fish fed the YC diet was greater than those of fish fed all other diets. Feed efficiency, protein efficiency ratio and protein retention of fish fed the YC diet was higher than those of fish fed all other diets. In the both 4^{th} and 8^{th} week infection trial, mortality of fish fed the Con diet was higher than that of fish fed all other diets. In conclusion, dietary inclusion of YC, GG and BB increased weight gain and SGR of fish. YC, GG and BB for 4 and 8 weeks lowered mortality of fish at occurrence of *E.tarda*.

3. Attractiveness/palatability of various protein sources and their function as feeding attractant to promote feed consumption, growth performance, feed utilization and body composition in juvenile rockfish *Sebastes schlegeli* through dietary supplementation

The attractability of various sources of feed ingredients was evaluated in juvenile rockfish, and their dietary supplementation effects on the growth, feed utilization and body composition of the fish were compared. The attractability of the top 5 selected feed ingredients showing high feeding attractant ability in fish was determined from among the 16 feed ingredients examined in the preliminary test. Three hundred sixty juvenile fish were distributed in 18 flow-through tanks for the feeding trial. Six experimental

diets were prepared: the control (Con) diet, and 5% jack mackerel meal (JM), sardine meal (SM), pollack meal (PM), squid meal (SQM) and shrimp meal (SHM) diets. Each diet was assigned in triplicate and hand-fed to satiation. The strongest feeding attractability of rockfish was obtained in JM, followed by SM, SQM, PM and SHM. The weight gain and specific growth rate (SGR) of fish fed the JM diet were greater than those of fish fed all other diets, followed by the SM, PM, SQM, SHM and Con diets. The feed consumption of fish fed the JM diet was higher than that of fish fed all other diets, followed by the SM, PM, SQM, SHM and Con diets. The condition factor (CF) of fish fed the JM diet was higher than that of fish fed all other diets, followed by the SM, PM, SQM, SHM and Con diet. In conclusion, the greatest feed consumption, weight gain, SGR and CF of fish were obtained in the JM diet, followed by the SM, PM, SQM, SHM and Con diets, in order.

Chapter 1.

General Introduction

Rockfish *Sebastes schlegeli* Hilgendorf 1880 has been one of the most commercially important marine aquaculture fish species in the Eastern Asia, such as Korea, Japan, and China since over three decades ago due to its fast growth and high resistance against disease. Its annual aquaculture production reached 17,996 tons in 2016 in Korea (KOSIS, 2017). Therefore, a variety of feeding trials has been performed with this species to determine dietary nutrient requirements (Kim et al., 2001; Lee et al., 2002; Yan et al., 2007), 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), and dietary additives to improve lysozyme activity and stress recovery (Hwang et al., 2013) and immune response (Kim et al., 1999).

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 a new natural source (plant-originated) of dietary additive that has unfavorable effect on environment and culturing fish to replace synthetic antibiotic keeps being needed.

Most rockfish aquaculture production in Korea has made by feeding either frozen raw fish (RF), such as sardines and mackerel, or RF-basal moist pellet (MP). Feeding fish either RF or MP is likely to cause several problems, such as severe water pollution resulting from a high leaching-out rate of nutrients in RF and MP, an unstable supply of RF or a depletion of the RF that are commonly used as fish meal sources in the wild. Alternatively, formulated feed (FF) should be developed to efficiently produce rockfish in an eco-friendly and cost-effective manner. Nevertheless, FF is still difficult rockfish farmers to adopt due to their biased perception of the superior production and feed consumption of fish fed either RF or MP over FF. A lower feed consumption of fish fed on FF compared to either RF or MP could result from a deficiency or lower amount of feeding attractants and/or stimulants in the former. Therefore, adding feed ingredients that contain high levels of rockfish attractants to FF is highly desirable for improving fish production.

The application of feeding attractants and/or stimulants in aquafeed has received considerable attention for a long time. The main reasons for their use are to improve the feed consumption of aquatic animals and to minimize feed wastage and water pollution sources (Tandler et al., 1982; Ellingsen and Døving, 1986; Takii et al., 1994). Fish detect chemical stimuli through at least two different chemoreception channels: olfaction (smell) and gustation (taste) (Hara, 1993). The chemical cues detected by olfactory receptors are conveyed to the brain through the olfactory nerve. Kohbara et al. (2000) showed that thresholds of adsorbate and non-adsorbate in the olfactory bulb response were approximately 10^{-5} and 10^{-8} and approximately 10^{-4} and 10^{-2} of the original concentration of the jack mackerel *Trachrus japonicus* extract, and in the gustatory nerve response of young yellowtail *Seriola quinqueradiata*, respectively. However, these results were obtained by studies determining the attractiveness of aquatic animals based on either omission tests or monitoring the physiological and electro(neuro)physiological responses of the components in the synthetic extracts of prey is a very complicate process. In addition, application of these results are very limited in either aquaculture industry or aquafeed formulation. No study has examined whether the addition of a selected feed ingredient affects fish performance based on its attractiveness. This application is simple, highly desirable and recommendable in formulating practical diets to improve the production of fish.

Therefore, the effect of dietary inclusion of natural sources of plant-originated ingredients on growth, body composition, and challenge test of juvenile rockfish against gram-positive *Streptococcus parauberis* in the first study. In the second study, dietary inclusion effect of the selected YC, GG and BB showing high growth performance and gram-positive disease resistance based on the first study on growth, body composition and challenge test of juvenile rockfish against gram-negative *Edwardsiella tarda* was investigated and compared to ethoxyquin (EQ). In the third study, the attractiveness of the selected feed ingredient among the various aquatic animal- and terrestrial plant-originated protein sources was evaluated by juvenile rockfish, and their dietary supplementation effects on growth performance, feed utilization and body composition of rockfish were determined.

Chapter 2.

Effect of various sources of dietary additive on growth, body composition and challenge test of juvenile rockfish *Sebastes schlegeli*

1. Introduction

In annual aquaculture production volume, the rockfish *Sebastes schlegeli* 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 alginlyticus* (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 the sespecies (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.

2. Materials and methods

2.1 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, Gyeongsangnma-do, Korea) twice a day at a ratio of 2-3% of fish body weight. A total of 1,680 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.

2.2 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,

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

	Experimental diets							
	Con	GG[1]	CJ[1]	BB[1]	PM[2]	TT[2]	BC[2]	YC[2]
Ingredients								
Anchovy meal[3]	58	58	58	58	58	58	58	58
Dehulled Soybean meal[4]	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
Additive powder		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[5]	1	1	1	1	1	1	1	1
Mineral premix[6]	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
Nutrients (%, DM)								
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

[1]GG (ginger), [1]CJ (cheonggukjang) and [1]BB (blueberry), and [2]PM (persimmon), [2]TT (tomato), [2]BC (broccoli) and [2]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

[3]Anchovy meal and [4]Dehulled soybean meal were purchased from Abank Co Ltd. (Seocho-gu, 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; ferriccitrate, 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.

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 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.

2.3 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.

2.4 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 1.2×10^5 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 × 100/total number of infected fish. Fish were starved throughout the 10-day challenge test.

2.5 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.

3. Results

3.1 Growth performance of fish

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 greater than that for fish fed the other diets.

3.2 Feed utilization

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 efficiency (FE) and protein efficiency ratio (PER) of fish fed the TT and YC diets were significantly higher compared to the other diets. 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.

3.3 Chemical composition of the whole body of fish

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

Table 2. Survival (%) and weight gain (g/fish) of juvenile rockfish 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^{bc}	100.0 ± 0.00	13.2 ± 0.15^{bc}
GG	3.0 ± 0.00	16.5 ± 0.07^{b}	99.0 ± 0.48	13.5 ± 0.07^{b}
CJ	3.0 ± 0.00	15.2 ± 0.09^{d}	100.0 ± 0.00	12.2 ± 0.09^{d}
BB	3.0 ± 0.00	16.3 ± 0.09^{bc}	99.5 ± 0.48	13.3 ± 0.09^{bc}
PM	3.0 ± 0.01	15.1 ± 0.12^{d}	100.0 ± 0.00	12.1 ± 0.12^{d}
TT	3.0 ± 0.00	16.0 ± 0.09^{c}	100.0 ± 0.00	13.0 ± 0.10^{c}
BC	3.0 ± 0.01	13.0 ± 0.17^{e}	99.0 ± 0.95	10.0 ± 0.18^{e}
YC	3.0 ± 0.01	17.4 ± 0.27^{a}	98.6 ± 1.43	14.4 ± 0.28^{a}

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.

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

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

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.

Table 4. Proximate analysis for composition (%) of the whole body of juvenile rockfish 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[cd]	15.6 ± 0.15[b]	7.8 ± 0.03[bc]	4.5 ± 0.07[a]
GG	71.8 ± 0.09[ab]	17.8 ± 0.26[a]	7.2 ± 0.09[d]	3.8 ± 0.03[de]
CJ	72.3 ± 0.52[a]	15.4 ± 0.22[b]	7.9 ± 0.03[ab]	3.8 ± 0.06[e]
BB	70.4 ± 0.66[bcd]	17.7 ± 0.21[a]	7.2 ± 0.03[d]	4.0 ± 0.06[c]
PM	70.0 ± 0.23[cd]	15.8 ± 0.07[b]	7.7 ± 0.12[c]	4.0 ± 0.03[cd]
TT	68.9 ± 0.32[d]	15.9 ± 0.12[b]	7.7 ± 0.03[c]	3.8 ± 0.03[de]
BC	69.9 ± 0.66[cd]	15.3 ± 0.26[b]	7.6 ± 0.06[c]	4.3 ± 0.06[b]
YC	70.9 ± 0.27[abc]	17.5 ± 0.20[a]	8.1 ± 0.09[a]	4.2 ± 0.03[b]

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.

YC diets. 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. 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.

3.4 Cumulative mortality of fish

Cumulative mortality of fish supplied with different experimental diets is shown in Fig. 1. Cumulative mortality of fish fed the Con diet was significantly higher than of other fish at 36 h post infection. Cumulative morality 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.

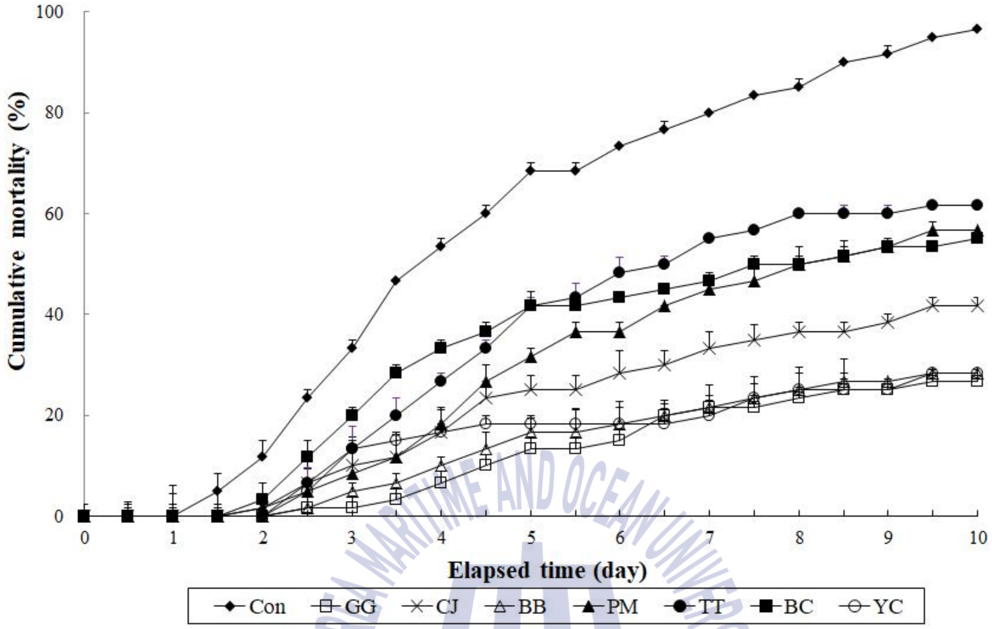


Fig. 1. Cumulative mortality (%) of juvenile rockfish fed the experimental diets containing the various sources of additives for 7 weeks, and then infected by *Streptococcus parauberis* for 10 days (means of triplicates ± SE). GG, CJ, BB, PM, TT, BC, and YC are explained in the footnotes of Table 1.

4. Discussion

Weight gain (g/fish) for fish fed the YC diet was 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 seabass 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., 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 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 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).

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 non specific 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 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 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 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 (Baeck et al., 2006). Cumulative morality 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 -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 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, YC showed the best results in improving weight gain in rockfish, and YC and TT were effective in improving feed efficiency and protein efficiency ratio. GG, BB and YC were effective in lowering the cumulative mortality of rockfish infected with *S. parauberis*.

Chapter 3.

Effects of dietary inclusion of yacon, ginger and blueberry on growth, body composition and challenge test of juvenile rockfish *Sebastes schlegeli* against *Edwardsiella tarda*

1. Introduction

Rockfish, *Sebastes schlegeli* Hilgendorf 1880, has been one of the most commercially important marine aquaculture fish species in Korea, Japan and China over two decades ago due to its fast growth and high resistance against disease. Its annual aquaculture production reached 18,774 tons in 2015 in Korea (MFAFF, 2016). Therefore, many feeding trials to determine dietary nutrient requirements (Kim et al., 2001; Lee et al., 2002; Yan et al., 2007), optimum dietary protein to lipid ratio (Lee and Kim, 2009), 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 (Mizanur et al., 2014) and frequency (Lee et al., 2000), and dietary additives to improve lysozyme activity and stress recovery (Hwang et al., 2013) and immune response against *Vibrio alginolyticus* infection (Kim et al., 1999) for rockfish have been performed.

Since mortality of fish occurs during year-round culture due to outbreak of disease, fish farmers can not avoid the economical loss. They commonly use antibiotics in feed to minimize it. However, as oral administration of the synthetic chemicals such as antibiotics in aquafeed is prohibited in some countries (Tang et al., 2001), development of the new natural source of dietary additive to replace antibiotics keeps being needed.

Yacon *Polymnia sonchifolia* Poeppig and Endlicher (YC) containing the polyphenols had an antioxidant activity (Kim et al., 2010a; Park et al., 2012) and its oral supplementation increased the antioxidant effect on animals (Kim, 2013). In addition, its leaves showed the antibacterial activity against *Staphylococcus aureus* (Choi et al., 2010). In Kim et al. (2016)'s study, yacon was the most effective additive to improve weight gain and feed utilization among the several feed additives, and YC, ginger (GG) and blueberry (BB) were equally effective to lower mortality of juvenile rockfish artificially infected by *Streptococcus parauberis* after 7-week feeding.

GG *Zingiber officinale* Roscoe (GG) containing gingerols and shogaols is known to have antibiotics effect (Sebiomo et al., 2011), antibacterial effect (Ekwenye and Elegalam, 2005), antimicrobial activity (Akintobi et al., 2013) and antioxidant activity (Balestra et al., 2011), and widely used for the treatments of several diseases (Ali et al., 2008). Later, Akintobi et al. (2013) reported that water and ethanol extracts of GG showed the highest inhibition against *Salmonella typhi* and *Proteus mirabilis*, respectively and concluded that the extracts of GG possessed antimicrobial compounds, which could be used as substitutes for the antibiotics. Dietary inclusion of GG effectively improved not only weight gain, but also disease resistance of rainbow trout *Oncorhynchus mykiss*, Asian seabass

Lates calcarifer and Nile tilapia *Oreochromis niloticus* (Nya and Austin, 2009; Talpur et al., 2013; Hassanin et al., 2014).

Blueberry *Vaccinium ashei* Reade (BB) containing anthocyanin had an antioxidant activity (Jeong et al., 2012; Vizzotto et al., 2013; Deng et al., 2014) and showed antioxidant effect on animals (Dunlap et al., 2006; Molan et al., 2008; Papandreou et al., 2009).

Therefore, these natural sources of plant-originated ingredients have high potential as feed additive to replace antioxidant/antibiotics in aquafeed. Ethoxyquin (6-ethoxy-2, 2, 4-trimethyl-1, 2-dihydroquinoline, EQ) is the most widely used feed additive in fish feed to prevent rancidity (Weil et al., 1968; FAO, 1970; Thorisson et al., 1992; Drewhurst, 1998). Blaszczyk et al. (2013) demonstrated that EQ cannot be used in any food for human consumption (except for spices, e.g., chili), but it can pass from feed to farmed fish, poultry, and eggs, so human beings can be exposed to this antioxidant. Recently, Wang et al. (2015) estimated optimal EQ concentration of 13.78 mg/kg diet for maximum growth of juvenile Japanese seabass *Lateolabrax japonicus*.

We hypothesized that oral administration of YC, GG and BB has desirable effect on growth performance of rockfish and lowering mortality at occurrence of disease. Since administration of dietary additive for desirable purpose costs more money to fish farmers, feeding fish on it shorter period of time, but inducing desirable effect on fish is more beneficial. In this study, therefore, the effects of dietary inclusion of various additives (YC, GG and BB) on growth, body composition and challenge test of juvenile rockfish against *Edwardsiella tarda* compared to EQ were investigated. In addition, cumulative mortality of rockfish fed the diets containing various additives for 4 and 8 weeks, and then infected *E. tarda* were compared.

2. Materials and methods

2.1 Fish and the experimental conditions

Juvenile rockfish were purchased from a private hatchery (Tongyeong City, Gyeongsangnam-do, Korea) and acclimated to the experimental conditions for 2 week before an initiation of the feeding trial. During the acclimation period, fish were hand-fed a commercial extruded pellet (Suhyup Feed Co. LTD, Gyeongsangnma-do, Korea) twice a day at a ratio of 2-3% body weight of fish. Three hundred sixty juvenile (an initial body weight of 16.1 g) fish were randomly chosen and distributed into 12 of 50-L flow-through tanks (water volume: 40 L) (thirty fish per tank). The flow rate of water into each tank was 1.42 L/min/tank. The water source was sand-filtered natural sea water and aeration was supplied into each tank. Water temperature monitored daily from 13.4 to 18.6°C (mean ± SD: 15.6 ± 1.65°C) and photoperiod followed natural conditions.

2.2. Preparation of the experimental diets

Four experimental diets were prepared; the control diet (Con) with 0.01% EQ, and YC, GG and BB diets (Table 5). 1% each additive, which is a commercially available product in Korea was included into the experimental diets at the expense of wheat flour. The Con diet was prepared to satisfy dietary nutrient requirements for rockfish (Kim et al., 2001, 2004). Fish and fermented soybean meals were used as the protein source in the experimental diets. Wheat flour, and squid liver and soybean oils were used

Table 5. Ingredients of the experimental diets containing the various sources of additives (DM basis, %)

	Experimental diets			
	Con	YC[1]	GG[1]	BB[1]
Ingredients (%)				
Anchovy meal[2]	63.5	63.5	63.5	63.5
Fermented soybean meal[3]	7.0	7.0	7.0	7.0
Wheat flour	22.99	22.0	22.0	22.0
Ethoxyquin[4]	0.01			
Additive powder		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[5]	1.0	1.0	1.0	1.0
Mineral premix[6]	1.0	1.0	1.0	1.0
Choline	0.5	0.5	0.5	0.5
Nutrients (%)				
Dry matter	90.9	90.8	90.8	90.4
Crude protein0	50.9	51.2	51.1	51.0
Crude lipid	10.3	10.1	10.1	10.1
Ash	11.0	11.2	10.9	10.9

[1]YC (yacon), [1]GG (ginger) and [1]BB (blueberry) were purchased from Tojongherb Co Ltd. (Dongdaemun-gu, Seoul, Korea).

[2]Anchovy meal was purchased from Abank Co Ltd. (Seocho-gu, Seoul, Korea).

[3]Fermented soybean meal was supplied by CJ CheilJedang Corp. (Jung-gu, Seoul, Korea).

[4]Ethoxyquin was supplied from Chunhajeil feed Co Ltd. (Daedeok-gu, Daejeon, 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.

as the carbohydrate and lipid sources in the experimental diets, respectively.

The ingredients of the experimental diets were well mixed with water at a ratio of 3:1 and pelletized by laboratory pellet extruder (Dongsung mechanics, Busan, Korea). The experimental diets were dried at room temperature over night and stored in -20°C until used. Each diet was randomly assigned to triplicate tanks of fish and hand-fed to satiation twice daily (09:00 and 17:00 h) for 7 day a week for 8 weeks.

2.3 Analytical procedures of the experimental diets and fish

Ten fish at the start and five 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. All methods were according to standard AOAC (1990) practices.

2.4 Challenge test

Ten and fifteen externally normal fish after the 4^{th} and 8^{th} week feeding trial, respectively, shown to be free from bacterial infection were selected from each tank and stocked into 12, static 50-l tanks. Ten fish used for challenge test on 4th week of the feeding trial was counted as the live fish at the end of the 8-week feeding trail. Fish was used for the *E. tarda* challenge and water was static. The bacteria used for the challenge were reference pathogenic strain of gram negative-*E. tarda* (ET-1) isolated from rockfish.

The culture suspension of *E. tarda* were grown on agar for 24 h, collected, washed and suspended in sterile 8.5% saline solution and counted. Then, fish were artificially infected by intraperitoneal injection with 0.1 mL of culture suspension of pathogenic *E. tarda* containing 1.2×10^5 cfu/mL. Fish were monitored for the following 10 days after *E. tarda* 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 10-day challenge test.

2.6 Calculation 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 (FE) = (final total weight of fish + total weight of 10 fish sacrificed for challenge test at 4th week − initial total weight of fish)/total feed consumed, protein efficiency ratio (PER) = weight gain of fish/protein consumed and protein retention (PR) = protein gain×100/protein consumed. Relative percent survival (RPS) of rockfish was calculated by formula (Amend, 1981): RPS = [1 − (% mortality of experimental fish/% mortality of the control fish)] ×100.

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.

3. Results

3.1 Growth performance of fish

All the fish remained alive at the end of the 8-week feeding trial. Weight gain and specific growth rate (SGR) of fish fed the YC diet was significantly (P < 0.05) greater than those of fish fed all other diets (Table 6). Weight gain and SGR of fish fed the GG and BB diets was also significantly (P < 0.05) greater than those of fish fed the Con diet.

3.2 Feed utilization

Feed consumption (g/fish) of fish fed the GG and BB diets was significantly (P < 0.05) higher than that of fish fed the Con and YC diets at the end of the 8-week feeding trial (Table 7). FE, PER and PR of fish fed the YC diet was significantly (P < 0.05) higher than those of fish fed all other diets. PER of fish fed the Con diet was significantly (P < 0.05) higher than that of fish fed the BB diet, but not significantly (P < 0.05) different from that of fish fed the GG diet. PR of fish fed the GG diet was significantly (P < 0.05) higher than that of fish fed the BB diet, but not significantly (P > 0.05) different from that of fish fed the Con diet.

3.3 Chemical composition of the whole body of fish

Moisture content of the whole body of fish fed the BB diet was significantly (P < 0.05) higher than that of fish fed all other diets (Table 8). Moisture content of the whole body of fish fed the Con and YC diets

Table 6. Survival (%), weight gain (g/fish) and specific growth rate (SGR) of rockfish fed the experimental diets containing the various sources of additives for 8 weeks

Experimental diets	Initial weight (g/fish)	Final weight (g/fish)	Weight gain (g/fish)	SGR[1] (%/day)
Con	16.1 ± 0.04	48.2 ± 0.17^{d}	32.0 ± 0.15^{d}	1.95 ± 0.005^{d}
YC	16.1 ± 0.01	51.9 ± 0.11^{a}	35.8 ± 0.10^{a}	2.09 ± 0.003^{a}
GG	16.1 ± 0.01	50.8 ± 0.10^{b}	34.7 ± 0.08^{b}	2.05 ± 0.002^{b}
BB	16.2 ± 0.02	50.0 ± 0.12^{c}	33.8 ± 0.13^{c}	2.01 ± 0.005^{c}

Values (means of triplicate ± 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 7. Feed consumption (g/fish), feed efficiency (FE), protein efficiency ratio (PER) and protein retention (PR) of rockfish fed the experimental diets containing the various sources of additives for 8 weeks

Experimental diets	Feed consumption (g/fish)	FE[1]	PER[2]	PR[3]
Con	32.2 ± 0.13^{b}	1.07 ± 0.012^{b}	1.95 ± 0.016^{b}	34.9 ± 0.34^{bc}
YC	33.4 ± 0.15^{b}	1.17 ± 0.008^{a}	2.09 ± 0.009^{a}	38.1 ± 0.14^{a}
GG	35.0 ± 0.30^{a}	1.07 ± 0.013^{b}	1.94 ± 0.021^{bc}	35.2 ± 0.46^{b}
BB	35.1 ± 0.28^{a}	1.04 ± 0.011^{b}	1.89 ± 0.008^{c}	33.8 ± 0.22^{c}

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.

Table 8. Proximate composition (%) of the whole body of rockfish fed the experimental diets containing the various sources of additives for 8 weeks

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
Con	68.6 ± 0.03^b	17.8 ± 0.03^b	8.8 ± 0.03^c	4.5 ± 0.03^a
YC	68.7 ± 0.03^b	18.1 ± 0.07^a	9.3 ± 0.03^a	4.1 ± 0.03^b
GG	69.2 ± 0.06^a	18.0 ± 0.03^a	9.1 ± 0.06^b	4.4 ± 0.03^a
BB	69.2 ± 0.03^a	17.9 ± 0.03^b	8.7 ± 0.03^c	4.4 ± 0.03^a

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

was also significantly (P < 0.05) higher than that of fish fed the GG diet. Crude protein content of the whole body of fish fed the YC and GG diets was significantly (P < 0.05) higher that of fish fed the Con and BB diets. Crude lipid content of the whole body of fish fed the YC diet was significantly (P < 0.05) higher that of fish fed all other diets. Crude lipid content of the whole body of fish fed the GG diet was also significantly (P < 0.05) higher that of fish fed the Con and BB diets. Ash content of the whole body of fish fed the Con, GG and BB diets was significantly (P < 0.05) higher that of fish fed the YC diet.

3.4 Challenge test

Cumulative mortality of rockfish fed the experimental diets containing various sources of additive for 4 and 8 weeks, and then infected with *E. tarda* for 10 days observation is depicted in Figs. 2 and 3, respectively.

In the 4^{th} week infection trial, mortality of fish fed the Con and YC diets started to appear at 90 h after infection. Mortality of fish fed the Con diet was significantly (P < 0.05) higher than that of fish fed the all other (YC, GG and BB) diets at 90 h and reached 80% at 10 day after infection. However, no significant difference in mortality was found in fish fed the YC, GG and BB diets throughout the 10-day observation.

In the 8^{th} week infection trial, mortality of fish fed the Con and BB diets started to appear at 90 and 96 h after infection, respectively. Mortality of fish fed the Con diet was significantly (P < 0.05) higher than that of fish fed the all other diets at 90 h and reached 93% at 10 day

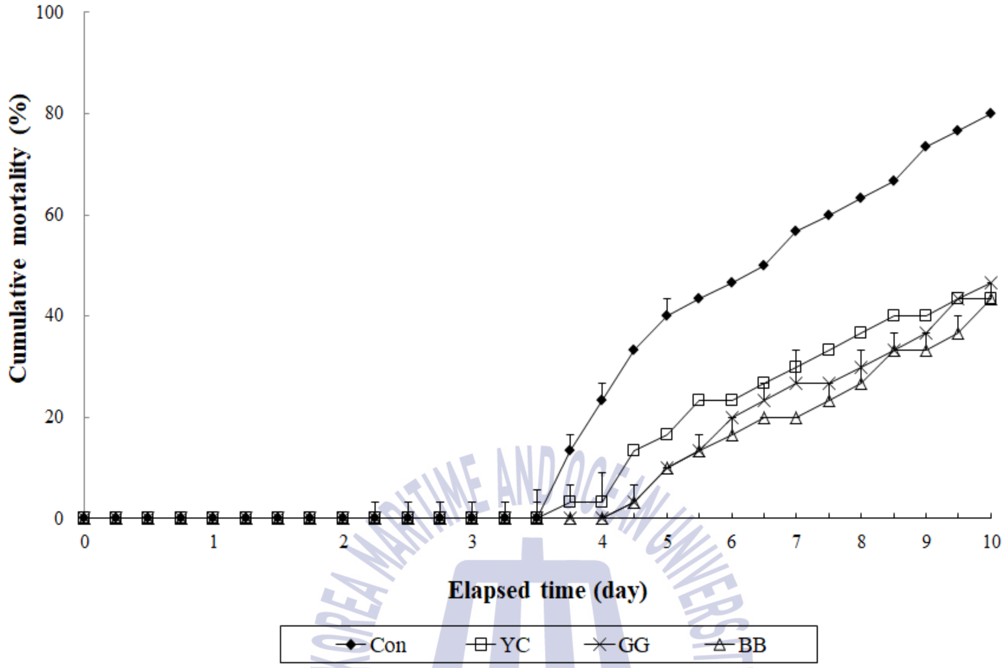


Fig. 2. Cumulative mortality (%) of juvenile rockfish fed the experimental diets containing various sources of additives for 4 weeks, and then infected by *Edwardsiella tarda* (means of triplicate ± SE). (Con, YC, GG and BB indicate that the control diet with 0.01% ethoxyquin, and the diets containing 1% yacon, ginger and blueberry, respectively).

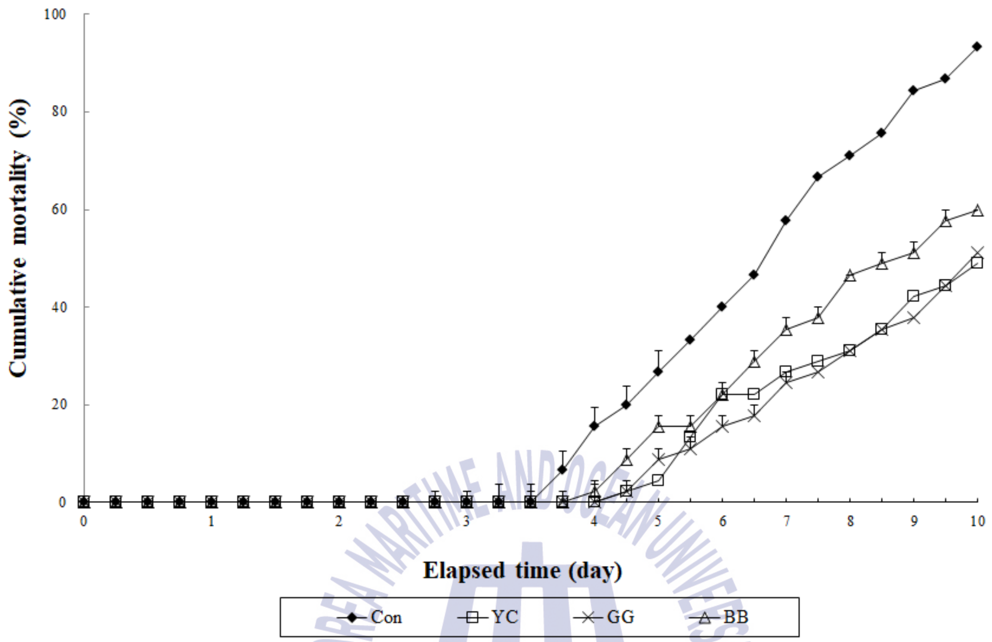


Fig. 3. Cumulative mortality (%) of juvenile rockfish fed the experimental diets containing various sources of additives for 8 weeks, and then infected by *E. tarda* (means of triplicate ± SE). (Con, YC, GG and BB indicate that the control diet with 0.01% ethoxyquin, and the diets containing 1% yacon, ginger and blueberry, respectively).

after infection. Cumulative mortality of fish fed the YC and GG diets was also significantly (P < 0.05) lower than that of fish fed the Con and BB diets since 156 h after infection throughout the 10-day observation.

RPS (%) of rockfish fed the experimental diets containing various sources of additive for 4 and 8 weeks, and then infected with *E. tarda* for 10 days observation was given in Table 9. In the 4th week infection trial, mortality of fish fed the Con, YC, GG and BB diets at 10-day post observation were 80.0, 43.3, 46.7 and 43.3%, respectively. In the 8th week infection trial, mortality of fish fed the Con, YC, GG and BB diets at 10-day post observation were 93.3, 48.9, 51.1 and 60.0%, respectively. RPS of fish increased by 44.7, 41.0 and 44.7%, and 47.4, 44.9 and 35.5% in fish fed the YC, GG and BB diets compared to the Con diet for 4 and 8 weeks, respectively, at 10-day post observation.

Table 9. Relative percent survival (RPS, %) of rockfish fed the experimental diets for 4 and 8 weeks, and artificially infected with *Edwardsiella tarda* at 5- and 10-day observation

Experimental diets	4 week		8 week	
	Day 5	Day 10	Day 5	Day 10
Con				
YC	54.4	44.7	84.9	47.4
GG	73.9	41.0	67.2	44.9
BB	73.9	44.7	36.7	35.5

Relative percent survival (RPS, %) was calculated by the formula: RPS = [1 − (% mortality of the experimental fish/% mortality of the control fish)] ×100.

4. Discussion

All feed additives used in this study achieved an improved growth performance (weight gain and SGR) of rockfish compared to the Con diet. The greatest weight gain and SGR was obtained in rockfish fed the YC diet agreed with Kim et al. (2016)'s study. Higher feed consumption of rockfish fed the GG and BB diets, but lower weight gain, SGR, FE, PER and PR compared to those of fish fed YC diet in this study indicates that YC is the most effective growth promoter in terms of improved growth performance and feed utilization.

An improved weight gain and SGR, but no difference in feed utilization (FE, PER and PR) of rockfish fed the GG diet compared to the Con diet in this study conflicted with other studies (Nya and Austin, 2009; Talpur et al., 2013; Hassanin et al., 2014) showing that weight gain and feed utilization (FE or PER) of rainbow trout, Asian sea bass and Nile tilapia were effectively improved when 1–10% GG was included into the diet. This could be resulted from freshness type of GG used. A commercially available GG was used in this study, but raw GG purchased from farm market was directly included into the diet with other feed ingredients while feed manufacturing in their study. However, GG, which is the same GG used in this study did not affect either weight gain or feed utilization of rockfish (Kim et al., 2016).

The plant-originated additives were effective to improve not only weight gain and feed utilization, but also disease resistance in some marine fish (Ji et al., 2007; Punitha et al., 2008; Talpur and Ikhwanuddin, 2013; Talpur et al., 2013; Talpur, 2014). When greasy grouper *Epinephelus tauvina* were fed on the diets supplemented with methanolic extract of each Indian herb of *Ocimum sanctum*, *Withania somnifera* or *Myristica fragrans* at the concentration of 0 (control), 100, 200, 400 and 800 mg/kg diet for 12 weeks, improved weight gain, FE and immune parameters (phagocytic activity, serum bacterial activity, albumin-globulin ratio and leukocrit) were observed in fish fed the diets supplemented with 100 and 200 mg/kg *O. sanctum* and *W. somnifera* compared to the control diet (Sivaram et al., 2004). Gabor et al. (2010) reviewed use of phytoadditives, such as garlic *Allium sativum*, onion *Allium cepa*, oregano *Origanum vulgare*, GG *Zingiber officinale*, Echinacea *Echinadea purpurea*, cinnamon *Cinnamomum verum* or nettle *Urtica dioica* in diet on performance of several fish and emphasized the major advantages in use of phytoadditives is the fact that they are natural substances and do not pose any threat to fish, man or environment.

The chemical composition of the whole body of rockfish was affected by dietary additive in this study. Similarly, dietary inclusion of different additives affected the whole body composition of fish (Bai et al., 2001; Yun et al., 2016). Dietary inclusion of YC increased the whole body lipid content of rockfish in this study and this conflicted with Kim et al. (2010b)'s study showing that dietary inclusion of YC could potentially 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 lowered total lipid content in rockfish

(Hwang et al., 2013).

The dead rockfish in the 4^{th} and 8^{th} week infection trials exhibited the typical symptoms of diseased olive flounder infected with *E. tarda*, such as reddish in abdomen and fins, darkening of the skin and swelling of abdomen with accumulation of ascites reported by Kusuda and Kawai (1998)'s study. Mortality of fish fed the Con diet was higher than that of fish fed the all other diets at 90 h throughout 10-day post observation in this study, but no difference in mortality was observed among fish fed the YC, GG and BB diets in the 4^{th} week infection trial. The cumulative mortality of fish fed the YC and GG diets was lower than that of fish fed the Con and BB diets at 156 h after infection in the 8^{th} week infection trial.

There is a quite similar trend toward increased mortality of rockfish over time, time to show initial mortality of fish receiving the Con diet, differences between the Con and experimental (YC, GG and BB) diets in this study. This indicates that 4 weeks of feeding rockfish with the experimental diets to lower mortality of fish at occurrence of *E. tarda* was comparable to 8 weeks of feeding fish. Unlike this study, however, Sivaram et al. (2004) reported that dietary inclusion of extract of 100 and 200 mg/kg diet *O. sanctum* and *W. somnifera* reduced mortality of greasy grouper at the end of 12 week feeding, but not either after 4- and 8-week feeding or fish receiving *M. fragrans* in any period of time when fish were fed on one of the diets supplemented with *O. sanctum*, *W. somnifera* or *M. fragrans* at the concentration of 0, 100, 200, 400 and 800 mg/kg diet for 4, 8 and 12 weeks, and then infected by *V. harveyi*. Punitha et al. (2008) also reported that greasy grouper did not effectively lowered mortality of fish after 20- or 40-day feeding, but fish receiving 100, 200, 400 and 800 mg/kg diet did compared to control diet only after 60-day feeding when fish were fed the diets containing extracts of herbal medicines (*Cynodon dactylon*, *Piper longum*, *Phyllanthus niruri*, *Tridax procumbens* and GG) at the concentration of 0 (control), 100, 200, 400 and 800 mg/kg diet for 20, 40 or 60 days, and then infected with *V. harveyi*.

Dietary inclusion of YC, GG and BB effectively lowered mortality of rockfish compared to the Con diet after the 4- and 8-week feeding trial in this study. This indicates that YC, GG and BB are all effective as immunostimulant to lower mortality of fish at occurrence of *E. tarda*. Similarly, Nya and Austin (2009) reported that mortality effectively lowered from 640 to 160, 40 and 0 g/kg in rainbow trout infected with *Aeromonas hydrophila* compared to fish receiving 0 (control), 10, 0.5 and 5 g/kg GG, respectively, when fish were fed with commercial diet supplemented with GG at the concentration of 0, 0.5, 1, 5 and 10 g/kg for two weeks prior to infection. In addition, Hassanin et al. (2014) reported that mortality of Nile tilapia lowered effectively when fish were fed with diets containing 1−10 g/kg GG for 10 weeks, and then infected with *A. hydrophila*. Mortality of Asian sea bass fed the diets containing 0.1−1 g/kg GG for 15 days, and then infected with *Vibrio harveyi* decreased effectively compared to the control diet without GG (Talpur et al., 2013).

Akintobi et al. (2013) showed that high antimicrobial activity of GG extract against six pathogenic microorganisms (*Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Bacillus subtilis* and *Salmonella typhi*) in vitro test and explained that GG can be used as substitutes for the antibiotics. In addition, aqueous extract of GG had little or no inhibiting effect on *Escherichia coli* and *Salmonellla typhi*, but ethanolic extract had a higher inhibitory effect (Ekwenye and Elegalam, 2005). Ohara et al. (2008) also reported that the GG family *Zingiberaceae* contains the most abundant and stable antibacterial components against *S. mutans* among 81 tested edible plants. Dietary supplementation of additives such as garlic, neem and peppermint have been reported successfully replace antibiotics in aquafeed and to lower the mortality of Asian seabass infected with *V. harveyi* (Talpur and Ikhwanuddin, 2012, 2013; Talpur, 2014). Vallejos-Vidal et al. (2016) reviewed several plants, herbs, algae extracts and pathogen associated molecular patterns as immunostimulants in aquafeeds.

In considering Kim et al. (2016)'s study showing that YC, GG and BB were effective to lower mortality of rockfish at occurrence *S. parauberis* (gram positive) (KCTC11980BP), which was first reported in rainbow trout *Oncorhynchus mykiss* in Japan (Hoshina et al., 1958) and this study showing that YC, GG and BB are all effective to lower mortality of rockfish at occurrence *E. tarda* (gram negative), which was first isolated from a pond-cultured eel (Hoshina, 1962), YC, GG and BB are all effective as immunostimulants for both bacterial pathogens. More studies to determine if antioxidant materials originated from additive to induce desirable effect on fish performance are found in fish body will need to be determined in future. Recently, Bulfon et al. (2015) also reviewed use of plan-derived products in farmed fish and demonstrated the desirable effects of plan extracts/products on fish growth, haematological profiles, immune responses and resistance to infectious diseases.

In conclusion, dietary inclusion of YC, GG and BB effectively increased weight gain and SGR of rockfish. However, YC improved FE, PER and PR of fish effectively. Oral administration of YC, GG and BB for 4 and 8 weeks effectively lowered mortality of rockfish at occurrence of *E. tarda*. A 4-week oral administration of additive (YC, GG and BB) was long enough to induce their desirable effect of lowering mortality of fish at occurrence of *E. tarda*.

Chapter 4.

Attractiveness/palatability of various protein sources and their function as feeding attractant to promote feed consumption, growth performance, feed utilization and body composition in juvenile rockfish *Sebastes schlegeli* through dietary supplementation

1. Introduction

Rockfish *Sebastes schlegeli* has been one of the most commercially important marine fish species for aquaculture in the Eastern Asian countries, such as Korea, Japan, and China, for over three decades due to its fast growth and high resistance against disease. Its annual aquaculture production reached 17996 tons in 2016 in Korea (KOSIS, 2017). Most rockfish aquaculture production in Korea has occurred by feeding either frozen raw fish (RF), such as sardines and mackerel, or RF-basal moist pellet (MP). Feeding fish either RF or MP is likely to cause several problems, such as severe water pollution resulting from a high leaching-out rate of nutrients in RF and MP, an unstable supply of RF or a depletion of the RF that are commonly used as fish meal sources in the wild. Alternatively, formulated feed (FF) should be developed to efficiently produce rockfish in an eco-friendly and cost-effective manner. Nevertheless, FF is still difficult rockfish farmers to adopt due to their biased perception of the superior production and feed consumption of fish fed either RF or MP over FF. A lower feed consumption of fish fed on FF compared to either RF or MP could result from a deficiency or lower amount of feeding attractants and/or stimulants in the former. Therefore, adding feed ingredients that contain high levels of rockfish attractants to FF is highly desirable for improving fish production. Although many studies have been performed on rockfish (Nakagawa et al., 2007; Jeon et al., 2014; Cho et al., 2015; Lee et al., 2016), little on the feeding attractants and/or stimulants of fish is known.

Feeding attractants and/or stimulants are usually low molecular-weight metabolites including amino acids, quaternary ammonium compounds, nucleosides and nucleotides, and organic acids, which are important tissue components of the main prey items and constitute the species' natural diet (Carr and Derby, 1986; Morais, 2017). However, feeding attractants and/or stimulants are known to vary depending on fish specifies, type of feeding attractants, and dose of feeding attractants (Tandler et al., 1982; Carr and Derby, 1986; Carr et al., 1996; Papatryphon and Soares, 2001; Xue and Cui, 2001; Kasumyan and Nikolaeva, 2002; Yacoob and Browman, 2007).

The application of feeding attractants and/or stimulants in aquafeed has received considerable attention for a long time. The main reasons for their use are to improve the feed consumption of aquatic animals and to minimize feed wastage and water pollution sources (Tandler et al., 1982; Ellingsen and Døving, 1986; Takii et al., 1994). Fish detect chemical stimuli through at least two different chemoreception channels: olfaction (smell) and gustation (taste) (Hara, 1993). The chemical cues detected by olfactory receptors are conveyed to the brain through the olfactory nerve. Kohbara et al. (2000) showed that thresholds of adsorbate and non-adsorbate in the olfactory bulb response were approximately 10^{-5} and 10^{-8} and approximately 10^{-4} and 10^{-2} of the original concentration of the jack mackerel *Trachurus japonicus* extract, and in the gustatory nerve response of young yellowtail *Seriola quinqueradiata*, respectively, and demonstrated that the gustatory receptors in yellowtail are involved in discriminating food items during feeding. Further, olfactory receptors may detect foods at some distance.

Amino acids are a major class of the olfactory stimuli that cause food search behavior in a variety of fishes (Jones, 1992; Kohbara et al., 2000; Yacoob and Browman, 2007). Takeda et al. (1984) showed that amino acid fractions were more stimulatory components than were either nucleotide fractions or other compounds in the extracts of a marine worm *Perinereis brevicirrus* for eel *Anguilla japonica* base on feed consumption. Carr et al. (1996) also reported that the extracts of tissues from 10 species of marine fish and 20 species of invertebrates contained high concentrations of five of the most frequently cited feeding stimulants (glycine, alanine, proline, arginine and betaine) base on feeding behavioral assay and concluded that glycine and alanine were major tissue components and were the two most frequently cited feeding stimulants for 35 teleost fishes. Glutamine and histidine served as feeding stimulants for yellowtail and olive flounder *Paralichthys olivaceus*, respectively (Kohbara et al., 2000; Ikeda et al., 2012). In an earlier study by Hara (1984), yellowtail achieved remarkable behavioral feeding attraction with histidine in the basic amino acid groups and glycine and threonine in the neutral amino acid groups in the extracts of squid *Todarodes pacificus*. Yacoob and Browman (2007) reported that the relative response magnitudes (expressed as a percentage of response to 10^{-3} M alanine) of 20 amino acids varied from 144% for methionine to 19% for aspartic acid in the olfactory sensitivity based on behavioral assay in Atlantic halibut *Hippoglossus hippoglossus*. Takaoka et al. (1995) showed that the amino acid fractions in the synthetic extracts of clam *Tapes japonicus* achieved a remarkably higher feeding stimulant response than did nucleotides and other chemical fraction based on daily feeding rate for tiger puffer *Takifugu rubripes*. Ellingsen and Døving (1986) reported that glycine was the most potent, followed by alanine, of the extracts of shrimp *Pandalus boreal*is for cod *Gadus morhua* and found the synergistic effect of 4 amino acids (alanine, arginine, glcycine and proline) was greater than the total amino acid pool in the shrimp extract according to bottom food search feeding behavior of fish. Papatryphon and Soares (2000) also demonstrated that the synergistic effects of L-alanine, L-serine, inosine-5′-monophosphate (IMP) and betaine stimulated feed intake for striped bass *Morone saxatilis* and that a mixture of all four compounds together achieved the maximum response. Later, Papatryphon and Soares (2001) showed that the inclusion of combined alanine, betaine, serine and IMP in a corn gluten-basal diet was as effective as a practical fish meal-basal diet for striped bass in terms of improved feed efficiency and growth performance.

In the nucleotide fraction of the extracts of krill *Euphausia pacifica*, inosine, in particular, was the most effective feeding stimulant in marbled rockfish *Sebastiscus marmoratus*, followed by the amino acid fraction showing a poor feeding activity in the fish (Takaoka et al., 1990). Hosokawa et al. (2001) also showed that nucleotides, especially, inosinic acid in the extracts of jack mackerel were the main compounds to achieve feeding responses in yellowtail. Betaine has also been identified as a feeding stimulant in a variety of fishes, such as pinfish *Lagodon rhomboides*, pigfish *Orthopristis chrysopterus*, Dover sole *Solea solea*, tiger puffer, striped bass and grouper *Epinephelus fuscoguttatus* (Carr, 1976; Carr et al., 1976; Mackie et al., 1980; Takaoka et al., 1995; Papatryphon and Soares, 2000; Lim et al., 2015).

Fish meal (Smith et al., 2005; Singh et al., 2006); crustacean meals, such as crab meal (Carr et al., 1977), krill meal (Takaoka et al., 1990; Shimizu et al., 1990; Suresh et al., 2011), shrimp head meal (Fox et al., 1994) and shrimp meal (Carr and Chaney, 1976); and mollusk meals, such as mussel meal (Nagel et al., 2014; Mongile et al., 2015), oyster meal (Carr et al., 1977), clam (Takaoka et al., 1995), squid liver meal (Suresh et al., 2011), squid extract (Toften et al., 1995; Xue and Cui, 2001) and squid meal (Naik et al., 2001), are known to have high attractiveness for several aquatic animals. In many of the above cases, however, omission tests were conducted to determine whether aquatic animals are attracted to the synthetic components based on a chemical analysis of the extracts of these ingredients. Determining the attractiveness of aquatic animals based on either omission tests or monitoring the physiological and electro(neuro) physiological responses of the components in the synthetic extracts of prey is a very complicate process.

No study has examined whether the addition of a selected feed ingredient affects fish performance based on its attractiveness. This application is simple, highly desirable and recommendable in formulating practical diets to improve the production of fish. Plant-originated protein sources, such as corn gluten meal (Lee et al., 1996) and soybean meal (Lim et al., 2004), have also been successfully used as alternative protein sources for fish meal in rockfish diets.

In this study, therefore, the attractiveness of the selected feed ingredient among the various aquatic animal- and terrestrial plant-originating protein sources was evaluated in juvenile rockfish, and their dietary supplementation effects on the growth performance, feed utilization and body composition of fish were determined and compared.

2. Materials and methods

2.1 Preliminary test of determination of attractiveness of various feed ingredients

2.1.1 Preparation of the experimental fish

Juvenile rockfish of similar size were purchased from a private hatchery (Tongyeong City, Gyeongsangnam-do, Korea) and acclimated to the experimental conditions for 2 weeks before the preliminary test. During the conditioning period, fish were hand-fed a commercial extruded pellet (Woosung Feed Co. Ltd., Daejeon, Korea) twice a day at a ratio of 2–3% of the body weight of the fish. Juveniles [average body weight of 3.6 ± 0.07 g (mean ± SE)] were used in the preliminary test. The water source was sand-filtered natural sea water. The water temperature was monitored in each trial and ranged from 15.7 to 20.0 °C (mean ± SD: 17.2 ± 1.84°C).

2.1.2 Feed ingredient used to determine attractivity of fish and its chemical composition

The sources of feed ingredients used to determine the attractiveness of fish and chemical composition are shown in Tables 10 and 11, respectively. Feed ingredients were analysed using the AOAC methods (1990). Crude protein was determined using 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,

Table 10. List of feed ingredient used in this study

Feed ingredient		Supply (nation)
Fish meal	Anchovy meal	Blumar (Santiago, Chile)
	Herring meal	FF Skagen (Skagen, Denmark)
	Jack mackerel meal	Foodcorp chile sa (Coronel, Chile)
	Hydrolyzed fish meal	Sopropêche (Wimille, France)
	Pollack meal	Kodiak fishmeal company (Alaska, USA)
	Sardine meal	Orizon S.A (Santiago, Chile)
Crustacean meal	Crab meal	Bigmama Seafood (Tongyeong city, Korea)
	Krill meal	Aker Biomarine ASA (Lysaker, Norway)
	Shrimp head meal	Harinesa (Guayas, Ecuador)
	Shrimp meal	Fortidex S.A (Guayas, Ecuador)
Mollusk meal	Mussel meal	Bigmama Seafood (Tongyeong city, Korea)
	Oyster meal	Bigmama Seafood (Tongyeong city, Korea)
	Squid liver meal	Dong Woo Ind Co (Bonghwa-gun, Korea)
	Squid meal	APM Logis (Seoul, Korea)
Plant meal	Corn gluten meal	FairBizKorea Co (Seoul, Korea)
	Defatted soybean meal	Dashmesh Global LLC (Illinois, USA)

Table 11. The proximate composition of feed ingredient (%, DM basis)

Feed ingredient		Nutrient			
		Moisture	Crude protein	Crude lipid	Ash
Fish meal	Anchovy meal	8.7	72.3	9.7	15.0
	Herring meal	6.7	66.8	8.8	15.9
	Jack mackerel meal	5.4	74.2	8.6	12.8
	Hydrolyzed fish meal	1.7	74.6	8.6	5.3
	Pollack meal	9.9	63.2	7.4	18.9
	Sardine meal	6.6	70.2	9.5	15.7
Crustacean meal	Crab meal	2.4	38.6	0.8	42.6
	Krill meal	4.7	59.5	19.8	10.3
	Shrimp head meal	8.5	50.5	8.8	20.6
	Shrimp meal	3.7	55.8	7.8	20.7
Mollusk meal	Mussel meal	1.9	61.5	11.7	8.3
	Oyster meal	10.8	47.9	1.0	8.1
	Squid liver meal	5.7	46.3	18.6	6.7
	Squid meal	7.0	70.7	1.9	5.1
Plant meal	Corn gluten meal	4.4	66.2	11.3	3.4
	Defatted soybean meal	5.2	58.9	2.5	8.1

Sweden); moisture was determined by oven drying at 105°C for 24 h; and ash was determined using a muffle furnace at 550°C for 4 h.

2.1.3 Preparation of apparatus to determine attractiveness of fish

Three reinforced acrylic tanks (1 m × 0.6 m × 0.5 m; water volume: 270 L) composed of three equally divided rectangular attracting chambers (0.6 m × 0.2 m × 0.5 m each) and an acclimatization chamber (0.4 m × 0.6 m × 0.5 m) were used to evaluate the attractiveness of feed ingredients to rockfish (Fig. 4). The flow rate of each attracting chamber was 3.24 L/min/chamber. A vertically movable acrylic shutter divided the attracting and acclimatization chambers. Each attracting chamber had a funnel-shaped entrance (10 and 5 cm in radius in and out, respectively) to allow fish free access to each feed ingredient placed in each attracting chamber. Funnel-shaped entrances were video-recorded to check the number of fish entering through the funnel-shaped entrance. Moderate aeration was supplied in each chamber, and the photoperiod followed natural conditions.

2.1.4 Evaluating the attractiveness of feed ingredients in the preliminary test

The attractiveness of feed ingredients to rockfish was determined by randomly selecting three kinds of feed ingredients at a time. Thirty randomly chosen fish were stocked in the acclimatization chamber for at least for 72 h before the test. Then, 20 g of different feed ingredient powders wrapped in 100 ㎛ (mesh size) micromesh gauze (Samjee Tech Co., Anyang city, Gyeonggi-do, Korea) was placed into three attracting chambers,

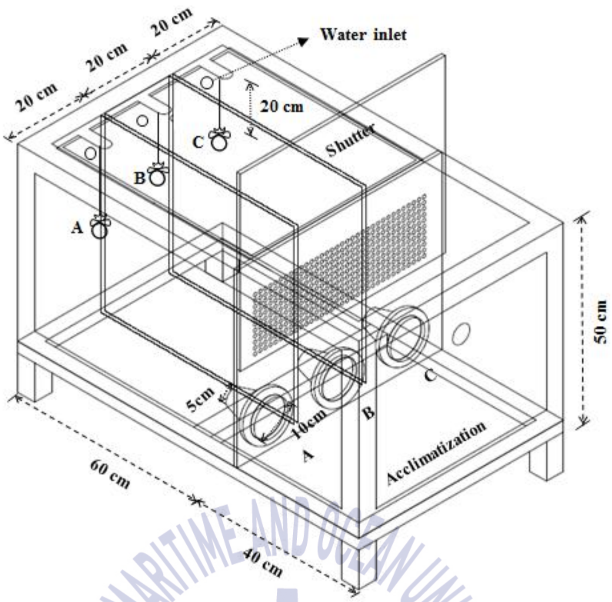


Fig. 4. Drawing of tank used to determine attractiveness of rockfish depending on the various feed ingredient.

each 20 cm below the top. Then, the shutter was raised to allow fish free access to each feed ingredient in the attracting chambers for 30 min. The raised shutter was returned to its original place to count the number of fish in each attracting chamber. To evaluate the attractiveness of feed ingredients to rockfish, tournament comparison was applied. From the 1st to 6th tests in the preliminary test, sixteen feed ingredients were compared to determine the highest attractiveness to fish. From the 7th to 12th tests in the preliminary test, the top 11 feed ingredients, in terms of attractiveness to fish, were chosen and evaluated by tournament comparison to determine the top 5 feed ingredients. Once fish were used to determine the attractiveness of a feed ingredient, they were never again used to determine attractiveness. The tank was cleaned by tap-water after every test and refilled with filtered seawater. New fish were used and acclimated to the experimental conditions for 72 h in each test. No replication to determine attractiveness by fish was made in the preliminary test.

2.2 Determination of attractiveness of the top 5 selected feed ingredients by rockfish

The top 5 selected feed ingredients showing high feeding attractiveness to rockfish, as determined in the preliminary test, were compared in triplicate. The observation of attractiveness to fish was monitored for 30 min after placing each feed ingredient in the attracting chamber. The attractiveness of feed ingredients to fish was evaluated using the same methods as in the preliminary test. The water temperature ranged from 17.2 to 22.3°C (mean ±

SD: 20.4 ± 2.76°C). Juvenile [average body weight of 3.9 ± 0.21 g (mean ± SE)] rockfish were used to determine the attractiveness of feed ingredients to rockfish.

2.3 Feeding trial of rockfish

2.3.1 The experimental conditions of the feeding trial

Three hundred sixty juvenile [average body weight of 5.3 ± 0.01 g (mean ± SE)] rockfish were randomly distributed into 18 50-L flow-through tanks (water volume: 40 L) (thirty fish per tank). The flow rate of water into each tank was 4.16 L/min/tank. The water source was sand-filtered natural seawater and aeration was supplied to each tank. The water temperature ranged from 17.6 to 23.9°C (mean ± SD: 20.9 ± 1.64°C) and the photoperiod followed natural conditions.

2.3.2 Preparation of the experimental diets

Six experimental diets were prepared (Table 12). A 55% anchovy meal which is being commonly used as fish meal source in commercial fish feed was included in the control (Con) diet. The 5% jack mackerel meal (JM), sardine meal (SM), pollack meal (PM), squid meal (SQM) and shrimp meal (SHM) were included in place of the anchovy meal. Anchovy meal showed a moderate feeding attractiveness to rockfish in the preliminary test, and fermented soybean meal was used as the protein source in the Con diet. Wheat flour and fish and soybean oils were used as the carbohydrate and lipid sources in the Con diet, respectively. All experimental diets were prepared

Table 12. Ingredient and chemical composition of the experimental diets containing feed ingredient with high feeding attractants (DM basis, %)

	Experimental diets					
	Con	JM	SM	PM	SQM	SHM
Ingredient (%)						
Anchovy meal	55	50	50	50	50	50
Jack mackerel meal (JM)		5				
Sardine meal (SM)			5			
Pollack meal (PM)				5		
Squid meal (SQM)					5	
Shrimp meal (SHM)						5
Fermented soybean meal[1]	10.5	10.5	10.5	10.5	10.5	10.5
Wheat flour	23	23	23	23	23	23
Fish oil	4.5	4.5	4.5	4.5	4.5	4.5
Soybean oil	4.5	4.5	4.5	4.5	4.5	4.2
Vitamin premix[2]	1	1	1	1	1	1
Mineral premix[3]	1	1	1	1	1	1
Choline	0.5	0.5	0.5	0.5	0.5	0.5
Nutrients (%)						
Dry matter	95.9	96.0	96.0	96.1	96.1	96.0
Crude protein	52.1	52.0	52.0	52.0	52.0	52.0
Crude lipid	15.7	15.9	15.9	15.5	15.6	15.5
Ash	10.5	10.4	10.5	10.5	10.4	10.5

[1]Fermented soybean meal was purchased from CJ CheilJedang Corp. (Jung-gu, Seoul, Korea).

[2]Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbicacid, 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.

[3]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; ferriccitrate, 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.

to satisfy the dietary nutrient requirements for rockfish (Kim et al., 2001, 2004).

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). The experimental diets were dried overnight at room temperature and stored at -20°C until use. Each diet was randomly assigned to the triplicate tanks of fish and hand-fed to satiation twice daily (07:00 and 17:00), 7 day a week for 8 weeks.

2.4 Analytical procedures of the experimental diets and fish

Ten fish at the start and from each tank at termination of the feeding trial were sampled and sacrificed for proximate analysis. The chemical composition of the experimental diets and fish were determined by the AOAC (1990) method. The amino acid (AA) compositions of the selected feed ingredients were determined using a high-speed AA analyser (Hitachi L-8800, Tokyo, Japan) after sample hydrolysis in 6 N HCl for 24 h at 110 °C.

2.5 Calculation of equation

The following equations were calculated: Attractiveness (%) of fish = number of fish moved into the each attracting chamber×100/total number of fish in acclimatization chamber. Specific growth rate (SGR, %/day) = (Ln final weight of fish − Ln initial weight of fish)×100/days of feeding trial; feed efficiency (FE) = weight gain of fish/feed consumed; protein efficiency ratio (PER) = weight gain of fish/protein consumed; protein retention (PR) = protein gain×100/protein consumed; condition factor (CF) = total body weight/total length^3; and hepatosomatic index (HSI) = liver weight/total body weight.

2.6 Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Significant differences ($P < 0.05$) among the means were determined using Duncan`s multiple range test (Duncan, 1955). All percentage data were arcsine-transformed prior to statistical analysis. Additionally, multiple regression analysis was performed between the essential amino acid content of the selected feed ingredients and the weight gain, SGR, feed consumption and CF of rockfish at the end of the 8-week feeding trial.

3. Results

3.1 Attractiveness of rockfish in the preliminary test

JM (40.0%), SM (33.3%), PM (40.0%), SHM (36.7%), mussel meal (40.0%) and oyster meal (43.3%) achieved the highest feeding attractiveness to rockfish in the 1st through 6th tests, respectively (Table 13). JM (40.0%), SHM (36.7%), SQM (33.3%), SM (40.3%), PM (40.0%) and PM (36.7%) achieved the highest feeding attractiveness to fish in the 7th through 12th tests, respectively.

3.2 Attractiveness of the top 5 selected feed ingredients to rockfish

Attractiveness of the top 5 selected feed ingredients to rockfish from the preliminary test is shown in Table 14. The number of fish that moved to the attracting chamber and the attractiveness of JM to fish was significantly (P < 0.007 and P < 0.006) higher than those of PM and SHM at 10 min after observation in the 1st trial. In the 2nd trial, the number of fish that moved to the attracting chamber and the attractiveness of JM to fish was significantly (P < 0.002 for both) higher than those of SM and SQM at 10 min after observation. The number of fish that moved to the attracting chamber and the attractiveness of SM to fish was also significantly (P < 0.05) higher than those of SQM at 10 min after observation in the 2nd trial. SM achieved a significantly (P < 0.005) higher number of fish that moved to the attracting chamber and attractiveness to fish than SQM and PM at 20 min

Table 13. Number of rockfish moved to the attracting chamber and the attractiveness of rockfish responding to various feed ingredients.

Test	Feed ingredient	Number of fish moved to attracting chamber	Attractiveness (%) of fish[1]
1st	Jack mackerel meal (JM)	12	40.0
	Anchovy meal	8	26.7
	Soybean meal	7	23.3
	FSA[2]	3	10.0
2nd	Sardine meal (SM)	10	33.3
	Squid liver meal	8	26.7
	Corn gluten meal	6	20.0
	FSA[2]	6	20.0
3rd	Pollack meal (PM)	12	40.0
	Squid meal (SQM)	8	26.7
	Herring meal	6	20.0
	FSA[2]	4	13.3
4th	Shrimp meal (SHM)	11	36.7
	Krill meal	8	26.7
	Shrimp head meal	7	23.3
	FSA[2]	4	13.3

5th	Mussel meal	12	40.0
	Hydrolyzed fish meal	9	30.0
	Crab meal	3	10.0
	FSA[2]	6	20.0
6th	Oyster meal	13	43.3
	Soybean meal	10	33.3
	Corn gluten meal	4	13.3
	FSA[2]	3	10.0
7th	Jack mackerel meal (JM)	12	40.0
	Pollock meal (PM)	6	20.0
	Sardine meal (SM)	6	20.0
	FSA[2]	6	20.0
8th	Shrimp meal (SHM)	11	36.7
	Oyster meal	10	33.3
	Mussel meal	5	16.7
	FSA[2]	4	13.3
9th	Squid meal (SQM)	10	33.3
	Hydrolyzed fish meal	8	26.7
	Soybean meal	7	23.3
	FSA[2]	5	16.7

10th	Sardine meal (SM)	12	40.3
	Squid meal (SQM)	10	33.3
	Shrimp meal (SHM)	4	13.3
	FSA[2]	4	13.3
11th	Pollock meal (PM)	12	40.0
	Oyster meal	8	26.7
	Hydrolyzed fish meal	5	16.7
	FSA[2]	5	16.7
12th	Pollock meal (PM)	11	36.7
	Shrimp head meal	9	30.0
	Krill meal	6	20.0
	FSA[2]	4	13.3

[1]Attractiveness (%) of fish = number of fish moved into each attracting chamber×100/total number of fish in acclimatization chamber

[2]FSA, number of fish stayed in acclimatization chamber after 30 min exposing to each ingredient.

Table 14. Number of rockfish moved to attracting chambers and the attractiveness of fish responding to the selected feed ingredient with time

Trial	Feed ingredient	Elapsed time (min)					
		10		20		30	
		Number of fish moved to attracting chamber	Attractiveness (%) of fish[1]	Number of fish moved to attracting chamber	Attractiveness (%) of fish[1]	Number of fish moved to attracting chamber	Attractiveness (%) of fish[1]
1st	Jack mackerel meal (JM)	11.3 ± 0.88[a]	37.8 ± 2.94[a]	12.0 ± 1.15[a]	40.0 ± 3.85[a]	12.0 ± 1.15[a]	40.0 ± 3.85[a]
	Pollock meal (PM)	6.0 ± 0.58[bc]	20.0 ± 1.92[bc]	7.3 ± 0.33[b]	24.4 ± 1.11[b]	7.7 ± 0.33[b]	25.6 ± 1.11[b]
	Shrimp meal (SHM)	4.3 ± 1.20[c]	14.4 ± 4.01[c]	6.0 ± 0.58[bc]	20.0 ± 1.92[bc]	6.0 ± 0.58[bc]	20.0 ± 1.92[bc]
	FSA[2]	8.3 ± 1.20[ab]	27.8 ± 4.01[ab]	4.7 ± 0.67[c]	15.6 ± 2.22[c]	4.3 ± 0.88[c]	14.4 ± 2.94[c]
	P-value	$P < 0.007$	$P < 0.006$	$P < 0.002$	$P < 0.002$	$P < 0.002$	$P < 0.002$
2nd	Jack mackerel meal (JM)	11.0 ± 0.58[a]	36.7 ± 1.92[a]	12.3 ± 0.88[a]	41.1 ± 2.94[a]	12.3 ± 0.88[a]	41.1 ± 2.94[a]
	Sardine meal (SM)	7.0 ± 0.00[b]	23.3 ± 0.00[b]	8.3 ± 0.33[b]	27.8 ± 1.11[b]	8.7 ± 0.33[b]	28.9 ± 1.11[b]
	Squid meal (SQM)	3.3 ± 1.20[c]	11.1 ± 4.01[c]	4.7 ± 0.88[c]	15.6 ± 2.94[c]	4.7 ± 0.88[c]	15.6 ± 2.94[c]
	FSA[2]	8.7 ± 0.88[ab]	28.9 ± 2.94[ab]	4.7 ± 0.33[c]	15.6 ± 1.11[c]	4.3 ± 0.33[c]	14.4 ± 1.11[c]
	P-value	$P < 0.002$	$P < 0.002$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

3rd	Sardine meal (SM)	10.0 ± 1.73[a]	33.3 ± 5.77[a]	12.0 ± 1.53[a]	40.0 ± 5.09[a]	12.0 ± 1.53[a]	40.0 ± 5.09[a]
	Squid meal (SQM)	7.3 ± 1.20[ab]	24.4 ± 4.01[ab]	7.7 ± 0.88[b]	25.6 ± 2.94[b]	7.7 ± 0.88[b]	25.6 ± 2.94[b]
	Pollock meal (PM)	4.0 ± 1.15[b]	13.3 ± 3.85[b]	4.7 ± 0.88[b]	15.6 ± 2.94[b]	4.7 ± 0.88[b]	15.6 ± 2.94[b]
	FSA[2]	8.7 ± 0.88[a]	28.9 ± 2.94[a]	5.7 ± 0.33[b]	18.9 ± 1.11[b]	5.7 ± 0.33[b]	18.9 ± 1.11[b]
	P-value	$P < 0.05$	$P < 0.05$	$P < 0.005$	$P < 0.005$	$P < 0.005$	$P < 0.005$

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

[1]Attractiveness (%) of fish = number of fish moved into each attracting chamber×100/total number of fish in acclimatization chamber.

[2]FSA, Number of fish stayed in the acclimatization chamber after 30 min exposing to each ingredient.

after observation in the 3rd trial. The strongest feeding attractant response of rockfish was observed in JM, followed by SM, SQM, PM and SHM, in order. Once fish moved to the attracting chamber from the acclimatization chamber through the funnel-shaped entrance, no fish returned during the 30-min observation.

3.3 Feeding trial of fish

No significant difference in the survival of fish was found at the end of the 8-week feeding trial since no mortality was observed in any treatment (Table 15). However, the weight gain and SGR of fish fed the JM diet were significantly ($P < 0.0001$) greater than those of fish fed all other diets, followed by the SM, PM, SQM, SHM and Con diets. The weight gain and SGR of fish fed the SM, PM, SQM and SHM diets were also significantly ($P < 0.05$) greater than those of fish fed the Con diet.

The feed consumption of fish fed the JM diet was significantly ($P < 0.0001$) higher than fish fed all other diets, followed by the SM, PM, SQM, SHM and Con diets, in order (Table 16). The feed consumption of fish fed the SM, PM, SQM and SHM diets was also significantly ($P < 0.05$) higher than that of fish fed the Con diet. However, FE, PER and PR were not significantly ($P > 0.05$) affected by the experimental diets. The CF of fish fed the JM diet was significantly ($P < 0.0001$) higher than that of fish fed all other diets, followed by the SM, PM, SQM, SHM and Con diet. The CF of fish fed the Con diet was significantly ($P < 0.05$) lower than that of fish fed the SM, PM and SQM diets but was not significantly ($P > 0.05$)

Table 15. Survival (%), weight gain (g/fish) and specific growth rate (SGR, %/day) of rockfish fed the experimental diets containing feed ingredient with high feeding attractants for 8 weeks

Experimental diets	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)	SGR[1] (%/day)
Con	5.3 ± 0.00[a]	23.1 ± 0.06[f]	100.0 ± 0.00[a]	17.8 ± 0.07[f]	2.64 ± 0.006[f]
JM	5.3 ± 0.01[a]	26.0 ± 0.04[a]	100.0 ± 0.00[a]	20.7 ± 0.03[a]	2.84 ± 0.002[a]
SM	5.3 ± 0.01[a]	25.6 ± 0.04[b]	100.0 ± 0.00[a]	20.3 ± 0.04[b]	2.81 ± 0.002[b]
PM	5.3 ± 0.01[a]	25.2 ± 0.09[c]	100.0 ± 0.00[a]	19.9 ± 0.09[c]	2.79 ± 0.007[c]
SQM	5.3 ± 0.00[a]	24.8 ± 0.06[d]	100.0 ± 0.00[a]	19.5 ± 0.06[d]	2.76 ± 0.003[d]
SHM	5.3 ± 0.00[a]	24.3 ± 0.12[e]	100.0 ± 0.00[a]	19.0 ± 0.12[e]	2.72 ± 0.008[e]
P-value		$P < 0.0001$		$P < 0.0001$	$P < 0.0001$

Values (means of triplicates ± 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 16. Feed consumption (g/fish), feed efficiency (FE), protein efficiency ratio (PER), protein retention (PR) condition factor (CF) and hepatosomatic index (HSI) of rockfish fed the experimental containing feed ingredient with high feeding attractants for 8 weeks

Experimental diets	Feed consumption (g/fish)	FE[1]	PER[2]	PR[3]	CF[4]	HSI[5]
Con	17.2 ± 0.05[f]	1.04 ± 0.006[a]	1.99 ± 0.011[a]	35.4 ± 0.18[a]	1.43 ± 0.005[d]	2.85 ± 0.005[a]
JM	20.1 ± 0.05[a]	1.03 ± 0.003[a]	1.99 ± 0.006[a]	35.2 ± 0.36[a]	1.50 ± 0.002[a]	2.85 ± 0.003[a]
SM	19.5 ± 0.08[b]	1.04 ± 0.003[a]	2.00 ± 0.006[a]	35.8 ± 0.16[a]	1.48 ± 0.003[b]	2.85 ± 0.006[a]
PM	19.2 ± 0.05[c]	1.04 ± 0.003[a]	2.00 ± 0.006[a]	35.5 ± 0.27[a]	1.47 ± 0.003[b]	2.84 ± 0.002[a]
SQM	18.8 ± 0.07[d]	1.04 ± 0.004[a]	1.99 ± 0.007[a]	35.2 ± 0.24[a]	1.45 ± 0.002[c]	2.85 ± 0.006[a]
SHM	18.3 ± 0.09[e]	1.03 ± 0.002[a]	1.99 ± 0.004[a]	34.6 ± 0.20[a]	1.44 ± 0.001[d]	2.83 ± 0.007[a]
P-value	$P < 0.0001$	$P > 0.5$	$P > 0.8$	$P > 0.08$	$P < 0.0001$	$P > 0.3$

Values (means of triplicates ± 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) = total body weight×100/total length^3

[5]Hepatosomatic index (HSI) = liver weight×100/total body weight

different from that of fish fed the SHM diet. The HSI of fish was not significantly ($P > 0.05$) affected by the experimental diets.

3.4 Amino acid composition of the selected feed ingredients

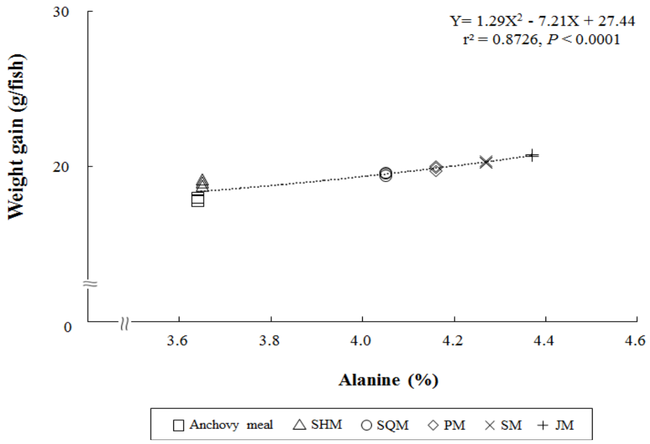
The amino acid profiles of the selected feed ingredients used in the 8-week feeding trial are given in Table 17. Aspartic and glutamic acids and glycine and lysine are present in high concentration among the nonessential and essential amino acids, respectively. The strong correlation between the contents of 3 essential amino acids (alanine, glycine and histidine) in the selected feed ingredients (SHM, SQM, PM, SM, JM and anchovy meal in the Con diet) and the weight gain (Fig. 5), SGR (Fig. 6), feed consumption (Fig. 7) and CF of fish (Fig. 8) were observed ($P < 0.0001$ for all criteria).

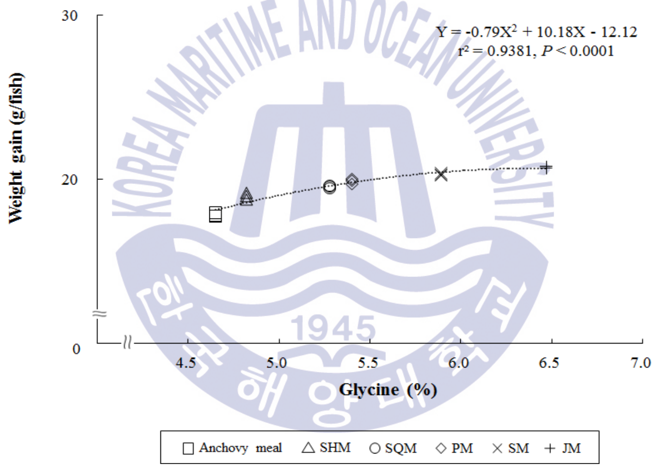
3.5 Proximate composition of the whole body of rockfish

The moisture and crude protein and lipid contents of the whole body of fish were not significantly ($P > 0.05$) affected by the experimental diets (Table 18). However, the ash content of the whole body of fish fed the Con diet was significantly ($P < 0.02$) higher than that of fish fed the SM, PM, SQM and SHM diets but not significantly ($P > 0.05$) different from that of fish fed the JM diet.

Table 17. Amino acid profiles of the selected feed ingredient (%, DM basis) used in the 8-week feeding trial

	Feed ingredient					
	JM	SM	PM	SQM	SHM	Anchovy meal
Alanine	4.37	4.27	4.16	4.05	3.85	3.64
Arginine	4.25	3.70	4.32	4.79	2.89	4.17
Aspartic acid	6.45	6.00	5.50	6.48	4.35	6.32
Cystine	0.96	0.80	0.72	0.86	0.54	0.85
Glutamic acid	13.81	13.74	11.88	14.84	11.93	16.23
Glycine	6.47	5.89	5.40	5.28	4.82	4.65
Histidine	2.83	2.33	1.63	1.30	1.21	1.13
Isoleucine	2.71	2.53	1.96	2.77	1.62	2.42
Leucine	4.95	4.82	3.95	4.98	3.92	4.89
Lysine	5.60	5.07	4.47	5.84	4.62	5.68
Methionine	2.12	1.88	1.63	1.68	1.21	1.98
Phenylalanine	2.78	2.70	2.17	2.48	2.23	2.89
Proline	3.25	3.08	2.79	2.89	2.53	3.16
Serine	2.94	2.71	3.29	2.97	1.98	2.99
Threonine	3.14	2.92	2.63	2.91	1.85	3.62
Tyrosine	2.00	1.91	1.52	1.88	1.70	1.93
Tryptophan	0.73	0.86	0.88	0.57	0.50	0.85
Valine	3.27	3.18	2.52	2.56	2.58	3.12


Y= 1.29X² - 7.21X + 27.44
r² = 0.8726, P < 0.0001
Weight gain (g/fish)
Alanine (%)
Anchovy meal
SHM
SQM
PM
SM
JM


Y = -0.79X² + 10.18X - 12.12
r² = 0.9381, P < 0.0001
Weight gain (g/fish)
Glycine (%)
Anchovy meal
SHM
SQM
PM
SM
JM

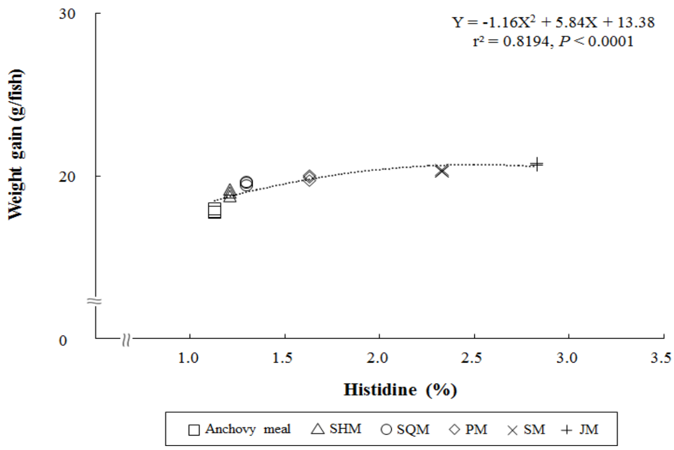
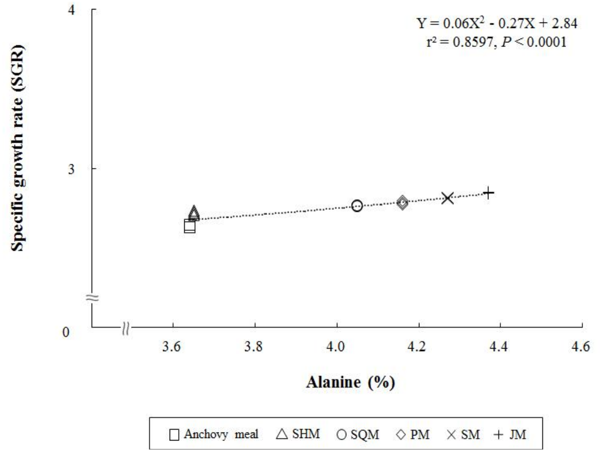
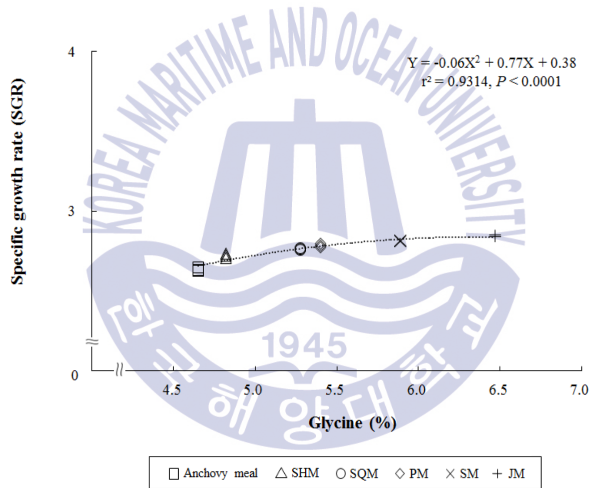

Y = -1.16X² + 5.84X + 13.38
r² = 0.8194, P < 0.0001
Weight gain (g/fish)
Histidine (%)
Anchovy meal
SHM
SQM
PM
SM
JM

Fig. 5. Correlation between alanine, glycine and histidine contents in the selected feed ingredient (X) showing high feeding attractants and weight gain (Y) of rockfish at the end of the 8-week feeding trial. The symbols □, △, ○, ◇, × and + indicate anchovy meal, shrimp meal (SHM), squid meal (SQM), pollack meal (PM), sardine meal (SM) and jack mackerel meal (JM), respectively.


Y = 0.06X2 - 0.27X + 2.84
r2 = 0.8597, P < 0.0001
Specific growth rate (SGR)
4
3
0
3.6
3.8
4.0
4.2
4.4
4.6
Alanine (%)
Anchovy meal
SHM
SQM
PM
SM
JM


Y = -0.06X2 + 0.77X + 0.38
r2 = 0.9314, P < 0.0001
Specific growth rate (SGR)
4
3
0
4.5
5.0
5.5
6.0
6.5
7.0
Glycine (%)
Anchovy meal
SHM
SQM
PM
SM
JM

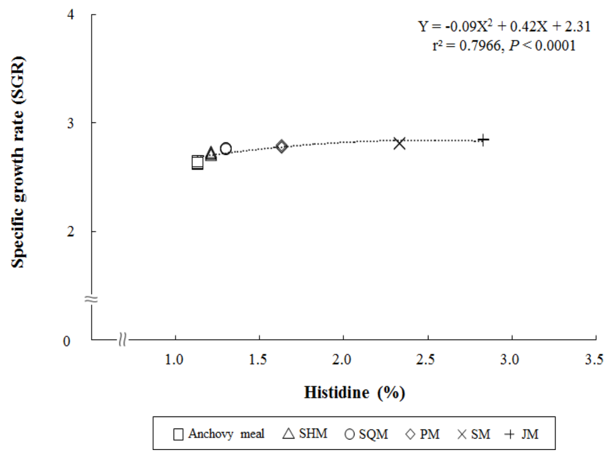
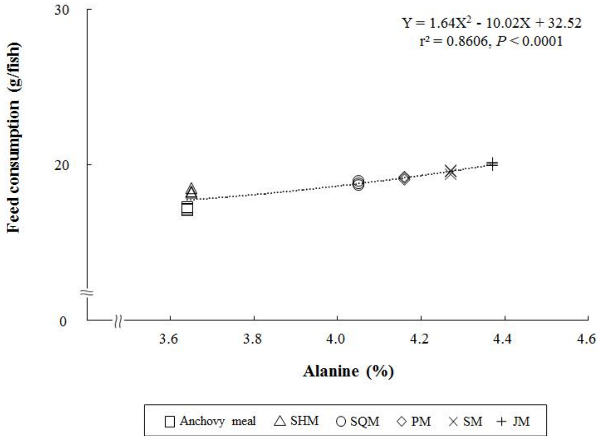
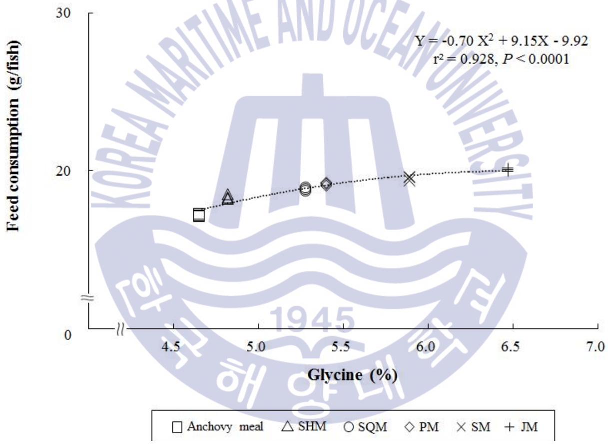

Y = -0.09X2 + 0.42X + 2.31
r2 = 0.7966, P < 0.0001
Specific growth rate (SGR)
4
3
2
0
1.0
1.5
2.0
2.5
3.0
3.5
Histidine (%)
Anchovy meal
SHM
SQM
PM
SM
JM

Fig. 6. Correlation between alanine, glycine and histidine contents in the selected feed ingredient (X) showing high feeding attractants and specific growth rate (SGR) (Y) of rockfish at the end of the 8-week feeding trial. For the symbols □, △, ○, ◇, × and + refer to Fig. 5.


Y = 1.64X² - 10.02X + 32.52
r² = 0.8606, P < 0.0001
Feed consumption (g/fish)
Alanine (%)
Anchovy meal
SHM
SQM
PM
SM
JM


Y = -0.70 X² + 9.15X - 9.92
r² = 0.928, P < 0.0001
Feed consumption (g/fish)
Glycine (%)
Anchovy meal
SHM
SQM
PM
SM
JM

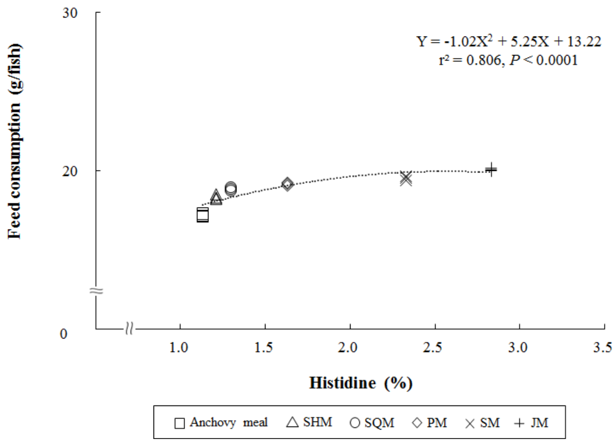
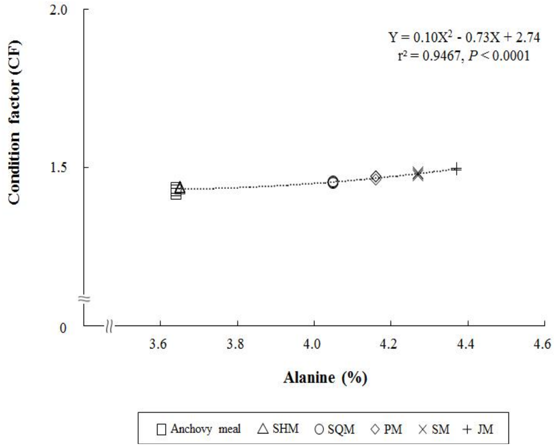
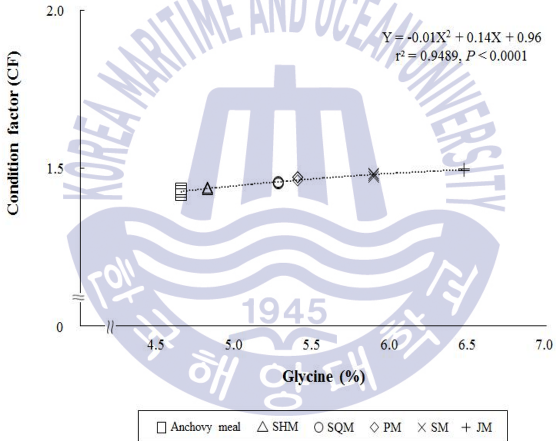

Y = -1.02X² + 5.25X + 13.22
r² = 0.806, P < 0.0001
Feed consumption (g/fish)
Histidine (%)
Anchovy meal
SHM
SQM
PM
SM
JM

Fig. 7. Correlation between alanine, glycine and histidine contents in the selected feed ingredient (X) showing high feeding attractants and feed consumption (Y) of rockfish at the end of the 8-week feeding trial. For the symbols □, △, ○, ◇, × and + refer to Fig. 5.


Y = 0.10X² - 0.73X + 2.74
r² = 0.9467, P < 0.0001
Condition factor (CF)
Alanine (%)
Anchovy meal SHM SQM PM SM JM


Y = -0.01X² + 0.14X + 0.96
r² = 0.9489, P < 0.0001
Condition factor (CF)
Glycine (%)
Anchovy meal SHM SQM PM SM JM

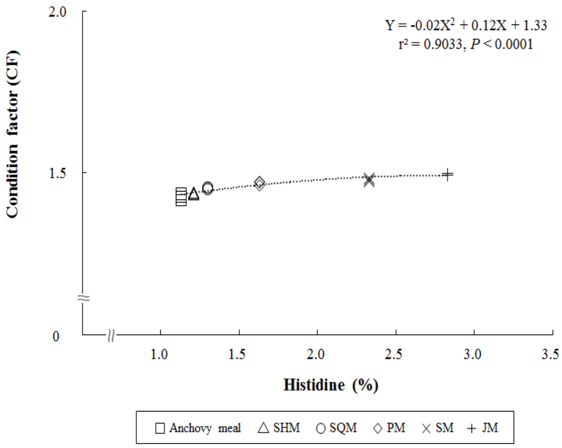

Y = -0.02X² + 0.12X + 1.33
r² = 0.9033, P < 0.0001
Condition factor (CF)
Histidine (%)
Anchovy meal SHM SQM PM SM JM

Fig. 8. Correlation between alanine, glycine and histidine contents in the selected feed ingredient (X) showing high feeding attractants and condition factor (Y) of rockfish at the end of the 8-week feeding trial. For the symbols □, △, ○, ◇, × and + refer to Fig. 5.

Table 18. Proximate composition (%) of the whole body of rockfish fed the experimental diets containing feed ingredient with high feeding attractants at the end of the 8-week feeding trial

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
Con	69.1 ± 0.16^{a}	17.4 ± 0.01^{a}	8.0 ± 0.08^{a}	4.6 ± 0.06^{a}
JM	68.8 ± 0.23^{a}	17.4 ± 0.10^{a}	8.0 ± 0.12^{a}	4.3 ± 0.14^{ab}
SM	68.9 ± 0.14^{a}	17.5 ± 0.06^{a}	8.0 ± 0.07^{a}	4.1 ± 0.18^{bc}
PM	68.8 ± 0.08^{a}	17.5 ± 0.07^{a}	8.0 ± 0.06^{a}	4.1 ± 0.14^{bc}
SQM	69.1 ± 0.08^{a}	17.3 ± 0.06^{a}	8.1 ± 0.12^{a}	3.9 ± 0.08^{c}
SHM	69.0 ± 0.11^{a}	17.2 ± 0.08^{a}	7.8 ± 0.03^{a}	3.9 ± 0.12^{bc}
P-value	$P > 0.4$	$P > 0.08$	$P > 0.4$	$P < 0.02$

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

4. Discussion

Responses of aquatic animals to feeding attractants and/or stimulants have been well studied, because they were closely related to understanding aquatic animals' feeding behaviors, aquatic ecology and aquaculture application (Carr et al., 1996; Kasumyan and Nikolaeva, 2002; Smith et al., 2005). Studies on feeding attractants and/or stimulants have also received substantial attention in recent years, as the reduced inclusion of fish meal in aquafeed is pursued to decrease both feed costs and the significant dependence on such an ingredient (NRC, 2011). Feed ingredients of marine animal origins, such as fish meal, fish hydrolysates, krill meal, shrimp meal, fish solubles, fish oil and various protein hydrolysates, are noted for their positive palatability to many species of fish (Barrow, 2000).

In the preliminary test (Table 13), in which rockfish were responded primarily based on olfactory stimulus from each ingredient in three divided attracting chambers, a strong feeding attractant response of rockfish was observed in three kinds of fish meal (JM, SM and PM), one mollusk meal (SQM) and one crustacean meal (SHM), but weak or moderate response were observed in the other plant-originated meal, fish meal, crustacean meal and mollusk meal. However, Barroso et al. (2013) showed that Senegalese sole *Solea senegalensis*, which is a good model species to show importance of olfaction in feeding behavior due to its feeding strategy and its well-developed olfactory system exhibited the highest acceptability index of behavioral response and feed intake to fish meal hydrolysate than to any other meal tested (fish, polychaete, mussel and squid meals) in the feeding trial. Barata et al. (2009) reported that olfaction played an important role in the food search behavior of Senegalese sole and that the hydrophilic fraction of a polychaete *Diopatra neapolitana* whole-body homogenate contained key substances (feeding attractants and/or stimulants) that affected sole food search behavior; moreover, food ingestion by sole was enhanced by the addition of polychaete homogenate to dry pellets.

Movement in most of the attracted rockfish from the acclimatization chamber to feed ingredient in the attracting chamber was made within 10 min in all trials (Table 14), in which rockfish were responded primarily based on olfactory stimulus from each ingredient placed in three divided attracting chambers, but few fish had moved to the attracting chamber by either 20 or 30 min after observation. However, there were always some fish that did not move to the attracting chamber from the acclimatization chamber after feed ingredients were placed in the attracting chamber for 30 min in this study. This probably occurred because the fish had not been well acclimated to the experimental conditions for 72 h before the determination of attractiveness of a feed ingredient to fish. The strongest feeding attractiveness to rockfish was obtained in JM, followed by SM, SQM, PM and SHM, in order based on behavioral response (movement to the attracting chamber from the acclimatization chamber) of fish in this study. Similarly, JM extracts were known to have high feeding attractants and/or stimulants, especially, IMP and lactic acid for yellowtail, and histidine for olive flounder, respectively based on feed consumption (Hidaka et al., 2000; Ikeda et al., 2012). Singh et al. (2006) reported that fry and juvenile sea bass *Lates calcarifer* showed high feeding activity towards Bombay duck meal over other fishery meals, such as anchovy, lesser sardine, ribbon fish and shrimp head.

Amino acids have been reported as effective feeding attractants and/or stimulants for several fishes (Takeda, 1980; Harada, 1985; Takeda et al., 1984; Ellingsen and Døving, 1986; Fukuda et al., 1989; Jones, 1992; Takaoka et al., 1995; Carr et al., 1996; Kohbara et al., 2000; Yacoob and Browman, 2007; Ikeda et al., 2012). Since amino acids were shown to be highly effective for the olfactory receptors of yellowtail (Kohbara et al., 2000), fish seem to rely on olfactory stimulants to find feed at some distance. In our study to determine effectiveness of feed ingredients, rockfish were attracted by mainly olfactory stimulants and approached to them over 50 cm apart away from the acclimatization chamber to the three divided attracting chambers in Fig. 4.

The strong second polynomial correlation (Figs 5, 6, 7, and 8) between the contents of 3 amino acids (alanine, glycine and histidine) in the selected feed ingredients (SHM, SQM, PM, SM, JM and anchovy meal in the Con diet) and weight gain, SGR, feed consumption and CF of rockfish in this study revealed that the alanine, glycine and histidine contents were consistent with the growth performance of fish, probably indicating that the alanine, glycine and histidine contents in the selected feed ingredients played an important role as feeding attractants and/or stimulants in rockfish in these experimental conditions. This was coincident with the studies of Carr et al.

(1996) and Ellingsen and Døving (1986) which showed that alanine and glycine were the two most effective feeing stimulants in several fishes. Carr et al. (1996) also demonstrated that some minor tissue components of prey, such as tryptophan, phenylalanine, aspartic acid, valine, and uridine 5′-monophosphate were important feeding stimulants for some fish species. However, Harada (1985) and Ikeda et al. (2012) reported that both histidine and glycine, and histidine, respectively, achieved the strong feeding attraction activities for yellowtail and olive flounder. When sea bream *Pagrus major* were fed diets supplemented with the extracts and non-muscle Antarctic krill *Euphausia superba* meal or white fish meal, the non-muscle krill meal elicited the strongest gustatory and olfactory responses (Shimizu et al., 1990). They analysed the amino acid profiles of the meals, tested several diets containing amino acids for feeding stimulants in sea bream and concluded that fish feeding was stimulated mainly by proline, glycine and glucosamine.

The experimental diets (JM, SM, PM, SQM and SHM diets) supplemented with the top 5 selected feed ingredients showing high feeding attractant ability at 5% all achieved outstanding improvements in the weight gain and SGR of rockfish over the anchovy meal in the Con diet (Table 15) in the 8-week feeding trial, in which rockfish consumed the experimental diets primarily based on the combined visual, olfactory and gustatory stimulants. This result indicated that adding the selected feed ingredients that possess high feeding attractiveness to rockfish to a diet could improve the growth performance of fish without the addition of synthetic feeding attractants and/or stimulants based on either the omission test of the component of the synthetic extracts of prey or the electro(neuro)physiological study of fish. This will be a very useful and practical technique for fish farm. Similarly, Lie et al. (1989) reported that the feed consumption and growth of cod improved when 10% of the saithe fillet was replaced with either cooked prawn or squid meal in the saithe fillet-basal diet.

The results for the attractiveness of the top 5 selected feed ingredients showing high feeding attractant ability was partially reconfirmed by the improved feed consumption of fish fed the JM, SM, PM, SQM and SHM diets compared to the Con diet in the 8-week feeding trial (Table 16) vs. JM, SM, SQM, PM and SHM, in order of the strong attractiveness to rockfish, based on behavioral response of fish as shown in Table 14. This difference could be a result of the olfactory response to fish (Table 14) and combined visual, olfactory and gustatory responses to rockfish (Table 16) to the feed ingredients. The attractiveness of feed ingredients to rockfish was made, primarily cued by olfactory stimulus at some distance in the specifically designed tank (Fig. 4) to determine attractiveness to fish (Tables 11 and 13), but the feed consumption of fish in the 8-week feeding trial was made simultaneously by olfactory and gustatory cues (Table 16). Kohbara et al. (2000) explained that gustatory receptors in yellowtail are involved in discriminating between food items during feeding whereas the olfactory receptors may detect foods at some distance. Goh and Tamura (1980) also proved that the feeding behavior of red sea bream *Pagrus major* was better harmonized with the electrical activity of the gustatory system than with the olfactory system when the supplemented effects of amino acids and betaine in a casein-base purified diet on the feeding behavior of fish were compared.

The improved feed consumption of fishes such as eel (Takeda et al., 1984), yellowtail (Harada, 1985; Fukuda et al., 1989; Hosokawa et al., 2001), olive flounder (Ikeda et al., 2012), marbled rockfish (Takaoka et al., 1990) and grouper (Lim et al., 2015) fed diets containing feeding attractants and/or stimulants was proved by an omission test of the component of the synthetic extracts in common prey. The distinctive improvement in the weight gain and SGR of rockfish fed the JM, SM, PM, SQM and SHM diets compared to the Con diet was a direct result of the improved feed consumption and palatability via the activation of the cephalic reflex that was induced by the smell and taste of the attractive substances in diets (Fange and Grove, 1979). However, this eventually led to no difference in the FE, PER and PR of rockfish fed the experimental diets due in this study. Unlike this study, however, Takii et al. (1986a, b) demonstrated that the inclusion of feeding stimulants (alanine, glycine, histidine, proline and uridine-5′-monophosphate) in diets increased not only the feeding activity of eels and promoted the digestion and absorption of the diet, but also the activities of the hepatic enzyme related to the carbohydrate and amino acid metabolism of fish. Later, Takii et al. (1994) showed that the addition of 1% of feeding stimulants (mixture of alanine, proline and IMP) to a fish meal-basal diet effectively improved the survival, weight gain, feed intake and feed utilization of yellowtail, as proved by the improvement in enzyme activities, digestion, absorption and nutrient retention of fish. The FE of striped bass *Morone saxatilis* fed a fish meal-basal diet supplemented with 2.7% of feeding attractants (mixture of several amino acids and betaine) improved (Papartyphon and Soares, 2001). The dietary inclusion of an alternative source (mussel meal) of fish meal improved the weight gain and feed efficiency of common sole *Solea solea*, which was a direct result of the improved feed consumption (Mongile et al., 2015).

An improved CF (fattier fish) in rockfish fed the JM, SM, PM and SQM diets compared to the Con diet seemed to be closely related to the improved growth performance and feed consumption of fish in this study. CF, which shows the degree of well-being of fish in their habitats, is calculated by a length–weight factor. This factor is a measure of various ecological and biological factors, such as the degree of fitness, gonad development and suitability of the environment with regard to the feeding condition (Mac Gregoer, 1959). When the CF value is higher, it means that a fish had attained a better condition (Nehemia et al., 2012). In addition, the CF of fish can be affected by a number of factors, such as stress, sex, season, availability of feeds, and other water quality parameters (Khallaf et al., 2003) and can be used to determine the degree of the feeding activity of a species to verify whether it is making good use of its feeding source (Weatherley, 1972). The HSI of rockfish, however, was not affected by the experimental diets in this study.

The chemical composition of the whole body of rockfish was not affected by the experimental diets, except for the ash content. There was no distinctive trend in ash content of the whole body of fish. Similarly, proximates of either the whole body or liver in yellowtail were not affected by adding feeding stimulants in diets, although fish achieved a distinctive difference in growth performance and feed utilization (Takii et al., 1994). Generally, the proximate composition of fish is commonly known to be influenced by several factors, such as sex maturity, stocking density, fish size (age), feeding rate (ratio), rearing temperature and feed composition (Srikar et al., 1979; Toko et al., 2007; Breck, 2014; Mizanur et al., 2014; Cho et al., 2015).

Many researchers and feed manufacturers have attempted to include feeding attractants and/or stimulants in aquafeed to improve the palatability and feed acceptance of aquatic animals. In particular, this application is important in larval and starter feeds, in which feed acceptability is a major concern. In addition, the supplementation of feed ingredients in diets based on the feeding attractiveness (behavior) of fish is a very useful technique, especially when low fish meal diets, which are likely to have low palatability, are being developed or when the electro(neuro)physiological study of the targeting fish is not well known. Fish commonly do not respond to the component of the synthetic extracts of prey or to real prey extracts. Xue and Cui (2001) showed that the supplementation of several feeding stimulants (betaine, glycine, L-lysine, L-methionine, L-phenylalanine or commercial squid extract) in a 26% fish meal-basal diet did not improve the feed consumption of gibel carp *Carassius auratus*, but a diet substituting 5% of fish meal with 6% of meat and bone meal did. Unlike, Xue and Cui

(2001), however, in this study, the feed consumption and growth performance of rockfish were outstandingly improved when anchovy meal-basal diets were incorporated with other feed ingredients (JM, SM, PM, SQM and SHM), showing strong feeding attractant ability at 5%. As the international market price of fish meal continues to increase, further studies are needed to determine the optimum level of feed ingredients showing strong feeding attractant and/or stimulant abilities in diets to maximize the performance of fish or benefits of fish farmers.

The addition of blue mussel meal was a very effective method, as a fish meal substitute and feeding attractant to improve the feed intake and growth performance of fish (Kikuchi et al., 2002; Kikuchi and Furuta, 2009; Nagel et al., 2014). Papatryphon and Soares (2001) also reported that the addition of feeding stimulants (alanine, betaine, serine and IMP) effectively improved the feed intake and performance of striped bass *Morone saxatilis* when fish meal was completely substituted with the combined plant (corn gluten and soybean meals) meal, and they concluded that combined plant meals with feeding stimulants could be used as a practical diet for striped bass culture. The addition of an attractant amino acid mixture into the plant-protein rich (soy protein concentrate) diet that completely substituted fish meal was effective to improve the weight gain, feed consumption and feed efficiency of European seabass *Dicentrarchus labrax* (Dias et al., 1997). However, Kubitza et al. (1997) reported that the addition of IMP and fish meal increased the feeding activity of largemouth bass *Micropterus salmoides*, whereas the addition of either an amino acid mixture (glycine, proline, serine, leucine, valine, histidine and tryptophan) or betaine did not, when fish meal was completely replaced by soybean meal. Inclusion of the selected feed ingredients showing high feeding attractiveness (behavior) to the targeting fish in diets seems to be a very easy and effective method for improving or maximizing the growth performance, feed consumption and CF of fish without conducting an omission test of the component of the synthetic extracts of prey or monitoring the electro physiological response of fish. In addition, the electro physiological study of fish can suggest if fish are able to be triggered by a given chemical cue with their chemoreceptors, but it does not always guarantee that the synthetic feeding attractants will bring about changes in feeding behavior or improve feed consumption.

In conclusion, the strongest attractiveness to rockfish was obtained in JM, followed by the SM, SQM, PM and SHM, in order, among the tested ingredients. The greatest feed consumption, weight gain and SGR of fish were obtained in the JM diet, followed by the SM, PM, SQM and SHM diets, in order. Adding the selected feed ingredients (JM, SM, PM and SQM) improved the CF of fish. Strong correlations between the alanine, glycine and histidine contents in the selected feed ingredients (SHM, SQM, PM, SM, JM and anchovy meal in the Con diet) and weight gain, SGR, feed consumption and CF of fish were observed.

Chapter 5.

General Discussion

Since mortality of rockfish frequently occurs year after year in Korea, development of dietary additive to minimize it is highly needed. Application of antibiotics (antioxidants) is one of the methods used in aquafeed. As oral administration of the synthetic chemicals such, as antibiotics in aquafeed, however, is prohibited in some countries including Korea.

Therefore, development of natural (phytoadditives) source of additive causing serious concern of either unfavorable effect on environment or fish-consumer is necessary. Recently, Vallejos-Vidal et al. (2016) reviewed the importance of natural sources, such as plant, herbs or algae extracts to replace antibiotics in aquafeed. Gabor et al. (2010) also demonstrated use of natural source, such as garlic *Alliumsativum*, onion *Allium cepa*, oregano *Origanum vulgare*, GG, Echinacea, *Echinadea purpurea*, cinnamon *Cinnamomum verum* or nettle *Urtica dioica* in diet for growth performance of several fish and emphasized the major advantages in the use of natural source is the fact that they are natural substances and do not pose any threat to fish, man or environment.

Responses of aquatic animals to feeding attractants and/or stimulants have been studied since long times ago because they were closely related to understanding aquatic animals' feeding behaviors, aquatic ecology and aquaculture application (Carr et al., 1996; Kasumyan and Nikolaeva, 2002;

Smith et al., 2005). Studies on feeding attractants and/or stimulants have also received substantial attention, as the reduced inclusion of fish meal in aquafeed is pursued to decrease both feed costs and the significant dependence on such an ingredient (NRC, 2011). Feed ingredients of marine animal origins, such as fish meal, fish hydrolysates, krill meal, shrimp meal, fish solubles, fish oil and various protein hydrolysates, are noted for their positive palatability to many species of fish (Barrow, 2000). Inclusion of the selected feed ingredient showing high feeding attractiveness (behavior) to the targeting fish in diets seems to be a very easy and effective method for improving growth performance and feed consumption of fish without conducting an omission test of the component of the synthetic extracts of prey or monitoring the electro(neuro)physiological response of fish.

I. Weight gain for fish fed the YC diet was greater than that for fish fed the other diets. 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). The weight gain of fish fed the CJ, PM and BC diets was lower compared to fish fed the Con diet. Feed consumption of the YC diet was higher compared to all other diets except for the BB diet. FE and PER of fish fed the TT and YC diets were higher compared to the other diets based on lower feed consumption but relative higher weight gain. Previous studies (Nya and Austin, 2009b; Talpur et al., 2013) similarly found that dietary inclusion of GG effectively improved FE and PER of fish. 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. Cumulative mortality of fish fed the Con diet was 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 *Streptococcus parauberis*. Cumulative mortality of fish fed the GG, BB and YC diets was lower compared to fish fed the other diets at day 5 post infection, but did not differ from each other. These results support that of the tested dietary supplements, YC showed the best results in improving weight gain in rockfish, and YC and TT were effective in improving feed efficiency and protein efficiency ratio. GG, BB and YC were effective in lowering the cumulative mortality of rockfish infected with *S. parauberis*.

II. Oral administration of feed additives (YC, GG and BB) achieved improved weight gain of rockfish compared to the Con diet. The greatest improvement in weight gain and FE was obtained in rockfish fed the YC diet agreed with Kim et al. (2016)'s study. Higher feed consumption of rockfish fed the GG and BB diets, but poorer weight gain, SGR, FE, PER and PR compared to those of fish fed YC diet in this study indicates that YC is the most effective growth promoter in terms of improved growth performance and feed utilization. The plant-originated additives were effective to improve not only weight gain and feed utilization, but also disease resistance in some marine fish (Ji et al., 2007; Punitha et al., 2008; Talpur and Ikhwanuddin, 2013; Talpur et al., 2013; Talpur, 2014). The dead rockfish in the 4th and 8th week infection trials exhibited the typical symptoms of diseased olive flounder infected with *E. tarda*, such as reddish in abdomen and fins, darkening of the skin and swelling of abdomen with accumulation of ascites reported by Kusuda and Kawai (1998)'s study. Mortality of fish fed the Con diet was higher than that of fish fed the all other diets at 90 h throughout 10-day post observation in this study, but no difference in mortality was observed among fish fed the YC, GG and BB diets in the 4th week infection trial. The cumulative mortality of fish fed the YC and GG diets was lower than that of fish fed the Con and BB diets at 156 h after infection in the 8th week infection trial. Dietary inclusion of YC, GG and BB effectively lowered mortality of rockfish compared to the Con diet after the 4- and 8-week feeding trial in this study. This indicates that YC, GG and BB are all effective as immunostimulant to lower mortality of fish at occurrence of *E. tarda*. In considering Kim et al. (2016)'s study showing that YC, GG and BB were effective to lower mortality of rockfish at occurrence *S. parauberis* and this study showing that YC, GG and BB are all effective to lower mortality of rockfish at occurrence *E. tarda*, YC, GG and BB are all effective as immunostimulants for both bacterial pathogens. Based on these results, dietary inclusion of YC, GG and BB effectively increased weight gain and SGR of rockfish. However, YC improved FE, PER and PR of fish effectively. Oral administration of YC, GG and BB for 4 and 8 weeks effectively lowered mortality of rockfish at occurrence of *E. tarda*. A 4-week oral administration of additive (YC, GG and BB) was long enough to induce their desirable effect of lowering mortality of fish at occurrence of *E. tarda*.

III. A strong feeding attractant response of rockfish was observed in three kinds of fish meal (JM, SM and PM), one mollusk meal (SQM) and one crustacean meal (SHM), but weak or moderate response was observed in the other plant-originated meal, fish meal, crustacean meal and mollusk meal. The strongest feeding attractiveness to rockfish was obtained in JM, followed by SM, SQM, PM and SHM, in order. Similarly, JM extracts were known to have high feeding attractants and/or stimulants, especially, IMP and lactic acid for yellowtail, and histidine for olive flounder, respectively (Hidaka et al., 2000; Ikeda et al., 2012). Amino acids have been reported as effective feeding attractants and/or stimulants for several fishes (Takeda, 1980; Harada, 1985; Takeda et al., 1984; Ellingsen and Døving, 1986; Fukuda et al., 1989; Jones, 1992; Takaoka et al., 1995; Carr et al., 1996; Kohbara et al., 2000; Yacoob and Browman, 2007; Ikeda et al., 2012). The strong second polynomial correlation (Figs 5, 6, 7, and 8) between the contents of 3 amino acids (alanine, glycine and histidine) in the selected feed ingredient (SHM, SQM, PM, SM, JM and anchovy meal in the Con diet) and weight gain, SGR, feed consumption and CF of rockfish in this study revealed that the alanine, glycine and histidine contents were consistent with the growth performance of fish, probably indicating that the alanine, glycine and histidine contents in the selected feed ingredient played an important role as feeding attractants and/or stimulants in rockfish in these experimental conditions. The experimental diets (JM, SM, PM, SQM and SHM diets) supplemented with the top 5 selected feed ingredient showing high feeding attractants and/or stimulants ability all achieved outstanding improvements in the weight gain and SGR of rockfish over the anchovy meal in the Con diet. This result indicated that adding the selected feed ingredients that possess high feeding attractiveness to rockfish to a diet could improve the growth performance of fish without the addition of synthetic feeding attractants and/or stimulants based on either the omission test of the component of the synthetic extracts of prey or the electro(neuro)physiological study of fish. Howver, this technique is either very complicate process or limited application in aquafeed industry. Nevertheless, the supplementation of feed ingredient in diet based on the feeding attractiveness (behavior) of fish is a very useful technique, especially when low fish meal diet, which are likely to have low palatability, are being developed or when the electro(neuro)physiological study of the targeting fish is not well known. Dietary administration of the selected feed ingredient showing high feeding attractiveness (behavior) to the targeting fish in diets seems to be a very easy and effective method for improving or maximizing the growth performance, feed consumption and CF of fish without conducting an omission test of the component of the synthetic extracts of prey or monitoring the electrophysiological response of fish. In addition, the electro(neuro)physiological study of fish can suggest if fish are able to be triggered by a given chemical cue with their chemoreceptors, but it does not always guarantee that the synthetic feeding attractants will bring about changes in feeding behavior or improve feed consumption. The strongest attractiveness to rockfish was obtained in JM, followed by the SM, SQM, PM and SHM, in order, among the tested ingredients. The greatest feed consumption, weight gain and SGR of fish were obtained in the JM diet, followed by the SM, PM, SQM and SHM diets, in order. In addition, dietray inclusion of the selected feed ingredients (JM, SM, PM and SQM) improved CF of fish. Strong correlations between the alanine, glycine and histidine contents in the selected feed ingredient (SHM, SQM, PM, SM, JM and anchovy meal in the Con diet) and weight gain, SGR, feed consumption and CF of fish were observed.

In conclusion, of the tested dietary additives, YC showed the best results in improving weight gain in rockfish, and YC and TT were effective in improving feed efficiency and protein efficiency ratio. Oral administration of YC, GG and BB for 8 weeks were effective in lowering the cumulative mortality of rockfish infected with gram-positive *S. parauberis* and gram-negative *E. tarda*. Especially, a 4−week oral administration of additive (YC, GG and BB) was long enough to induce their desirable effect of lowering mortality of fish at occurrence of *E. tarda*. The strongest attractiveness to rockfish was obtained in JM, followed by the SM, SQM, PM and SHM, in order, among the various protein sources. The greatest feed consumption, weight gain and SGR of fish were obtained in the JM diet, followed by the SM, PM, SQM and SHM diets, in order. Dietary administration of the selected feed ingredients (JM, SM, PM and SQM) improved CF of fish. Strong correlations between the alanine, glycine and histidine contents in the selected feed ingredients (SHM, SQM, PM, SM, JM and anchovy meal in the Con diet) and weight gain, SGR, feed consumption and CF of fish were observed. Therefore, it is expected that dietary administration of the additives (YC, GG and BB) and feed ingredient showing strong feeding attractants/stimulants, such as JM, SM, PM, SQM and SHM improve growth performance of juvenile rockfish effectively.

Acknowledgments

I would like to express my genuine gratitude to professor Sung Hwoan Cho for his precious advice and constant encouragement throughout this study. I wish to thanks to my committee, professor Sang-Min Lee and Ph.D. Jin-Hyung Yoo, Ph.D. Bo-Young Jee and Ph.D. Mun-Gyeong Kwon for their critical advices for my Ph.D. dissertation, and to professors Young Wan Seo and Sun Young Lim for their considerate advices and interests in this dissertation. I also wish to thank all staffs in Gangneung-Wonju National University for their suppling experimental facilities.

For my Lab. seniors and juniors, Sang Mok Lee, Bum Hee Park, Sung Choon Ji, Chung Il Kim, Young Jin Cho, In-Cheol Choi, Kyoung Tae Kim, Sung Hyo Myung, Won Gwan Jung, Ye Eun Kim, Ki Wook Lee, Hyeon Jong Kim, Dong Gyu Choi, Bok Il Jang, Ah-Young Yun, June Kim and Hae Seung Jung at the Feed Nutrition and Engineering Lab. in Korea Maritime and Ocean University. I warmly thank for their help at practical things as well as sharing the good and bad moments of research and life.

Finally, I would like to express my cordial thanks to my family who have continuously loved, encouraged and supported me.

References

Akintobi, O.A., Onoh, C.C., Ogele, J.O., Idowu, A.A., Ojo, O.V. and Okonko, I.O. 2013. Antimicrobial activity of *Zingiber officinale* (ginger) extract against some selected pathogenic bacteria. Nature and Science, 11:7-15.

Alderman, D.J. and Hastings, T.S. 1998. Antibiotic use in aquaculture: development of resistance-potential for consumer health risks. International Journal of Food Science and Technology, 33:139-155.

Ali, B.H., Blunden, G., Tanira, M.O. and Nemmar, A. 2008. Some phytochemical pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. Food and Chemical Toxicology, 46:409-420.

Amend, D.F. 1981. Potency testing of fish vaccines. Developments in Biological Standardization, 49:447-454.

AOAC (Association of Official Analytical Chemists), 1990. Official Methods of Analysis, 15th edn. Arlington, VA, USA.

Baeck, G.W., Kim, J.H., Gomez, D.K. and Park, S.C. 2006. Isolation and characterization of *Streptococcus* sp. from diseased flounder (*Paralichthys olivaceus*) in Jeju island. Journal of Veterinary Science, 7:53-58.

Bai, S.C., Koo, J., Kim, K. and Kim, S. 2001. Effects of Chlorella powder as a feed additive on growth performance in juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Aquaculture Research. 32 (Suppl. 1):92-98

Balestra, F., Cocci, E., Pinnavaia, G. and Romani, S. 2011. Evaluation of antioxidant, rheological and sensorial properties of wheat flour dough and bread containing ginger powder. LWT-Food Science and Technology, 44:700-705.

Banerjee, R., Verma, A.K., Das, A.K., Rajkumar, V., Shewalkar, A.A. and Narkhede, H.P. 2012. Antioxidant effects of broccoli powder extract in goat meat nuggets. Meat Science, 91:179-184.

Barata, E.N., Hubert, F., Conceição, L.E.C., Velez, Z., Rema, P., Hubbard P.C. and Canário, A.V.M. 2009. Prey odour enhances swimming activity and feed intake in the Senegalese sole. Aquaculture. 293:100-107.

Barroso, F.G., Rodiles, A., Vizcaino, A.J., Marítinez, T.F. and Alarcón, F.J. 2013. Evaluation of feed attractants in juvenile Senegalese sole, *Solea senegalensis*. Journal of the World Aquaculture Society, 44:682-693.

Barrow, F.T. 2000. Feed additives. Encyclopedia of Aquaculture, Stickney, R.R. edition. New York, John Wiley and Sons Inc. pp. 335-340.

Blaszczyk, A., Augustyniak, A. and Skolimowski, J. 2013. Ethoxyquin: an antioxidant used in animal feed. International Journal of Food Science, 1-12.

Bobek, P. 1999. Dietary tomato and grape pomace in rats: effect on lipids in serum and liver, and on antioxidant status. British Journal of Biomedical Science, 56:109-113.

Breck, J.E. 2014. Body composition in fishes: body size matters. Aquaculture, 433:40-49.

Bulfon, C., Volpatti, D. and Galeotti, M. 2015. Current research on the use of plant-derived products in farmed fish. Aquaculture Research, 46:513-551.

Carr, W.E.S. 1976. Chemoreception and feeding behavior in the pigfish, *Orthopristis chrysopterus*: Characterization and identification of stimulatory substances in a shrimp extract. Comparative Biochemistry and Physiology - Part A: Physiology, 55:153-157.

Carr, W.E.S. and Chaney, T.B. 1976. Chemical stimulation of feeding behavior in the pinfish, *Lagodon rhomboides*: characterization and identification of stimulatory substances extracted from shrimp. Comparative Biochemistry and Physiology - Part A: Physiology, 54:437-441.

Carr, W.E.S. and Derby, C.D. 1986. Chemically stimulated feeding behavior in marine animals. Importance of chemical mixtures and involvement of mixture interactions. Journal of Chemical Ecology, 12:989-1011.

Carr, W.E.S., Blumenthal, K.M. and Netherton, J.C. 1977. Chemoreception in the pigfish, *Orthopristis chrysopterus*: The contribution of amino acids and betaine to stimulation of feeding behavior by various extracts. Comparative Biochemistry and Physiology - Part A: Physiology, 58:69-73.

Carr, W.E.S., Netherton, J.C., Gleeson, R.A. and Derby, C.D. 1996. Stimulants of feeding behavior in fish: analyses of tissues of diverse marine organism. The Biological Bulletin, 190:149-160.

Chen, X., Hu, W., Xie, Y., Li, Y., Guo, L. and Jin, W. 2012. Antioxidant research of persimmon extraction in ionizing radiation mice. Advanced

Materials Research, 343-344:1198-1206.

Choi, J.G., Kang, O.H., Lee, Y.S., Oh, Y.C., Chae, H.S., Obiang-Obounou, B., Park, S.C., Shin, D.W., Hwang, B.Y. and Kwon, D.Y. 2010. Antimicrobial activity of the constituents of *Smallanthus sonchifolius* leaves against methicillin-resistant *Staphylococcus aureus*. European Review for Medical and Pharmacological Sciences, 14:1005-1009.

Defoirdt, T., Sorgeloos, P. and Bossier, P. 2011. Alternatives to antibiotics for the control of bacterial disease aquaculture. Current Opinion in Microbiology, 14:251-258.

Deng, Y., Yang, G., Yue, J., Qian, B., Liu, Z., Wang, D., Zhong, Y. and Zhao, Y. 2014. Influence of ripening stages and extracting solvents on the polyphenolic compounds, antimicrobial and antioxidant activities of blueberry leaf extracts. Food Control, 38:184-191.

Dias, J., Gomes, E.F. and Kaushik, S.J. 1997. Improvement of feed intake through supplementation with an attractant mix in European seabass feed plant-protein rich diets. Aquatic Living Resources, 10:385-389.

Drewhurst, I. 1998. EQ. JMPR Evaluations 1998 Part II Toxicological. Joint meeting of the FAO panel of experts on pesticide residues in food and the environment and the WHO Core Assessment Group, Rome. pp. 21-30.

Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics, 11:1-42.

Dunlap, K.L., Reynolds, A.J. and Duffy, L.K. 2006. Total antioxidant power in sled dogs supplemented with blueberries and the comparison of blood parameters associated with exercise. Comparative Biochemistry and

Physiology - Part A: Molecular & Intergrative Physiology, 143:429-434.

Ekwenye, U.N. and Elegalam, N.N. 2005. Antibacterial activity of ginger (*Zingiber officinale* Roscoe) and garlic (*Allium sativum* L.) extracts on *Escherichia coli* and *Salmonella typhi*. International Journal of Molecular Medicine and Advance Sciences, 1:411-417.

Ellingsen, O.F. and Døving, K.B. 1986. Chemical fractionation of shrimp extracts inducing bottom food search behavior in cod (*Gadus morhua* L.). Journal of Chemical Ecology, 12:155-168.

Fange, R. and Grove, D.S. 1979. Digestion in Fish Physiology, Vol. VIII. Acad. Press, NY. pp. 162-260.

FAO. 1970. PL: 1969/M/17/1, WHO/FOOD./70.38. Pesticide residues in food. Report of the 1969 joint meeting of the FAO working party of experts on pesticide residues and WHO expert group on pesticide residues, FAO Agricultural Studies, No. 84 WHO Technical Report Series, No. 458, 159 EQ, Rome.

Fox, C.J., Blow, P., Brown, J.H. and Watson, I. 1994. The effect of various processing methods on the physical and biochemical properties of shrimp head meals and their utilization by juvenile *Penaeus monodon* Fab. Aquaculture, 122:209-226.

Fukuda, K., Kohbara, J., Zeng, C. and Hidaka, I. 1989. The feeding-stimulatory effects of squid muscle extracts on the young yellowtail *Seriola quinqueradiata*. Nippon Suisan Gakkaishi, 55:791-797.

Gabor, E., Sara, A. and Barbu. A. 2010. The effects of some phytoadditives on growth, health and meat quality on different species of fish. Animal

Science and Biotechnologies, 43:61-65.

Goh, Y. and Tamura, T. 1980. Effect of amino acids on the feeding behavior in red sea bream. Comparative Biochemistry and Physiology - Part C: Comparative Pharmacology, 66:225-229.

Goyal, S., Verma, N. and Jinddal, V. 2013. To evaluate the protective effect of *Vitis vinifera* and *Solanum lycopersicum* in diet induced obesity in rats. Toxicology Letters, 221:S253.

Han, S.Y., Kang, B.K., Kang, B.J., Kim, J.M., Han, J.E., Kim, J.H., Choresca Jr, C., Shin, S.P., Jun, J.W. and Park, S.C. 2011. Protective efficacy of a combined vaccine against *Edwardsiella tarda*, *Streptococcus iniae* and *Streptococcus parauberis* in farmed olive flounder *Paralichthys olivaceus*. Fish Pathology, 46:108-111.

Hara, T.J. 1993. Role of olfaction in fish behavior. In: Pitcher, T.J. (Ed.), behavior of Teleost Fishes. Chapman & Hall, London, pp. 171-199.

Harada, K. 1985. Feeding attraction activities of amino acids and lipids for juvenile yellowtail. Bulletin of the Japanese Society for the Science of fish, 51:453-459.

Hassanin, M.E., Hakim, Y. and Badawi, M.E. 2014. Dietary effect of ginger (*Zingiber officinale* Roscoe) on growth performance, immune response of Nile tilapia (*Oreochromis niloticus*) and disease resistance against *Aeromonas hydrophila*. Abbassa International Journal for Aquaculture **7**:35-52.

Hidaka, I., Kohbara, J., Araki, T., Morishita, T., Miyajima, T., Shimizu, S. and Kuriyama, I. 2000. Identification of feeding stimulants from a jack mackerel muscle extract for young yellowtail *Seriola quinqueradiata.* Aquaculture, 181:115-126.

Hoshina, T. 1962. On a new bacterium, *Paracolobactrum anguillimortiferum*. Bulletin of the Japanese Society for the Science of Fish, 28:162-164.

Hoshina, T., Sano, T. and Morimoto, Y. 1958. A *Streptococcus* pathogenic for fish. Journal of Tokyo University of Fisheries, 44:57-58.

Hosokawa, H., Takii, K., Shimeno, S. and Takeda, M. 2001. Identification of feeding stimulants for yellowtail in muscle extract of jack mackerel. Aquaculture Science, 49:225-229.

Hwang, I., Jeong, M. and Chung, S. 2011. The physicochemical properties and the antioxidant activities of persimmon peel powder with different particle sizes. Journal of the Korean Society for Applied Biological Chemistry, 54:442-446.

Hwang, J., Lee, S., Rha, S., Yoon, H., Park, E., Han, K. and Kim, S. 2013. Dietary green tea extract improves growth performance, body composition, and stress recovery in the juvenile black rockfish, *Sebastes schlegeli*. Aquaculture International, 21:525-538.

Ikeda, I., Okamoto, Y. and Oda, K. 2012. Identification of feeding stimulants for Japanese flounder in muscle extract of jack mackerel. Aquaculture Science, 60:195-198.

Jeon, G.H., Kim, H.S., Myung, S.H. and Cho, S.H. 2014. The effects of the dietary substitution of fishmeal with tuna by-product meal on growth, body composition, plasma chemistry and amino acid profiles of juvenile Korean rockfish (*Sebastes schlegeli*). Aquaculture Nutrition, 20:753-761.

Jeong, H., Jo, Y., Jeong, J., Kim, H. and Heo, H.J. 2012. Nutritional composition and *in vitro* antioxidant activities of blueberry (*Vaccinium ashei*) leaf. Korean Journal of Food Preservation, 19:604-610.

Ji, S., Takaoka, O., Jeong, G., Lee, S., Ishimaru, K., Seoka, M. and Takii, K. 2007. Dietary medicinal herbs improve growth and some non-specific immunity of red sea bream *Pagrus major*. Fisheries Science, 73:63-69.

Jones, K.A. 1992. Food search behavior in fish and the use of chemical lures in commercial and sports fishing. In: Hara, T.J. (Ed.), Fish Chemoreception. Chapman & Hall, London, pp. 288-320.

Kasumyan, A.O. and Nikolaeva, E.V. 2002. Comparative analysis of taste preferences in fishes with different ecology and feeding. Journal of Ichthyology, 42:S203-S214.

Khallaf, E.A., Galal, M. and Authman, M. 2003. The biology of *Oreochromis niloticus* in a polluted canal. Ecotoxicology, 12:405-416.

Kikuchi, K. and Furuta, T. 2009. Use of defatted soybean meal and blue mussel meat as substitute for fishmeal in the diet of tiger puffer, *Takifugu rubripes*. Journal of the World Aquaculture Society, 40:472-482.

Kikuchi, K., Ueda, A., Sugita, H. and Takeda, S. 2002. Effect of dietary inclusion of blue mussel extracts on growth and body composition of Japanese flounder *Paralichthys olivaceus*. Journal of the World Aquaculture Society, 33:41-47.

Kim, Y. 2013. Effects of dietary supplementation of yacon (*Polymnia sonchifolia*) by-products on performance and physico-chemical properties of chicken thigh meat. Korean Journal of Poultry Science, 40:1-9.

Kim, H. and Chin, K. 2011. Physico-chemical properties and antioxidant activity of pork patties containing various tomato powders of solubility. Korean Journal for Food Science of Animal Resources, 31:436-441.

Kim, K.H., Hwang, Y.J. and Bai, S.C. 1999. Resistance to *Vibrio alginolyticus* in juvenile rockfish (*Sebastes schlegeli*) fed diets containing different doses of aloe. Aquaculture, 180:13-21.

Kim, K., Wang, X.J. and Bai, S.C. 2001. Reevaluation of the optimum dietary protein level for the maximum growth of juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Aquaculture Research, 32 (Suppl. 1):119-125.

Kim, H., Lee, M., Jeon, S. and Choi, M. 2003. The effect of supplementation of persimmon leaf extract on lipid and antioxidant metabolism in rats fed a high-cholesterol diet. Nutritional Sciences, 6:141-147.

Kim, K., Wang, X., Han, K. and Bai, S.C. 2004. Optimum dietary protein level and protein-to-energy ratio for growth of juvenile Korean rockfish *Sebastes schlegeli*. Journal of the World Aquaculture Society, 35:305-314.

Kim, A., Lee, J., Chang, H. and Lee, M. 2009. Antioxidant effects of chungkukjang fermented using *Bacillus subtilis* DJI in rats fed a high cholesterol diet. Journal of the Korean Society of Food Science and Nutrition, 38:1699-1706.

Kim, A.R., Lee, J.J., Jung, H.O. and Lee, M.Y. 2010a. Physicochemical composition and antioxidative effects of yacon (*Polymnia sonchifolia*). Journal of Life Science, 20:40-48.

Kim, A., Lee, J., Lee, Y., Jung, H. and Lee, M. 2010b. Cholesterol-lowering and anti-obesity effects of *Polymnia sonchifolia* Poepp. & Endl. powder in rats fed a high fat-high cholesterol diet. Journal of the Korean Society of Food Science and Nutrition, 39:210-218.

Kim, S., Pham, M.A., Kim, K., Son, M. and Lee, K. 2010c. Effects of microbial fermentation of soybean on growth performances, phosphorous availability, and antioxidant activity in diets for juvenile olive flounder (*Paralichthys olivaceus*). Food Science and Biotechnology, 19:1605-1610.

Kim, E.O., Lee, H., Cho, C.H., Kim, Y.J. and Kim, D. 2011. Antioxidant capacity and anti-inflammatory effect of the ethyl acetate fraction of dried persimmon *(Diospyros kaki* Thumb.) on THP-1 human acute monocytic leukemia cell line. Journal of the Korean Society for Applied Biological Chemistry, 54:606-611.

Kim, J., Harikrishnan, R., Kim, M., Balasundaram, C. and Heo, M. 2012. *Broussonetia kazinoki* as a feed additive enhances disease resistance against *Streoptococcus parauberis* in *Paralichthys olivaceus*. Fish Pathology, 47:20-22.

Kim, H.S., Kim, H.J., Choi, D.G., Jang, B., Cho, S.H., Kwon, M., Min, B. and Kim, D.S. 2016. Effect of various sources of dietary additives on growth, body composition, and challenge test survival of juvenile rockfish *Sebastes schlegeli*. Turkish Journal of Fisheries and Aquatic Sciences, 16:759-766.

Kohbara, J., Hidaka, I., Morishita, T. and Miyajima, T. 2000. Gustatory and olfactory sensitivity to extracts of jack mackerel muscle in young yellow

Seriola quinqueradiata. Aquaculture, 181:127-140.

Kota, N., Krishna, P. and Polasa, K. 2008. Alterations in antioxidant status of rats following intake of ginger through diet. Food Chemistry, 106: 991-996.

Kubitza, F., Lovshin, L.L. and Lovell, R.T. 1997. Identification of feed enhancers for juvenile largemouth bass *Micropterus salmoides*. Aquaculture, 148:191-200.

Kusuda, R. and Kawai, K. 1998. Bacterial diseases of cultured marine fish in Japan. Fish Pathology, 33:221-227.

Lee, S. 2002. Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*). Aquaculture, 207:79-95.

Lee, S. and Kim, K. 2009. Effects of dietary carbohydrate to lipid ratios on growth and body composition of juvenile and grower rockfish, *Sebastes schlegeli*. Aquaculture Research, 40:1830-1837.

Lee, S., Yoo, J. and Lee, J.Y. 1996. The use of soybean meal, corn gluten meal, meat meal, meat and bone meal, or blood meal as a dietary protein source replacing fish meal in Korean rockfish (*Sebastes schlegeli*). Korean Journal of Animal Nutrition and Feedstuffs, 20:21-30.

Lee, S., Hwang, U. and Cho, S.H. 2000. Effects of feeding frequency and dietary moisture content on growth, body composition and gastric evacuation of juvenile Korean rockfish (*Sebastes schlegeli*). Aquaculture, 187:399-409.

Lee, S., Jeon, I.G. and Lee, J.Y. 2002. Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish (*Sebastes schlegeli*). Aquaculture, 211:227-239.

Lee, S., Azarm, H.M. and Chang, K.H. 2016. Effects of dietary inclusion of fermented soybean meal on growth, body composition, antioxidant enzyme activity and disease resistance of rockfish (*Sebastes schlegeli*). Aquaculture, 459:110-116.

Lee, S.D., Kim, J.H., Jung, H.J., Kim, Y.H., Kim, I.C., Kim, S.B., Lim, S.Y., Jung, W.S., Lee, S. and Kim, Y.J. 2013. The effect of ginger extracts on the antioxidant capacity and IgG concentrations in the colostrum and plasma of neo-born piglets and sows. Livestock Science, 154:117-122.

Lie, Ø., Lied, E. and Lambertsen, G. 1989. Feed attractants for cod (*Gadus morhua*). Fiskeri Direktoratets Skrifter Serie Ernaering, 7:227-233.

Lim, L.S., Ebi, I., Chor, W.K., Kawamura, G. and Shapawi, R. 2015. Determination on the possibility of dietary betaine supplementation to improve feed intake of soybean meal-based diet in the juvenile grouper (*Epinephelus fuscoguttatus*): a pilot study. Malaysian Applied Biology Journal, 44:137-141.

Lim, S.R., Choi, S.M., Wang, X.J., Kim, K.W., Shin, I.S., Min, T.S. and Bai, S.C. 2004. Effects of dehulled soybean meal as a fish meal replacer in diets for fingerling and growing Korean rockfish *Sebastes schlegeli*. Aquaculture, 231:457-468.

MacGregoer, J.S. 1959. Relation between fish condition and population size in the sardine (*Sardinops caerulea*). U.S. Fishery Wild Service, Fish Bulletin, 60:215-230.

Mackie, A.M., Adron, J.W. and Grant, P.T. 1980. Chemical nature of feeding stimulants for the juvenile Dover sole, *Solea solea* (L). Journal of Fish Biology, 16:701-708.

Ministry for food, agriculture, forestry and fisheries (MFAFF). 2014. Ministry for food, agriculture, forestry and fisheries. Korea Statistical Yearbook 2013.

Ministry for food, agriculture, forestry and fisheries (MFAFF). 2016. Ministry for food, agriculture, forestry and fisheries. Korea. Statistical Yearbook 2015.

Mizanur, R.M., Yun, H., Moniruzzaman, M., Ferreira, F., Kim, K. and Bai, S.C. 2014. Effects of feeding rate and water temperature on growth and body composition of juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf 1880). Asian-Australasian Journal of Animal Sciences, 27:690-699.

Molan, A.L., Lila, M.A. and Mawson, J. 2008. Satiety in rats following blueberry extract consumption induced by appetite-suppressing mechanisms unrelated to *in vitro* or *in vivo* antioxidant capacity. Food Chemistry, 107:1039-1044.

Mongile, F., Mandrioli, L., Mazzoni, M., Pirini, M., Zaccaroni, A., Sirri, R., Parma, L., Gatta, P.P., Sarli, G. and Bonaldo, A., 2015. Dietary inclusion of mussel meal enhances performance and improves feed and protein utilization in common sole (*Solea solea*, Linnaeus, 1758) juveniles. Journal of Applied Ichthyology, 31:1077-1085.

Morais, S. 2017. The physiology of taste in fish: potential implications for feeding stimulation and gut chemical sensing. Reviews in Fisheries Science & Aquaculture, 25:133-149.

Moreira, E.A.M., Fagundes, R.L.M., Filho, D.W., Neves, D., Sell, F., Bellisle, F. and Kupek, E. 2005. Effects of diet energy level and tomato powder consumption on antioxidant status in rats. Clinical Nutrition, 24:1038-1046.

Mueller, K., Blum, N.M., Kluge, H. and Mueller, A.S. 2012. Influence of broccoli extract and various essential oils on performance and expression of xenobiotic- and antioxidant enzymes in broiler chickens. British Journal of Nutrition, 108:588-602.

Nagel, F., von Danwitz, A., Schlachter, M., Kroeckel, S., Wagner, C. and Schulz, C. 2014. Blue mussel meal as feed attractant in rapeseed protein-based diets for turbot (*Psetta maxima* L.). Aquaculture Research, 45:1964-1978.

Naik, S.D., Sahu, N.P. and Jain, K.K. 2001. Use of squilla (*Orato squilla neap*), squid (*Sepia pharonis*) and clam (*Katelysia opima*) meal alone or in combination as a substitute for fish meal in the postlarval diet of *Macrobrachium rosenbergii*. Asian-Australasian Journal of Animal Sciences, 14:1272-1275.

Nakagawa, M., Okouchi, H., Adachi, J., Hattori, K. and Yamashita, Y. 2007. Effectiveness of stock enhancement of hatchery-released black rockfish *Sebastes schlegeli* in yamada bay-evaluation by a fish market survey. Aquaculture, 263:295-302.

Nehemia, A., Maganira, J.D. and Rumisha, C. 2012. Length-weight relationship and condition factor of tilapia species grown in marine and fresh water ponds. Agriculture and Biology Journal of North America, 3:117-124.

NRC. 2011. National Research Council. Feed Additives, National Academy Press, Washington DC. USA.

Nya, E.J. and Austin, B. 2009a. Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Disease, 32:963-970.

Nya, E.J. and Austin, B. 2009b. Use of dietary ginger, *Zingiber officinale* Roscoe, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Disease, 32:971-977.

Ohara, A., Saito, F. and Matsuhisa, T. 2008. Screening of antibacterial activities of edible plants against *Streptococcus mutans*. Food Science and Technology Research, 14:190-193.

Papandreou, M.A., Dimakopoulou, A., Linardaki, Z.I., Cordopatis, P., Klimis-Zacas, D., Margarity, M. and Lamari, F.N. 2009. Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. Behavioural Brain Research, 198:352-358.

Papatryphon, E. and Soares, J.H.Jr. 2000. Identification of feeding stimulants for striped bass *Morone saxatilis*. Aquaculture, 185:339-352.

Papatryphon, E. and Soares, J.H.Jr. 2001. Optimizing the levels of feeding stimulants for use in high-fish meal and plant feedstuff-based diets for striped bass, *Morone saxatilis*. Aquaculture, 202:279-288.

Park, J.S., Kim, H.S. and Chin, K.B. 2012. The antioxidant activity of yacon (*Polymnia sonchifoliaty)* and its application to the pork patties as a natural antioxidant. Korean Journal for Food Science of Animal Resources, 32:190-197.

Punitha, S.M.J., Babu, M.M., Sivaram, V., Shankar, V.S., Dhas, S.A., Mahesh, T.C., Immanuel, G. and Citarasu, T. 2008. Immunostimulating influence of herbal biomedicines on non specific immunity in grouper *Epinephelus tauvina* juvenile against *Vibrio harveyi* infection. Aquaculture International, 16:511-523.

Sebiomo, A., Awofodu, A.D., Awosanya, A.O., Awotona, F.E. and Ajayi, A.J. 2011. Comparative studies of antibacterial effect of some antibiotics and ginger (*Zingiber officinale*) on two pathogenic bacteria. Journal of Microbiology and Antimicrobials, 3:18-22.

Shimizu, C., Ibrahim, A., Tokoro, T. and Shirakawa, Y. 1990. Feeding stimulation in sea bream, *Pagrus major*, fed diets supplemented with Antarctic krill meals. Aquaculture, 89:43-53.

Shon, M., Lee, J., Choi, J., Choi, S., Nam, S., Seo, K., Lee, S., Sung, N. and Park, S. 2007. Antioxidant and free radical scavenging activity of methanol extract of *chungkukjang.* Journal of Food Composition and Analysis, 20: 113-118.

Singh, R.K., Balange, A.K., Khandagale P.A. and Chavan, S.L. 2006.

Evaluation of fish meals as natural feed stimulants on the feed behavior of fry and juveniles of *Lates calcarifer* (Bloch). Asian Fisheries Science Journal, 19:97-106.

Sivaram, V., Babu, M.M., Immanuel, G., Murugadass, S., Citarasu, T. and Marian, M.P. 2004. Growth and immune response of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemented diets against *Vibrio harveyi* infections. Aquaculture, 237:9-20.

Smith, D.M., Tabrett, S.J., Barclay, M.C. and Irvin, S.J. 2005. The efficacy of ingredients included in shrimp feeds to stimulate intake. Aquaculture Nutrition, 11:263-272.

Srikar, L. N., Keshavanath, P. and Peter, M. 1979. Changes in biochemical composition of *Clarias batrachus* (Linn.) before and after spawning. Mysore Journal of Agricultural Sciences, 13:87-88.

Suresh, A.V., Kumaraguru vasagam, K.P. and Nates, S. 2011. Attractability and palatability of protein ingredients of aquatic and terrestrial animal origin, and their practical value for blue shrimp, *Litopenaeus stylirostris* fed diets formulated with high levels of poultry byproduct meal. Aquaculture, 319:132-140.

Takaoka, O., Takii, K., Nakamura, M., Kumai, H. and Takeda, M. 1990. Identification of feeding stimulants for marbled rockfish. Nippon Suisan Gakkaishi, 56:345-351.

Takaoka, O., Takii, K., Nakamura, M., Kumai, H. and Takeda, M. 1995. Identification of feeding stimulants for tiger puffer. Fisheries Science, 61:833-836.

Takeda, M. 1980. Feeding stimulants for fish. Heredity, 34:45-51.

Takeda, M., Takii, K. and Matsui, K. 1984. Identification of feeding stimulants for juvenile eel. Bulletin of the Japanese Society of Scientific Fisheries, 50:645-651.

Takii, K., Shimeno, S., Takeda, M. and Kamekawa, S. 1986a. The effects of feeding stimulants in diet on digestive enzyme activities of eel. Bulletin of the Japanese Society of Scientific Fisheries, 52:1449-1454.

Takii, K., Shimeno, S. and Takeda, M. 1986b. The effect of feeding stimulants in diet on some hepatic enzyme activities of eel. Bulletin of the Japanese Society of Scientific Fisheries, 52:2131-2134.

Takii, K., Shimeno, S., Akutsu, M. and Takeda, M. 1994. Dietary supplement of feeding stimulants on performance and digestive function of yellowtail, *Seriola quinqueradiata*. Bulletin of the Fisheries Laboratory of Kinki University, 4:127-137.

Talpur, A.D. 2014. *Mentha piperita* (Peppermint) as feed additive enhanced growth performance, survival, immune response and disease resistance of Asian seabass, *Lates calcarifer* (Bloch) against *Vibrio harveyi* infection. Aquaculture, 420-421:71-78.

Talpur, A.D. and Ikhwanuddin, M. 2012. Dietary effects of garlic (*Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection in Asian seabass, *Lates calcarifer* (Bloch). Aquaculture, 364-365:6-12.

Talpur, A.D. and Ikhwanuddin, M. 2013. *Azadirachta indica* (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *Lates calcarifer* challenged with *Vibrio harveyi*. Fish Shellfish Immunology, 34:254-264.

Talpur, A.D., Ikhwanuddin, M. and Bolong, A.A. 2013. Nutritional effects of ginger (*Zingiber officinale* Roscoe) on immune response of Asian sea bass, *Lates calcarifer* (Bloch) and disease resistance against *Vibrio harveyi*. Aquaculture, 400-401:46-52.

Tandler, A., Berg, B.A., Kissil, G.W. and Mackie, A.M. 1982. Effect of food attractants on appetite and growth rate of gilthead bream, *Sparus aurata* L. Journal of Fish Biology, 20:673-681.

Tang, S., Kerry, J.P., Sheehan, D. and Buckley, D.J. 2001. A comparative study of tea catechins and α-tocopherol as antioxidants in cooked beef and chicken meat. European Food Research and Technology, 213:286-289.

Thorisson, S., Gunstone, F. and Hardy, R. 1992. The antioxidant properties of ethoxiquin and of some of its oxidation products in fish oil and meal. Journal of American Oil Chemists' Society, 69:806-809.

Toften, H., Jørgensen, E.H. and Jobling, M. 1995. The study of feeding preferences using radiography: oxytetracycline as a feeding deterrent and squid extract as a stimulant in diets for Atlantic salmon. Aquaculture Nutrition, 1:145-149.

Toko, I., Fiogbe, E.D., Koukpode, B. and Kestemont, P. 2007. Rearing of African catfish (*Clarias gariepinus*) and vundu catfish (*Heterobranchus longifilis*) in traditional fish ponds (whedos): Effect of stocking density on growth, production and body composition. Aquaculture, 262:65-72.

Tomofuji, T., Ekuni, D., Azuma, T., Irie, K., Endo, Y., Yamamoto, T., Ishikado, A., Sato, T., Harada, K., Suido, H. and Morita, M. 2012. Supplementation of broccoli or *Bifidobacterium longum*-fermented broccoli suppresses serum lipid peroxidation and osteoclast differentiation on alveolar bone surface in rats fed a high-cholesterol diet. Nutrition Research, 32:301-307.

Vallejos-Vidal, E., Reyes-López, F., Teles, M. and MacKenzie, S. 2016. The response of fish to immunostimulant diets. Fish & Shellfish Immunology 56:34-69.

Vizzotto, M., da Rosa Fetter, M., Dutra Corbelini, D., Couto Pereira, M. and Nogueira Gonzales, T. 2013. Bioactive compounds and antioxidant activity of blueberry (*Vaccinium ashei* Reade). Acta Horticulturae, 972:111-116.

Wang, J., Ai, Q., Mai, K., Xu, H., Zuo, R., Xu, W., Zhang, W. and Zhang, C. 2015. Effects of dietary ethoxyquin on growth, feed utilization and residue in the muscle of juvenile Japanese seabass, *Lateolabrax japonicus*. Aquaculture Research, 46:2656-2664.

Weatherley, A.H., 1972. Growth and ecology of fish populations. Academic Press, London, pp.293.

Weil, J.T., Van der Veen, J. and Olcott, H.S. 1968. Stable nitroxides as lipid antioxidants. Nature, 219:168-169.

Xue, M. and Cui, Y. 2001. Effect of several feeding stimulants on diet preference by juvenile gibel carp (*Carassius auratus gibelio*), fed diets with or without partial replacement of fish meal by meat and bone meal. Aquaculture, 198:281-292.

Yacoob, S.Y. and Browman, H.I. 2007. Olfactory and gustatory sensitivity to some feed-related chemicals in the Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture, 263:303-309.

Yamanoi, H., Momoyama, K., Yasunobu, H. and Muroga, K. 1988. *Vibrio anguillarum* infection in flounder (*Paralichthys olivaceus*) fingerlings. Fish Pathology, 23:69-70.

Yan, Q., Xie, S., Zhu, X., Lei, W. and Yang, Y. 2007. Dietary methionine requirement for juvenile rockfish, *Sebastes schlegeli*. Aquaculture Nutrition, 13:163-169.

Yun, A., Kim, H.S., Seo, Y., Cho, S.H. and Bae, J.Y. 2016. Effects of dietary antioxidant supplementation on the growth, serum chemistry, body composition and challenge test results of juvenile rockfish *Sebastes schlegeli*. Korean Journal of Fisheries and Aquatic Sciences, 49:323-329.

Zhang, Y., Lu, X., Fu, Z., Wang, Z. and Zhang, J. 2011. Sulphated modification of a polysaccharide obtained from fresh persimmon (*Diospyros kaki* L.) fruit and antioxidant activities of the sulphated derivatives. Food Chemistry, 127:1084-1090.