Thesis for the Degree of Master of Fisheries Science

Effects of Dietary Yucca Meal on Growth, Hematology, Non-specific Immune Responses and Disease Resistance in Juvenile Nile Tilapia, (*Oreochromis niloticus*)

by

Grace Wanjiru Njagi

KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

Pukyong National University

February 2016

Effects of Dietary Yucca Meal on Growth, Hematology, Non-specific Immune Responses and Disease Resistance in Juvenile Nile Tilapia, *Oreochromis niloticus*

치어기틸라피아사료내사포닌함유유카추출물의첨가가성장, 면역및질병저항성에미치는영향

Advisor: Prof. Sungchul Charles Bai

by

Grace Wanjiru Njagi

A thesis submitted in partial fulfilment of the requirement
for the degree of Master of Fisheries Science
in KOICA-PKNU International Graduate Program of Fisheries Science
Pukyong National University
26 February 2016

Effects of Dietary Yucca Meal on Growth, Hematology, Non-specific Immune Responses and Disease Resistance in Juvenile Nile Tilapia, *Oreochromis niloticus.*

A dissertation

by

Grace Wanjiru Njagi

Approved by :

(Chairman) Prof. Yang-Bong Lee

(Member) Prof. In-Soo Kong

(Member) Prof. Sungchul C. Bai

February 26 2016

Dedication

I heartily dedicate this dissertation to

the love and memory of my beloved husband,

Anthony, who passed on while I was undertaking

this graduate studies. You gave me your unconditional love

and unwavering support and I will

always be grateful for having

you in my life.

I will always cherish your memories.

Table of Contents

List of Tables

List of Figures

Effects of Dietary Yucca Meal on Growth, Hematology, Non-specific Immune Responses and Disease Resistance in Juvenile Nile Tilapia, ***Oreochromis niloticus.***

Grace Wanjiru Njagi

KOICA-PKNU International Graduate Program of Fisheries Science

Pukyong National University

Abstract

A 10-weeks feeding trial was conducted to determine the effects of dietary Yucca meal supplementation (YMS) on growth performance, hematology, non-specific immune responses and disease resistance in juvenile Nile tilapia, *Oreochromis niloticus*. Six isonitrogenous (40% crude protein) and isoenergetic (16.39 kJ available energy g^{-1}) experimental diets were formulated to contain 0% (YMS_0), 0.1% ($YMS_{0.1}$), 0.3% ($YMS_{0.3}$), 0.5% ($YMS_{0.5}$), 1% ($YMS_{1.0}$) and 2% ($YMS_{2.0}$) dietary Yucca meal on a dry-weight basis. A diet without Yucca meal supplementation (YMS_0) was used as the control diet.

At the end of the 10 weeks feeding trial; weight gain (WG) of fish fed on diet $YS_{0.1}$ was significantly higher than those of fish fed on $YMS_{0.5,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets ($P<0.05$). Specific growth rate (SGR) of fish fed on diet $YMS_{0.1}$ was significantly higher than that of fish fed on $YMS_{0.5,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets. Feed efficiency (FE) of fish fed on diet

$YMS_{0.1}$ was significantly higher than that of fish fed on $YMS_{0,}$ $YMS_{0.5,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets. There was no significant difference in protein efficiency ratio and survival across all the treatment groups. Fish fed on diet $YMS_{0.1}$ had significantly higher whole body protein content than did fish fed on diets $YMS_{0,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets. Whole body ash content increased significantly with increasing Yucca meal levels. There was no significant difference in whole body lipid and moisture content across all the treatment groups.

Glutamic oxaloacetic transaminase (GOT) levels were significantly higher in fish fed on $YMS_{0.1,}$ $YMS_{0.3}$ and $YMS_{1.0}$ diets than those of fish fed on YMS_0 and $YMS_{2.0}$ diets ($P<0.05$). Glutamic pyruvic transaminase (GPT) levels in fish fed on the control diet (YMS_0) were significantly lower than those of fish fed on all the other experimental diets. There was no significant difference in glucose and total protein levels across all the treatment groups.

Lysozyme activity of fish fed on $YMS_{0.1}$ was significantly higher than that of fish fed on YMS_0, $YMS_{0.5}$ and $YMS_{1.0}$ diets ($P<0.05$). Nitro blue tetrazolium (NBT) absorbance's of fish fed on $YMS_{0.1}$, $YMS_{0.3}$ and $YMS_{0.5}$ were significantly higher than those of fish fed on diet $YMS_{2.0}$. After the 14 days of the challenge test, cumulative survival rates of fish fed on diet $YMS_{0.1}$ were significantly higher than those of fish fed on $YMS_{0,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets.

In conclusion, dietary Yucca meal improved growth, promoted non-specific immune responses and enhanced disease resistance against *A. hydrophila* bacterial challenge in

juvenile Nile tilapia. In the current study, the best results were obtained at Yucca meal inclusion level of 0.1 % (23.9 mg kg^{-1} saponin) in the diet YMS_0.

I. Introduction

Aquaculture production has seen a tremendous increase in the recent past due to increased demand for food fish. In 2012, while global marine capture fishery production was stable at about 80 million tonnes, global aquaculture production set another all-time high at more than 90 million metric tonnes (FAO, 2014) with per capita fish consumption increasing from 10 kg in the 1960s to more than 19 kg in 2012.

Fish is a key source of proteins, essential amino acids and minerals, especially in low-income, food-deficit countries (FAO, 2009). Increasing evidence has also shown that the consumption of fish enhances brain development and learning in children, protects vision and eye health, and offers protection from cardiovascular diseases and some cancers. The fats and fatty acids in fish, particularly the long chain ώ-3 poly-unsaturated fatty acids (ώ -3 PUFA), are highly beneficial and difficult to obtain from other food sources. Increased demand for fish has been brought about by the need to feed the ever growing world population which was estimated to be over 7

billion people in 2011, is projected to surpass 9 billion people by 2050 and to exceed 10 billion in 2100 (UN, 2010).

However, fish production from capture fisheries world over is on the decline and most of the fisheries resources are fully exploited, overexploited, depleted or recovering from depletion. This has largely been attributed to the negative impacts of climate change on fisheries resources (Brander, 2010) such as changes in temperature, oxygen, salinity, winds, vertical mixing and pH. Climate change is an additional pressure in the face of the many (fishing, mortality, loss of habitat, pollution, disturbance by introduced species) which fish stocks already experience.

Natale et al. (2013) observed that the average yearly growth rate for aquaculture production between 1970 and 2009 was 8.3%, compared to 4.9% for poultry, 2.9% for pig, 1.8% for sheep and goats, 1.4% for cattle and 1.2% for capture fisheries. While the productivity gain after 'green revolution' in agriculture and livestock is slowing down, aquaculture is yet to realize its full potential and thus seen as one of the tools that will play a critical role in mitigating climate change and effects of global warming on food production.

This will be achieved through domestication of new fish species and diversification coupled with intensification of culture systems.

Nile tilapia is one of the most important and commonly cultured species among the Tilapias. This has been attributed to its favourable characteristics for culture such as rapid growth, good survival in high-density culture, tolerance to a wide range of environmental conditions, stress and disease resistance, ability to reproduce in captivity and high adaptation to a wide range of supplemental feeding (El-Sayed, 2006). Consequently, this has made Nile tilapia a good candidate for culture in a wide range of systems such as semi-intensive, intensive grow-out, and cage culture especially in developing countries. Global aquaculture production of Nile tilapia rose from 970,641 metric tonnes in year 2000 to 2,537,461 metric tonnes in year 2010, with a high production recorded in year 2012 at 3,197,330 metric tonnes (FAO FishStat 2014), representing a 26% increase in just two years.

The need to meet the increased demand for fish has promoted intensification in aquaculture production which has consequently led to increased demand for aquaculture inputs such as feeds. Notably, the availability of quality and

affordable feeds plays a central role in the sustainability of any aquaculture venture. Cho and Slinger (1979) reveals that feed costs usually accounts for more than 50 % of the total production costs in any aquaculture venture. This implies that a reduction in feed cost will have a direct positive influence on the profitability of any aquaculture venture (Francis et al., 2005). As a result, the need to improve the performance of the existing feeds and development of new, more efficient, quality and affordable feeds has become necessary.

Intensification and commercialization of culture production systems is also closely linked to elevation of stressors for fish, physiologically and environmentally. Increased stress leads to possible suppression of the immune system which subsequently increases fish susceptibility to pathogens and infectious diseases. The aquaculture industry has been overwhelmed with its share of diseases and problems caused by viruses, bacteria, fungi, parasites and other undiagnosed and emerging pathogens. Disease is now a primary constraint to the culture of many aquatic species, impeding both economic and social development in many countries

(Bondad-Reantaso et al., 2012; Murray and Peeler, 2012; Stentiford et al., 2013; Rico et al., 2013).

Over the decades, the sector has faced significant problems with disease outbreaks and epidemics which caused significant economic losses (Kim et al., 2009; Murray et al., 2012; Kumar et al., 2014). The major causes of economic losses in tilapia culture are bacterial diseases mostly caused by the genera *Aeromonas, Pseudomonas, Vibrio, Flavobacterium, Edwardsiella and Streptococcus*. Epizootiologically, *Aeromonas hydrophila* is perhaps the most important cause of severe disease outbreaks in pond-cultured and wild freshwater fishes (Roberts, 2012). As pointed out, the use of chemical drugs, largely antibiotics to treat and prevent disease outbreaks has resulted to multiple negative impacts both environmentally and socially on human health (Reverter et al., 2014).

Some of the highlighted negative effects include increase in bacterial resistant strains which have rendered use of antibiotics ineffective (Cabello, 2006, 2013; Sapkota et al., 2008). The horizontal transfer of resistance determinants to human pathogens constitutes important threats to public

health and the presence of antibiotic residues in aquaculture products has been noted as one of the major factors that leads to customer reluctance in consumption of such products (Defoirdt et al., 2007).

There has been widespread ban on the use of antibiotics in animal production. In June 2012, the Government of South Korea announced a total ban on inclusion of non-therapeutic antibiotics in feed for livestock. The move by the Ministry of Food, Agriculture, Forestry and Fisheries (MFAFF) in S. Korea was similar to the ban on antibiotic growth promoters issued in January 2006 in the EU. Such restrictions are not uncommon with bans having been imposed by Nordic countries between 1986 and 1998 and also by Switzerland in 1999 (Johnson, 2011; Shane, 2014). This has subsequently led to trade impediments with a recent scenario in Nov. 2014 where United States Food and Drug Administration (FDA)'s refused shrimp imports from Vietnam and Malaysia due to contamination by antibiotics banned in the US.

Thus, the need for quality improvement in feeds coupled with the need for environmental friendly, affordable, safe and long-lasting disease

management methods in aquaculture have led to the exploration of different sources of feed additives for use in fish feeds. Barrow (2000) defined feed additives as non-nutritive ingredients or components of ingredients that are included in formulations to either influence the physical or chemical properties of the diet or affect fish performance or quality of resulting products. The feed additives improve the immunity, productivity and economic efficiency of fish via its improvement of fish body weight (Carnevali et al., 2006), weight gain (Venkat et al., 2004) feed conversion ratio and efficiency (Abdel and Mohamed, 2008).

Plant products, mainly from traditional medicinal herbs have been reported to stimulate appetite and promote weight gain, to act as immunostimulants modulating specific and non-specific immune responses, and to have antibacterial and anti-parasitic properties in fish and shellfish aquaculture (Yin et al., 2006, 2009; Zhang et al., 2009; Zaki et al., 2013; Lee et al., 2013; Talpur, 2014; Wang et al., 2015). Bricknell and Dalmo (2005) defined an immunostimulant as a naturally occurring compound that modulates the immune system by increasing the host's resistance against diseases that in most circumstances are caused by pathogens. This has been attributed to

their active molecules such as alkaloids, terpenoids, saponins and flavonoids (Reverter et al., 2014). As earlier observed by Harikrishnan et al. (2011) immunostimulants mainly facilitate the function of phagocytic cells. This is achieved through their increased bactericidal activities, stimulation of the natural killer cells, complemented lysozyme activity, and antibody responses in fish and shellfish which confer enhanced protection from infectious diseases.

Non-specific immune responses are the first line of defence against pathogen infestation in any organism; which consist of neutrophil activation, production of peroxidise and oxidative radicals and initiation of other inflammatory factors. Wang et al. (2015) found out that juvenile common carp fed on diets supplemented with *Rehmannia glutinosa* (plant used for Chinese herbal medicine) root powder had significantly higher growth performance, improved non-specific immune parameters such as lysozyme and leucocyte phagocytic activity, and had higher survival rates when challenged with *Aeromonas hydrophila.* Nile tilapia and common carp fed on diets supplemented with extracts from Chinese herbs were also found to

have enhanced resistance to *Aeromonas hydrophila* (Ardo et al., 2008; Yin et al., 2009; Tang et al., 2014)

Yucca schidigera, commonly known as Yucca, is a plant which belongs to the Agavaceae family, and is native to the South-Western United States and Mexico. Yucca has high levels of saponin and polyphenolics, with saponin being the major active compound (Cheeke, 1996). Consequently this has led to the wide exploitation of Yucca for the commercial extraction of saponin for use in human herbal medicinal therapy, pharmaceuticals and cosmetic industry, and as an additive in animal feeds (Piacente et al., 2005).

Yucca extracts have in the past been utilized in animal feeds where it was shown to affect the pH and the concentration of micro-organisms such as protozoa, bacteria and fungi in the rumen (Eryavuz and Dehority, 2014; Aregheore, 2005). Also in a study by (Santacruz-Reyes and Chien, 2009, 2012) to investigate the potential and effectiveness of Yucca extract in shrimp farming using effectiveness analysis and empirical modelling, it was elucidated that Yucca extract was a suitable candidate for water quality management (ammonia treatment) in aquaculture.

Saponins are amphipathic steroid or tritepenoid glycosides phenomenically grouped by the soap-like foaming they produce when shaken in aqueous solutions, and have one or more hydrophilic moiety combined with a lipophilic triterpene (Cheok et al., 2014). Naturally, triterpenes are hydrocarbons produced by plants and some insects, often strong smelling and have a protective role. Saponins have been found to have pharmaceutical properties of haemolytic, molluscicidal, anti-inflammatory, antifungal or antiyeast, antibacterial or antimicrobial, antiparasitic, antitumor, and antivirals (Sparg et al., 2004). Yucca saponins are steroidal saponins possessing spirostanol and furostanol aglycones.

Numerous studies have been carried out on the effects of saponin from different plants on various species of shellfish and fish. These include giant freshwater prawn, *Macrobrachium rosenbergii* (Yeh et al., 2006), common carp and Nile tilapia (Francis et al., 2001, 2002a, 2005) and juvenile striped catfish, *Pangasianodon hypophthalmus* (Güroy et al., 2014). In the current study, it was hypothesised that dietary Yucca meal supplementation is likely to improve growth performance, promote non-specific immune responses and enhance disease resistance against *A. hydrophila* bacterial challenge in

juvenile Nile tilapia, *O. niloticus*. Thus, the objectives of the current experiment was to determine the effects of dietary Yucca meal supplementation on the growth performance, whole body proximate composition, hematological parameters, non-specific immune responses and disease resistance against *A. hydrophila* bacterial challenge in juvenile Nile.

II. Materials and methods

a. Experimental diets

A powder form of De-Odorase®, a commercial product from Alltech Co. Ltd. (Alltech Korea, Seocho-Gu, Seoul, South Korea), containing 30% *Yucca schidigera* extract, and with a saponin concentration of 2.39% was utilized in this study (here after referred to as Yucca meal). Six diets were formulated to be isonitrogenous and isoenergetic and to contain 40% crude protein and 16.39 kJ available energy g^{-1} (NRC, 2011) for use in the experiment. A basal diet without Yucca meal supplementation (YMS_0) was used as control diet, and five other diets were prepared by supplementing cellulose with dietary Yucca meal in the formulated diets at 0.1% ($YMS_{0.1}$), 0.3% ($YMS_{0.3}$), 0.5% ($YMS_{0.5}$), 1% ($YMS_{1.0}$) and 2% ($YMS_{2.0}$) on a dry-weight basis.

Fish meal, wheat gluten meal, spray dried blood meal, and dehulled soybean meal were used as protein source, wheat flour and corn starch as carbohydrate source, and soybean oil and fish oil as lipid source. Essential vitamins and minerals were provided in the additional vitamin and mineral

premixes. Proximate analyses of the formulated experimental diets are shown in Table 1.

Procedures for diet preparation and storage were followed as previously described by Bai and Kim (1997). Briefly, diets were prepared by mixing all the dry, powder ingredients in an electric mixer, followed by the addition of oil and water at a ratio of 50 mL water/100 g feed. The mixture was formed into dough with soft, smooth consistency and moist pellets were made by passing the dough through a screw-type laboratory pelletizing machine. The pellets were then air dried for approximately 48 hours. After drying, the pellets were broken up using a grinding machine, sieved into the proper diameter size, packaged in zip-lock polythene bags, labelled and stored at −20˚C until use for feeding trial

b. Experimental fish and feeding trial

The experiment was carried out at the Feeds and Foods Nutrition Research Centre (FFNRC), Pukyong National University, Busan, South Korea.

Table 1. Ingredient composition and proximate analysis of the experimental diets.

Ingredients	Diets					
	YMS_0	$YMS_{0.1}$	$YMS_{0.3}$	$YMS_{0.5}$	$YMS_{1.0}$	$YMS_{2.0}$
Fish meal[1]	10.0	10.0	10.0	10.0	10.0	10.0
Wheat gluten meal[1]	10.0	10.0	10.0	10.0	10.0	10.0
Blood meal spray dried[1]	11.4	11.4	11.4	11.4	11.4	11.4
Dehulled soybean meal[1]	23.1	23.1	23.1	23.1	23.1	23.1
Wheat flour[1]	22.2	22.2	22.2	22.2	22.2	22.2
Corn starch[1]	8.6	8.6	8.6	8.6	8.6	8.6
Soybean oil[1]	3.4	3.4	3.4	3.4	3.4	3.4
Fish oil[2]	3.4	3.4	3.4	3.4	3.4	3.4
Vitamin Premix[3]	2.0	2.0	2.0	2.0	2.0	2.0
Mineral Premix[4]	2.0	2.0	2.0	2.0	2.0	2.0
Calcium phosphate[5]	2.0	2.0	2.0	2.0	2.0	2.0
Yucca meal[6]	0.0	0.1	0.3	0.5	1.0	2.0
Cellulose[7]	2.0	1.9	1.7	1.5	1.0	0.0
Proximate analysis(% of DM)						
Moisture	9.20	9.25	10.2	9.52	9.92	10.8
Ash	6.69	6.45	6.28	6.36	6.39	6.24
Lipid	6.32	6.59	6.42	6.65	6.63	6.56
Protein	41.4	41.2	40.2	40.3	40.8	40.5

[1] Suhyup Feed Company, Uiryeong, Korea
[2] E-Wha Oil Co. Ltd, Busan, Korea
[3]Contains (as mg/kg mixture): Ascorbic acid, 100; dl-Calcium Pantothenate, 150; Choline bitartrate, 3,000; Inositol, 150; Menadione, 6; Niacin, 150; Pyridoxine.HCl, 15; Riboflavin, 30; Thiamine mononitrate, 15; dl-α-Tocopherol Acetate, 100; Retinyl acetate, 6; Biotin, 1.5; Folic acid, 5.4; Cobalamin, 0.06; Cholecalciferol, 2.4.
[4]Contains (as mg/kg mixture): NaCl, 35.88; MgO, 11.50; $NaH_2PO_4.2H_2O$, 20.00; KCl, 29.86; K_2HPO_4, 92.80; $ZnSO_4.7H_2O$, 1.60; Fe-citrate, 2.53; $CaCO_3$, 17.32; $AlCl_3.6H_20$, 0.14; KIO_3, 0.08; $CuCl_2$, 0.17; $MnSO_4.H_2O$, 0.54; $CoCl_2.6H_2O$, 0.54.
[5]Sigma-Aldrich Korea, Yongin, Korea
[6](as De-Odorase®) Alltech Korea, Seocho-Gu, Seoul, South Korea
[7]United States Biochemical, Cleveland, OH 44122

Juvenile Nile tilapias were obtained from Kunsan University Aquaculture Farm. Prior to the start of the feeding trial, the Nile tilapia were fed on a Yucca meal free basal formulated diet, twice daily to apparent satiation for a week to acclimate them to the experimental diets and conditions. At the start of the experiment, twenty fish with an initial weight averaging 3.8 ± 0.05 g (mean ± SD) were randomly selected and distributed into each of the 18 tanks. Each tank was then randomly assigned one of the three replicates of the six dietary treatments. Fish were fed on twice daily (0900hr and 1800 hr), six days a week for ten weeks at a fixed rate of 4% of wet body weight (BW/day). Total fish weight in each aquarium was determined every fortnight and feeding rates was adjusted accordingly.

The feeding trial was conducted using a semi-recirculating system with 18 tanks (40L) receiving filtered seawater at the rate of 2 L/min from the central tank. Supplemental aeration was provided to maintain the dissolved oxygen near saturation during the feeding trial. Water temperature and pH during the experiment were maintained at 27±0.1°C and 7.45±0.3, respectively. Fifty percent of water was exchanged everyday using pre-heated filtered tap water. Siphoning was conducted daily to remove fish

wastes in the rearing tanks. Mortality was also monitored daily and any dead fish was removed, weighed and recorded accordingly. A photoperiod of 12hr light: 12 hr dark was used throughout the experiment

c. Sample collection and analysis

i. Growth Performance

At the end of the feeding trial, fish were starved for 24 hours, and the total number and weight of fish in each tank was determined for calculation of weight gain (WG), feed efficiency (FE), specific growth rate (SGR), protein efficiency ratio (PER) and survival rate (SR).

ii. Proximate analysis

A total of 15 fish randomly selected from the initial pool at the beginning of the feeding trial, and 4 fish per tank at the end of the feeding trial, were sampled for freeze-drying and subsequent whole body proximate analysis. All proximate analysis for the formulated diets and whole body composition were conducted in accordance to the standard procedures (AOAC, 2000). Samples of fish and diets were dried to constant weight at 105^{o}C to determine their moisture content. Crude ash was determined by incineration

of the samples at 550^{o}C for 3.5 hours in a muffle furnace. Protein content was determined using the Kjeldahl method (N x 6.25) after acid digestion. Crude lipid was determined using the soxhlet extraction method which utilizes ether extraction using the Soxtec system 1046 (Tecator AB, Hoganas, Sweden) after freeze drying the samples for 12 hours.

iii. Haematological parameters

At the end of the feeding trial, six fish per tank were randomly captured, anesthetized with MS222 at a concentration of 100mg/L and blood samples collected from the caudal fin using heparinized syringes for determination of respiratory burst (NBT). After the above mentioned measurement with whole blood, plasma was separated by centrifugation at 5000 x g for 10 minutes and stored at -70^{o}C as separate aliquots for determination of biochemical parameters including plasma total protein, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), cholesterol and glucose. These were measured by a chemical analyzer (Fuji DRI-CHEM 3500i, Fuji Photo Film Ltd, Tokyo, Japan)

iv. Non-specific immune responses

Another set of blood samples of the same fish was taken without heparin and allowed to clot for 30 minutes. The serum was then separated by centrifugation at 5,000 x g for 10 minutes and stored at -70^{o}C for the analysis of the non-specific immune responses including lysozyme and superoxide dismutase (SOD) activities.

A turbidometric assay was used for the determination of serum lysozyme in accordance with Hultmark et al., (1980) method, though slightly modified. Micrococcus lysodeikticus (0.75mg ml^{-1}) was suspended in sodium phosphate buffer (0.1 M, pH 6.4), 200 μl of suspension was placed in each well of 96-well plates, and 20 μl serum was subsequently added. The reduction in absorbance of the samples was recorded at 570 nm after incubation at room temperature for 0 and 30 minutes in a microplate reader (UVM 340, Biochrom, Cambridge, UK). A reduction in absorbance of 0.001 min^{-1} was regarded as one unit of lysozyme activity.

Superoxide dismutase (SOD) activity was measured by the percentage of reaction inhibition rate of the enzyme with WST-1 (Water Soluble Tetrazolium dye) substrate and xanthine oxidase using an SOD Assay kit (Enzo ADI-900-157, Enzo Life Sciences, Inc.) in accordance with

manufacturer's instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the coloured product of WST-1 reaction with superoxide) after 20 minutes of reaction time at 37^{o}C. The inhibition percentage was normalized by mg protein and presented as SOD activity units

Oxidative radical production by phagocytosis during respiratory burst was measured through the Nitro Blue Tetrazolium (NBT) assay described by (Anderson and Siwicki, 1995). Briefly, blood and NBT (0.2%, Sigma-Aldrich, St. Louis, MO, USA) were mixed in equal proportion (1:1 ratio) and incubated for 30 minutes at room temperature. Then 50 µl was taken out and dispensed into glass tubes. One millilitre of dimethylformamide (Sigma –Aldrich) was added and centrifuged at 2,000 x g for 5 minutes. Finally, the optical density of supernatant was measured at 540 nm using spectrophotometer (Genesys 10UV, Rochester, NY, USA). Dimethylformamide was used as the blank.

d. Bacterial challenge test

At the end of the feeding trial, fish were redistributed based on their earlier dietary treatment groups in 40L tanks as triplicate groups of 5 fish and allowed to stabilize for 24 hours. Bacteria species, *Aeromonas hydrophila* were obtained from the Department of Biotechnology, Pukyong National University, Busan, Korea. Fish from each experimental group were injected intraperitoneally with 0.1 ml of the bacterial suspension per fish at a concentration of 2 x 10^7colony-forming units (CFU) ml^{-1}. After twenty four hours of challenge, the flow rate was established (2L/min) and mortality of the challenged fish from each tank was monitored up to 14 days.

e. Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using SAS Program Version 9.1 for Windows to test the effects of the dietary treatment with Yucca meal across the treatment groups. When a significant treatment effect was observed, a Least Significant Difference (LSD) test was used to compare the treatment means. Treatment effects were considered at $P<0.05$ level of significance. The breakpoint for optimum

maximum and upper limit dietary Yucca meal inclusion level was estimated by polynomial and broken line linear regression models.

III. Results

a. Growth performance

Growth performance and feed efficiency of Nile tilapia fed on the different experimental diets are shown in Table 2. At the end of the 10 weeks feeding trial; weight gain (WG) of fish fed on diet $YMS_{0.1}$ was significantly higher than those of fish fed on $YMS_{0.5,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets ($P<0.05$). However, there was no significant difference in WG among fish fed on diet $YMS_{0,}$ $YMS_{0.1}$ and $YMS_{0.3}$ diets. Equally, there was no significant difference in WG among fish fed on diet $YMS_{0.5,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets. The graphical presentation of weight gain is as shown in Figure 1.

Specific growth rate (SGR) of fish fed on diet $YMS_{0.1}$ was significantly higher than those of fish fed on $YMS_{0.5,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets ($P<0.05$). However, there was no significant difference in SGR among fish fed on diet $YMS_{0,}$ $YMS_{0.1,}$ and $YMS_{0.3.}$ Equally, there was no significant difference in SGR among fish fed on $YMS_{0.5,}$ $YSM_{1.0}$ and $YMS_{2.0}$ diets. The graphical presentation of specific growth rate is as shown in Figure 2.

Table 2. Growth performance of juvenile Nile tilapia fed on the experimental diets for 10 weeks[1]

	Diets[2]						Pooled
	YMS_0	$YMS_{0.1}$	$YMS_{0.3}$	$YMS_{0.5}$	$YMS_{1.0}$	$YMS_{2.0}$	SEM[3]
WG (%) [4]	368^{ab}	384^{a}	365^{ab}	347^{b}	346^{b}	347^{b}	4.23
SGR (%) [5]	2.75^{ab}	2.82^{a}	2.74^{ab}	2.67^{b}	2.67^{b}	2.67^{b}	0.02
FE (%)[6]	72.4^{b}	76.4^{a}	73.7^{ab}	70.7^{b}	70.7^{b}	70.6^{b}	0.62
PER[7]	0.50^{a}	0.54^{a}	0.52^{a}	0.51^{a}	0.53^{a}	0.50^{a}	0.06
Survival (%) [8]	95.0^{a}	98.3^{a}	100^{a}	98.3^{a}	98.3^{a}	96.7^{a}	0.73

[1]Values are means from triplicate groups of fish where values in each row with a different superscript are significantly different (p 0.05).

[2] Refer to Table 1

[3] Pooled standard error of mean: SD/√n

[4]Weight gain (WG, %) = (final weight-initial weight)×100 / initial weight

[5]Specific growth rate (SGR, %) = (ln final weight – ln initial weight) X 100 / days

[6]Feed Efficiency (FE, %) = (wet weight gain / dry feed intake) x 100

[7]Protein Efficiency Ratio (PER) = (wet weight gain/protein intake)

[8]Survival rate (%) = (total fish-dead fish) × 100 / total fish

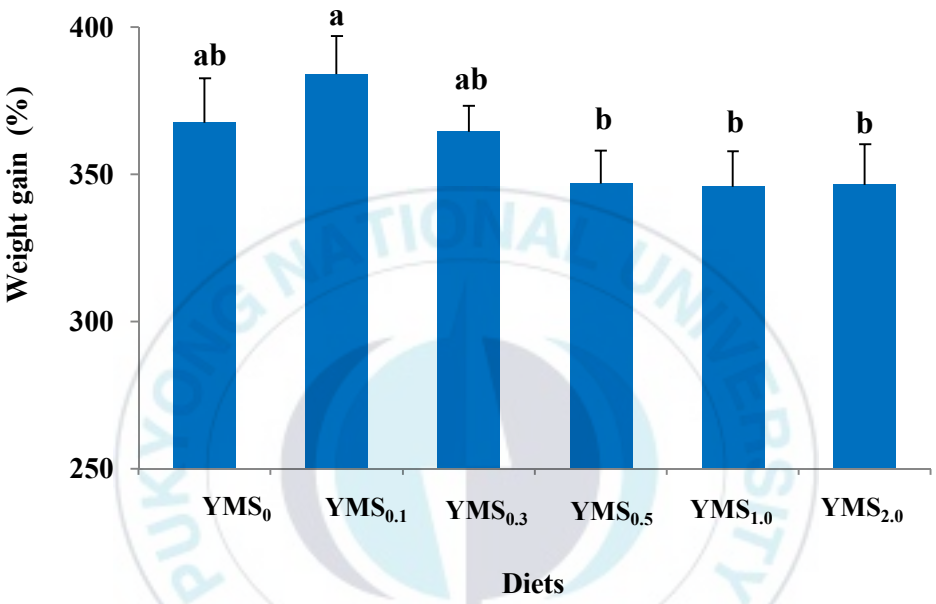

Fig. 1. Weight gain of juvenile Nile tilapia fed on the experimental diets for 10 weeks

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

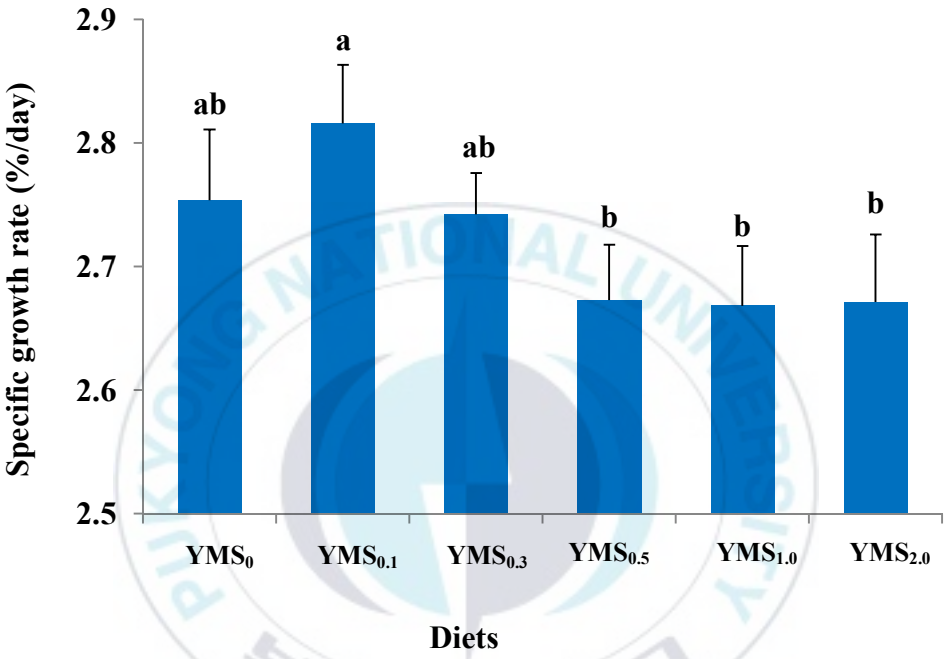

Fig. 2. Specific growth rate of the juvenile Nile tilapia fed on the experimental diets for 10 weeks.

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

Feed efficiency (FE) of fish fed on diet $YS_{0.1}$ was significantly higher than those of fish fed on $YMS_{0,}$ $YMS_{0.5,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets ($P<0.05$). However, there was no significant difference in FE among fish fed on diet YMS_0 and $YMS_{0.1}$. Equally, there was no significant difference in FE among fish fed on YMS_0, $YMS_{0.5,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets. The graphical presentation of feed efficiency is as shown in Figure 3. There was no significant difference in protein efficiency ratio (PER) and survival rate across all the treatment groups. The graphical presentation of protein efficiency ratio and survival rate is as shown in Figure 4 and Figure 5 respectively.

Polynomial regression analysis of weight gain indicated the optimum maximum level of Yucca meal inclusion for improved performance in Nile tilapia as 0.14% in the diet as shown in Figure 6. **($y = -233.9 * x^2 + 63.59 * x + 372.0$ $R^2=0.829$)**

Polynomial and broken line linear regression analysis of weight gain indicated the upper limit level of Yucca meal inclusion for improved performance in Nile tilapia as 0.58 % in the diet as shown in Figure 7. **($y = -217.4 * x^2 + 54.167 * x^2 + 378.03$ $R^2=0.6187$)**

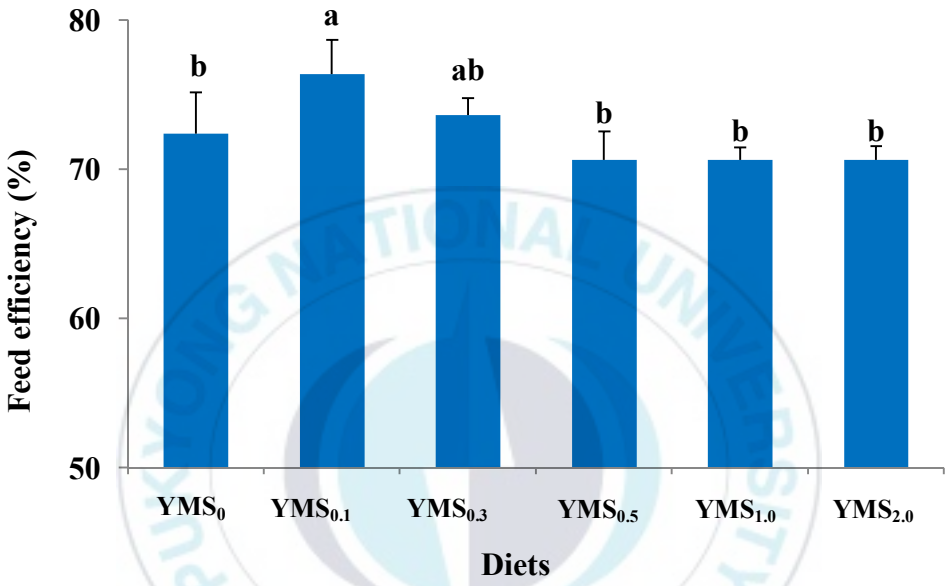

Fig. 3. Feed efficiency of juvenile Nile tilapia fed on the experimental diets for 10 weeks

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

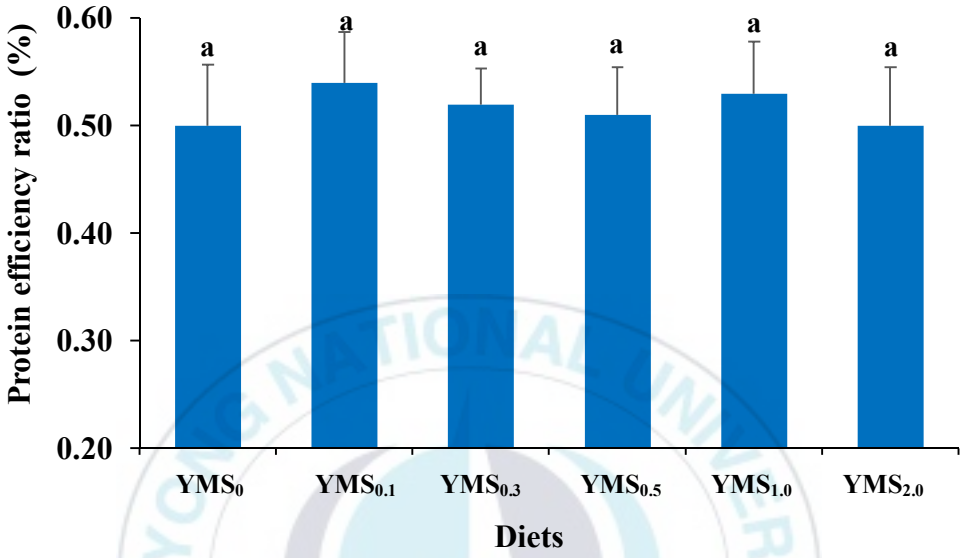

Fig. 4. Protein efficiency ratio of juvenile Nile tilapia fed on the experimental diets for 10 weeks

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

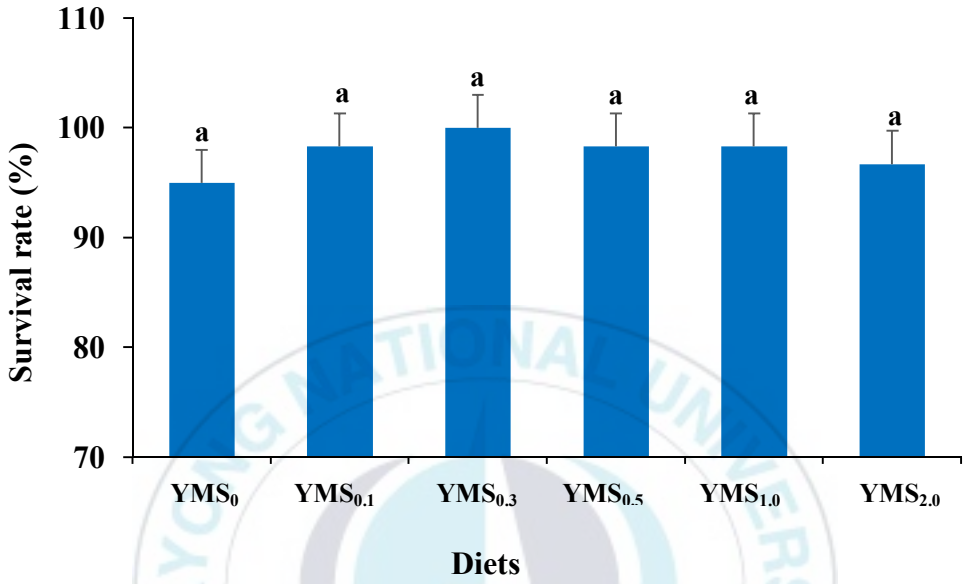

Fig. 5. Survival rate of the juvenile Nile tilapia fed on the experimental diets for 10 weeks

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

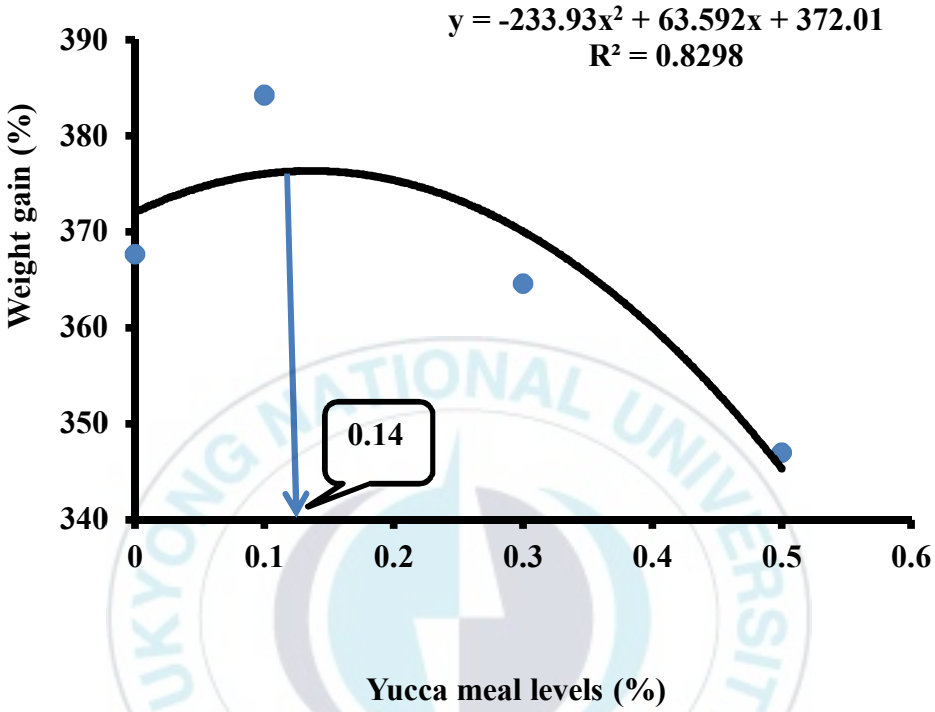

Fig. 6. Polynomial regression analysis of weight gain.

Each point represents the mean of three triplicate groups

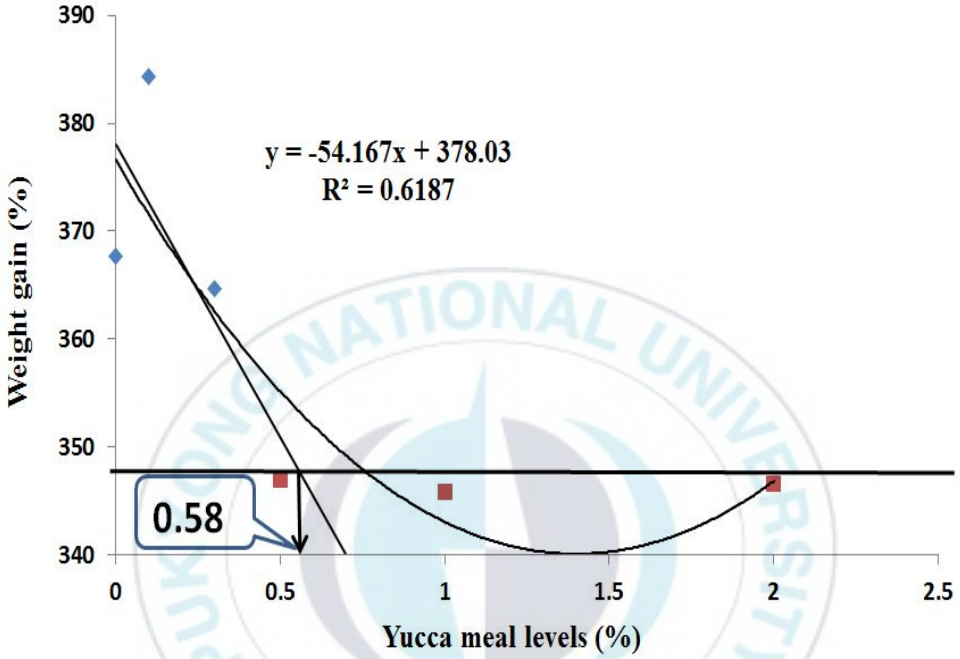

Fig. 7. Polynomial & Broken line linear regression analysis of weight gain

Each point represents the mean of three triplicate group

b. Proximate composition

Whole body proximate composition of fish fed on the experimental diets is as shown in Table 3. At the end of the 10 weeks feeding trial; fish fed on diet $YMS_{0.1}$ had significantly higher whole body protein content than did fish fed on $YMS_{0,}$ $YMS_{1.0}$ and $YMS_{2.0}$. There was however no significant difference in whole body protein content among fish fed on $YMS_{0.1,}$ $YMS_{0.3}$ and $YMS_{0.5}$ diets. Equally there was no significant difference in whole body protein among fish fed on $YMS_{0,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets.

Whole body ash content increased significantly with increasing Yucca meal inclusion levels. Fish fed on diet $YMS_{2.0}$ had significantly higher whole body ash content than did fish fed on diet $YMS_{0,}$ $YMS_{0.1,}$ $YMS_{0.3}$ and $YMS_{0.}5$. There was however no significant difference in whole body ash content among fish fed on $YMS_{2.0,}$ and $YMS_{1.0}$ diets. Equally there was no significant difference in whole body ash content among fish fed on $YMS_{0,}$ $YMS_{0.1,}$ $YMS_{0.3}$ and $YMS_{0.5}$ diets. Whole body crude lipid and moisture contents among fish fed on all the experimental diets had no significant difference. The graphical presentation of whole body protein, lipid, ash and moisture content is as shown in Figure 8,9,10 and 11, respectively.

Table 3. Whole-body proximate composition of juvenile Nile tilapia fed on the experimental diets for 10 weeks (% dry matter basis)[1]

	Diets[2]						Pooled
Content (%)	YMS_0	$YMS_{0.1}$	$YMS_{0.3}$	$YMS_{0.5}$	$YMS_{1.0}$	$YMS_{2.0}$	SEM[3]
Protein	57.6^{bc}	60.9^{a}	58.3^{ab}	58.3^{ab}	56.8^{bc}	55.9^{c}	0.50
Lipid	6.48	6.57	6.49	6.60	6.56	6.61	0.03
Ash	13.8^{b}	14.2^{b}	14.1^{b}	13.7^{b}	14.5^{ab}	15.3^{a}	0.18
Moisture	74.3^{a}	74.0^{a}	73.6^{a}	73.25^{a}	73.4^{a}	74.7^{a}	0.21

[1]Values are means from triplicate groups of fish where values in each row with a different superscript are significantly different (p 0.05).

[2]Refer to Table 1

[3]Pooled standard error of mean: SD/√n.

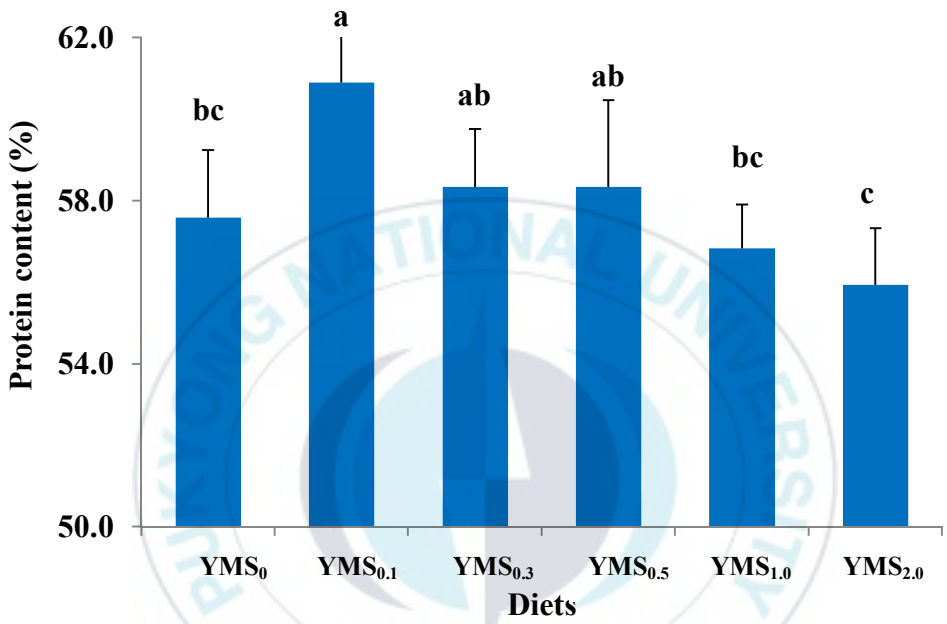

Fig. 8. Whole-body protein content of juvenile Nile tilapia fed on the experimental diets for 10 weeks.

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

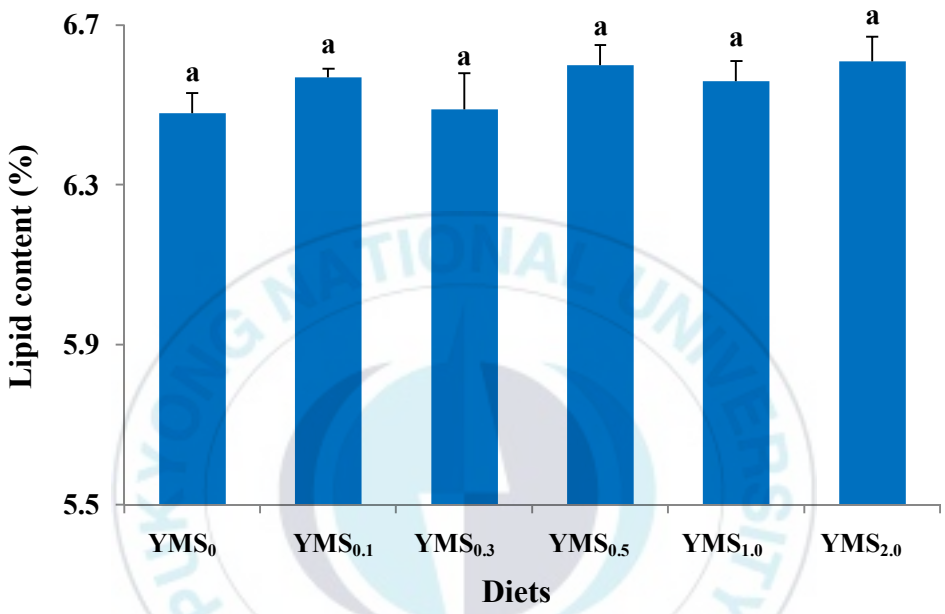

Fig. 9. Whole-body lipid content of juvenile Nile tilapia fed on the experimental diets for 10 weeks

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

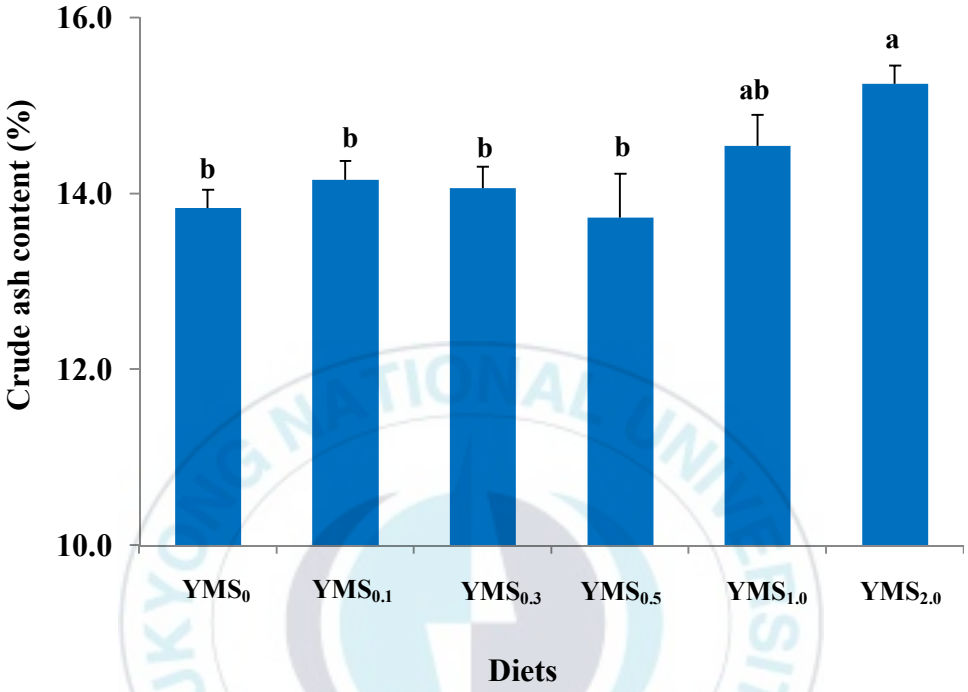

Fig. 10. Whole-body ash content of juvenile Nile tilapia fed on the experimental diets for 10 weeks

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

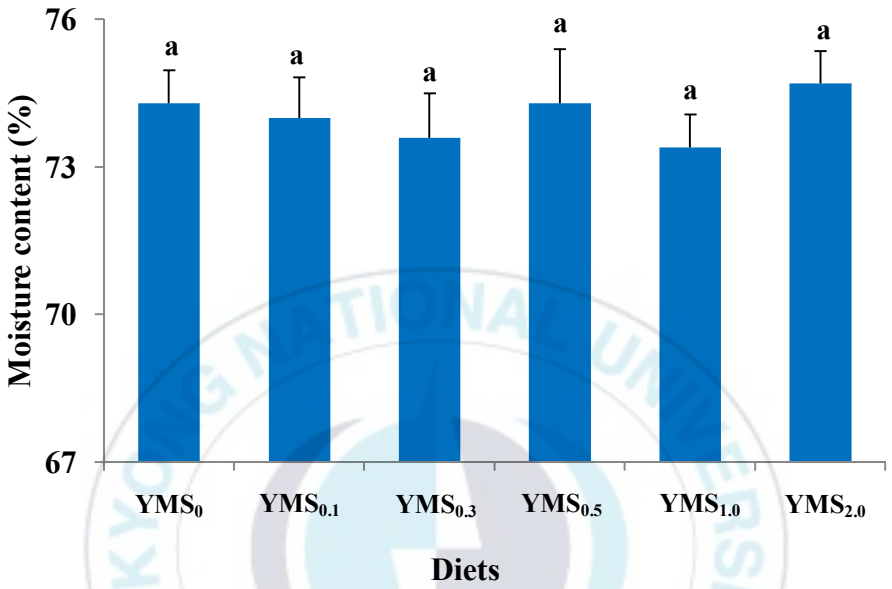

Fig. 11. Whole-body moisture content of juvenile Nile tilapia fed on the experimental diets for 10 weeks.

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

c. Hematological parameters

Hematological parameters of juvenile Nile tilapia fed on the experimental diets for 10 weeks are as shown in Table 4. Glutamic oxaloacetic transaminase (GOT) levels were significantly higher in fish fed on diets $YMS_{0.1,}$ $YMS_{0.3}$ and $YMS_{1.0}$ than those of fish fed on YMS_0 and $YMS_{2.0}$ diets ($P<0.05$). However, there was no significant difference in GOT levels among fish fed on diets $YMS_{0.1,}$ $YMS_{0.3,}$ $YMS_{0.5}$ and $YMS_{1.0}$. Equally, there was no significant difference in GOT levels among fish fed on YMS_0 and $YMS_{2.0}$ diets. The graphical presentation of GOT levels across the treatment groups is as shown in Figure 12.

Glutamic pyruvic transaminase (GPT) levels in fish fed on control diet (YMS_0) were significantly lower than those of fish fed on all the other experimental diets. However, there was no significant different in GPT levels in fish fed on all the Yucca meal supplemented diets. The graphical presentation of GOT levels across the treatment groups is as shown in Figure 13.

Table 4. Biochemical parameters of juvenile Nile tilapia fed on the experimental diets for 10 weeks[1]

	Diets[2]						Pooled
	YMS_0	$YMS_{0.1}$	$YMS_{0.3}$	$YMS_{0.5}$	$YMS_{1.0}$	$YMS_{2.0}$	SEM[5]
GOT (U/I)[3]	34.3^{b}	41.5^{a}	43.0^{a}	40.6^{ab}	41.3^{a}	37.6^{b}	0.45
GPT (U/I)[4]	9.00^{b}	11.0^{a}	11.5^{a}	11.5^{a}	11.2^{a}	11.5^{a}	0.12
T-Cholesterol(mg/dl)	167^{b}	174^{ab}	191^{ab}	190^{a}	173^{ab}	169^{b}	0.45
Glucose(mg/dl)	48.3^{a}	52.7^{a}	50.7^{a}	47.3^{a}	46.7^{a}	47.0^{a}	0.71
T-Protein(g/dl)	3.00^{a}	3.03^{a}	3.17^{a}	3.07^{a}	3.17^{a}	2.93^{a}	0.01

[1]Values are means from triplicate groups of fish where values in each row with a different superscript are significantly different (p 0.05).

[2] Refer to Table 1

[3]GOT (U/L): Glutamatic oxaloacetic transaminase

[4] GPT (U/L): Glutamatic pyruvic transaminase

[5]Pooled standard error of mean: SD/√n.

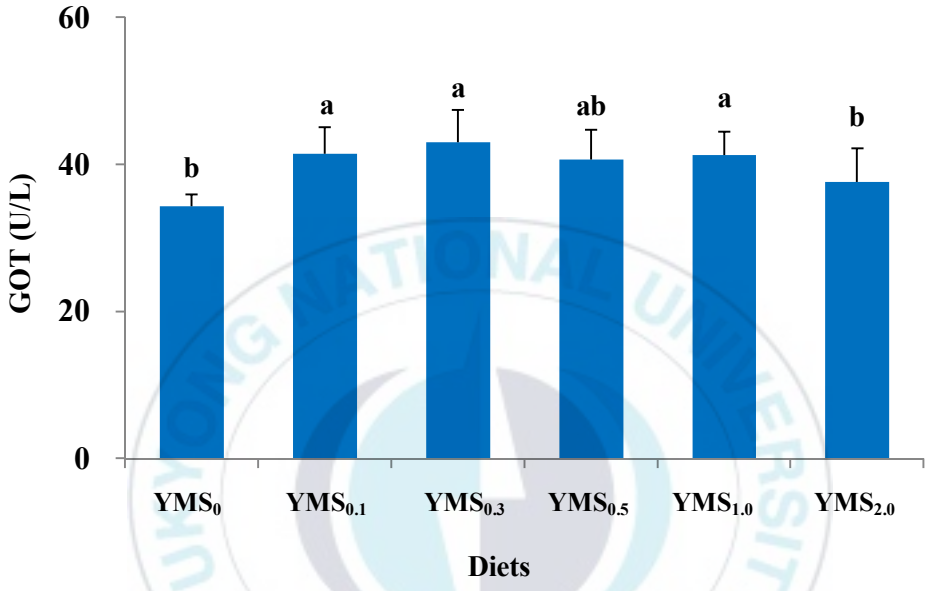

Fig. 12. Glutamic oxaloacetic transaminase (GOT) activity of juvenile Nile tilapia fed on the experimental diets for 10 weeks.

$YMS_{0,}$ $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

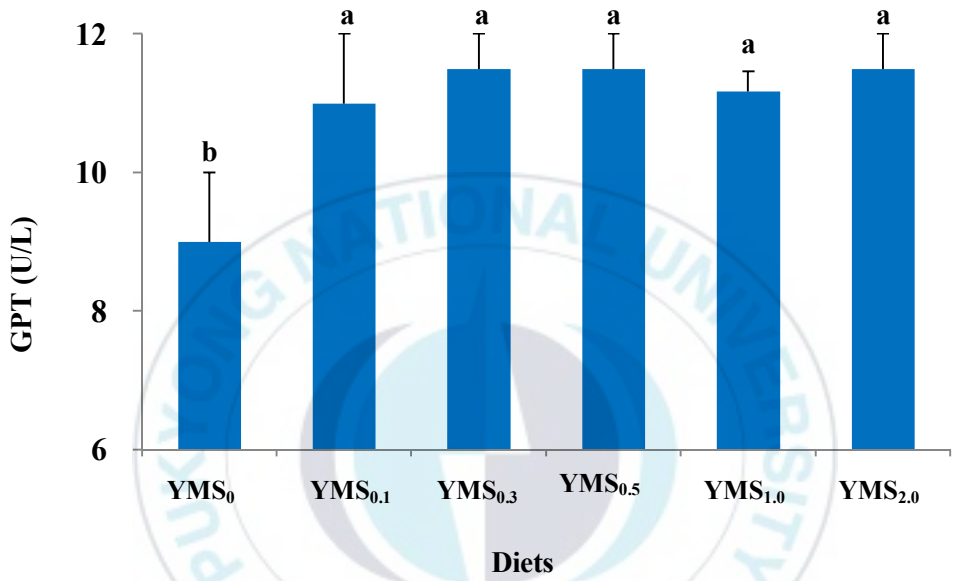

Fig. 13. Glutamic pyruvic transaminase (GPT) activity of juvenile Nile tilapia fed on the experimental diets for 10 weeks. $YMS_{0,}$ $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

Fish fed on diet $YMS_{0.5}$ had significantly higher total cholesterol than those of fish fed on YMS_0 and $YMS_{2.0}$ diets. However, there was no significant difference in cholesterol levels among fish fed on diets $YMS_{0.1,}$ $YMS_{0.3,}$ $YMS_{0.5}$ and $YMS_{1.0}$. Equally, there was no significant difference in cholesterol levels among fish fed on YMS_0, $YMS_{0.1,}$ $YMS_{0.3,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets. The graphical presentation of cholesterol levels across the treatment groups is as shown in Figure 14.

There was no significant difference in plasma glucose and total protein levels among fish fed on all the experimental diets. The graphical presentation of glucose and total protein levels across the treatment groups is as shown in Figure 15 and Figure 16, respectively.

d. Non-specific immune parameters

Non-specific immune responses of the fish fed on the experimental diets for 10 weeks are as shown in Table 5.

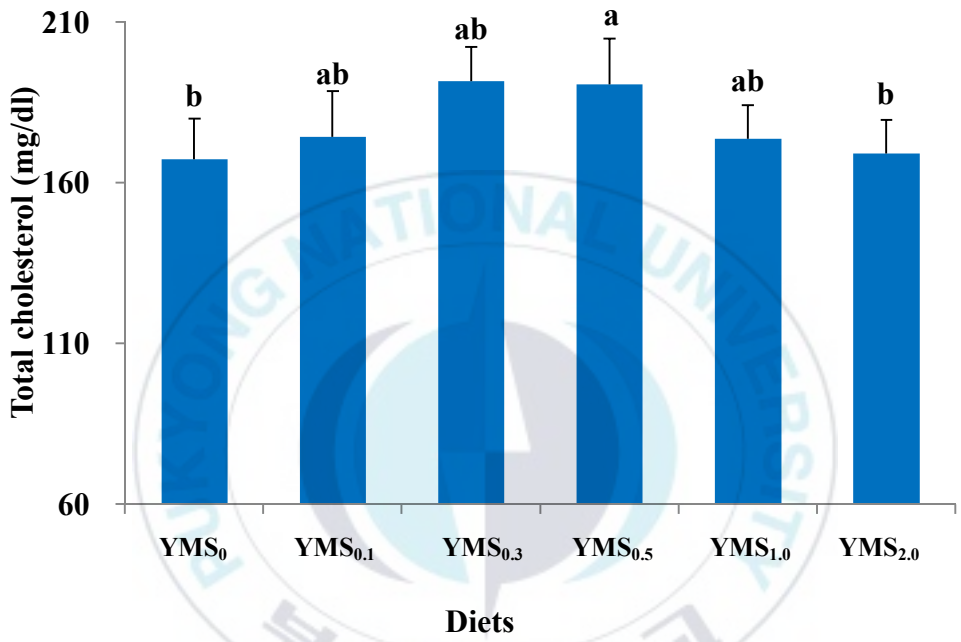

Fig. 14. Total cholesterol of juvenile Nile tilapia fed on the experimental diets for 10 weeks.

$YMS_{0,}$ $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

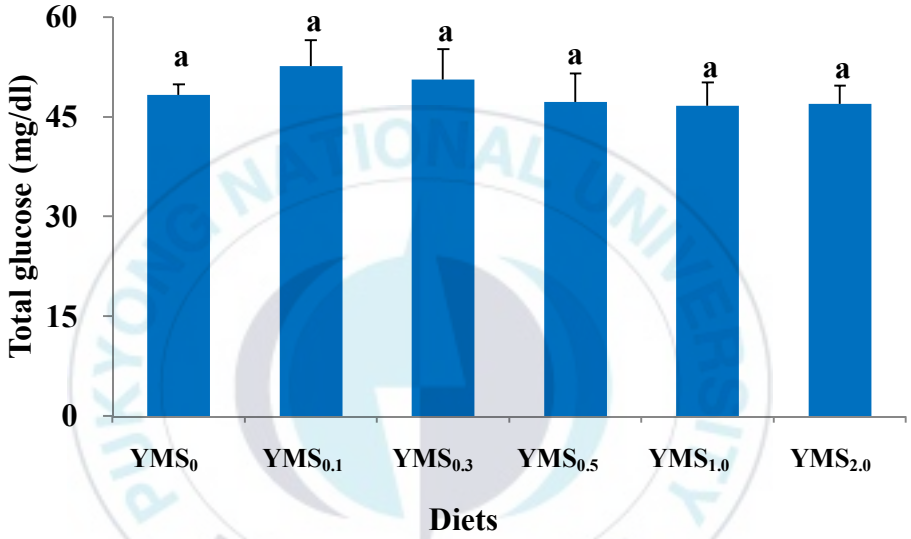

Fig. 15. Total glucose of juvenile Nile tilapia fed on the experimental diets for 10 weeks.

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

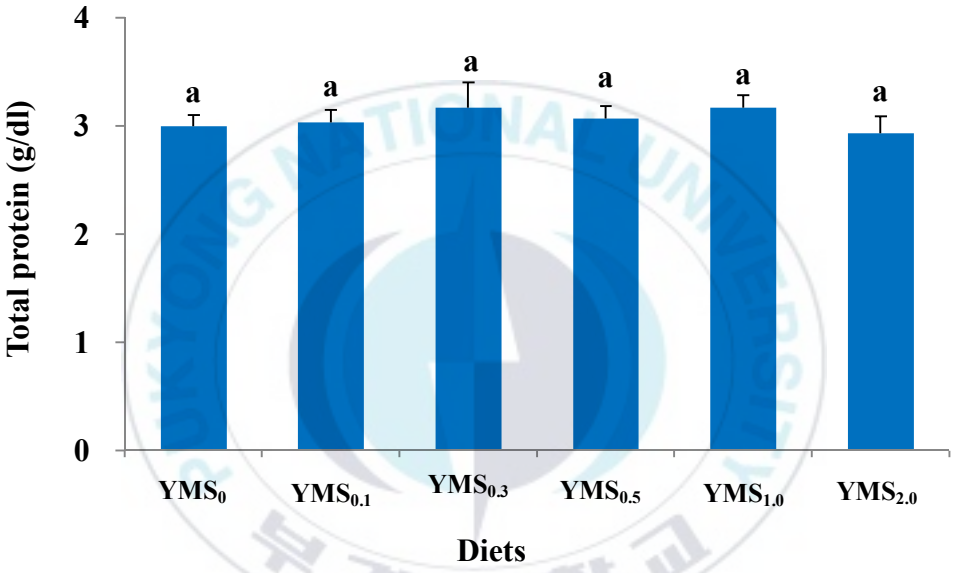

Fig. 16. Total protein of juvenile Nile tilapia fed on the experimental diets for 10 weeks

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively

Table 5. Non-specific immune responses of juvenile Nile tilapia fed on the experimental diets for 10 weeks[1]

	Diets[2]						Pooled
	YMS_0	$YMS_{0.1}$	$YMS_{0.3}$	$YMS_{0.5}$	$YMS_{1.0}$	$YMS_{2.0}$	SEM[5]
Lysozyme	0.56^{b}	0.68^{a}	0.64^{ab}	0.53^{b}	0.54^{b}	0.59^{ab}	0.03
NBT[3]	0.97^{ab}	1.02^{a}	1.01^{a}	0.98^{a}	0.91^{ab}	0.84^{b}	0.03
SOD[4]	24.4^{c}	26.8^{a}	26.7^{ab}	25.4^{bc}	25.5^{bc}	25.1^{c}	0.39

[1]Values are means from triplicate groups of fish where values in each row with a different superscript are significantly different (p 0.05).

[2] Refer to Table 1

[3] NBT: Nitro blue tetrazolium

[4]SOD: Superoxide dismutase

[5]Pooled standard error of mean: SD/√n.

Lysozyme activity of fish fed on diet $YMS_{0.1}$ was significantly higher than of fish fed on YMS_0, $YMS_{0.5}$ and $YMS_{2.0}$ diets ($P<0.05$). However, there was no significant difference in lysozyme activity among fish fed on $YMS_{0.1,}$ $YMS_{0.3}$ and $YMS_{2.0}$ diets. Equally, there was no significant difference in lysozyme activity among fish fed on $YMS_{0,}$ $YMS_{0.3}$ $YMS_{0.5,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets. The graphical presentation of lysozyme activity across the treatment groups is as shown in Figure 17.

Nitro blue tetrazolium (NBT) absorbances of fish fed on diets $YMS_{0.1}$, $YMS_{0.3}$ and $YMS_{0.5}$ were significantly higher than those of fish fed on diet $YMS_{2.0}$ ($P<0.05$). However, there was no significant difference in NBT absorbance among fish fed on $YMS_{0,}$ $YMS_{0.1,}$ $YMS_{0.3,}$ $YMS_{0.5}$ and $YMS_{1.0}$ diets. Equally, there was no significant difference in NBT absorbances among fish fed on $YMS_{0,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets. The graphical presentation of NBT absorbance across the treatment groups is as shown in Figure 18.

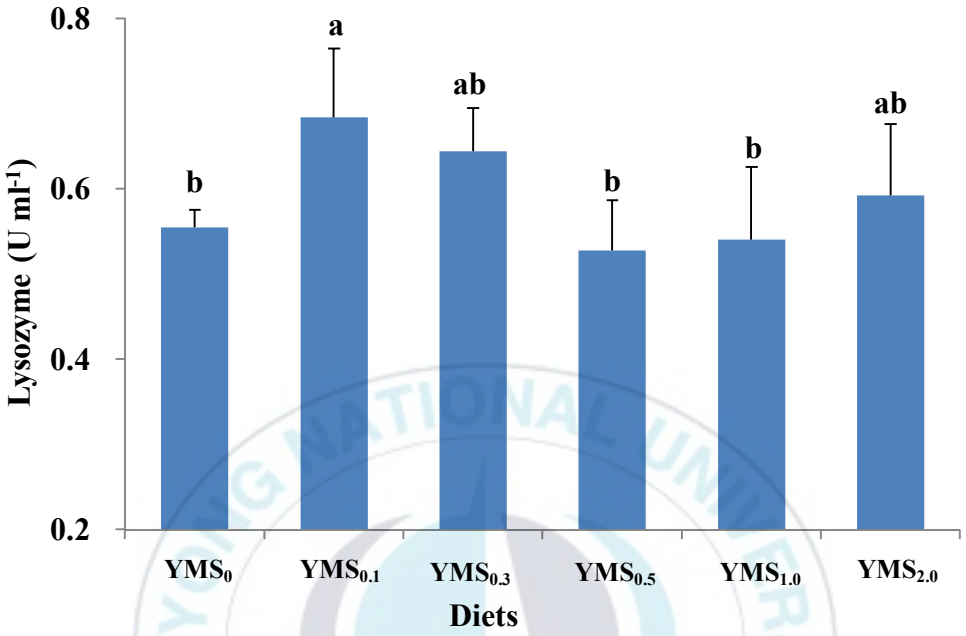

Fig. 17. Lysozyme activity of juvenile Nile tilapia fed on the experimental diets for 10 weeks.

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively.

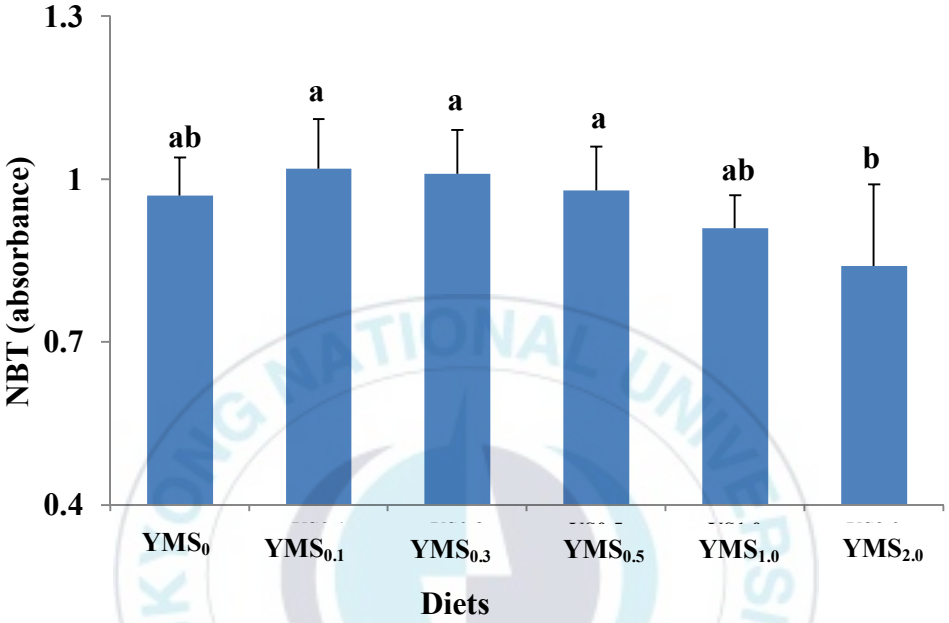

Fig. 18. Nitro blue tetrazolium activity of juvenile Nile tilapia fed on the experimental diets for 10 weeks

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively

Superoxide dismutase (SOD) inhibition of fish fed on diet $YMS_{0.1}$ was significantly higher than those of fish fed on diets $YMS_{0,}$ $YMS_{0.5,}$ $YMS_{1.0}$ and $YMS_{2.0}$ ($P<0.05$). However, there was no significant difference in SOD inhibition among fish fed on $YMS_{0.3,}$ $YMS_{0.5,}$ and $YMS_{1.0}$ diets. Equally, there was no significant difference in SOD inhibition among fish fed on $YMS_{0,}$ $YMS_{0.5,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets. The graphical presentation of SOD inhibition across the treatment groups is as shown in Figure 19.

Bacterial challenge test

Cumulative survival rate due to *Aeromonas hydrophila* of juvenile Nile tilapia fed on the experimental diets is shown in Figure 20. During *A. hydrophila* challenge test, the first mortality occurred on day 2. After the 14 days of the challenge test, cumulative survival rate of fish fed on diet $YMS_{0.1}$ was significantly higher than those of fish fed on $YMS_{0,}$ $YMS_{1.0}$ and $YMS_{2.0}$ diets ($P<0.05$). However, there was no significant difference in cumulative survival rates among fish fed on $YMS_{0.1,}$ $YMS_{0.3}$ and $YMS_{0.5}$ diets, after 14 days of challenge test.

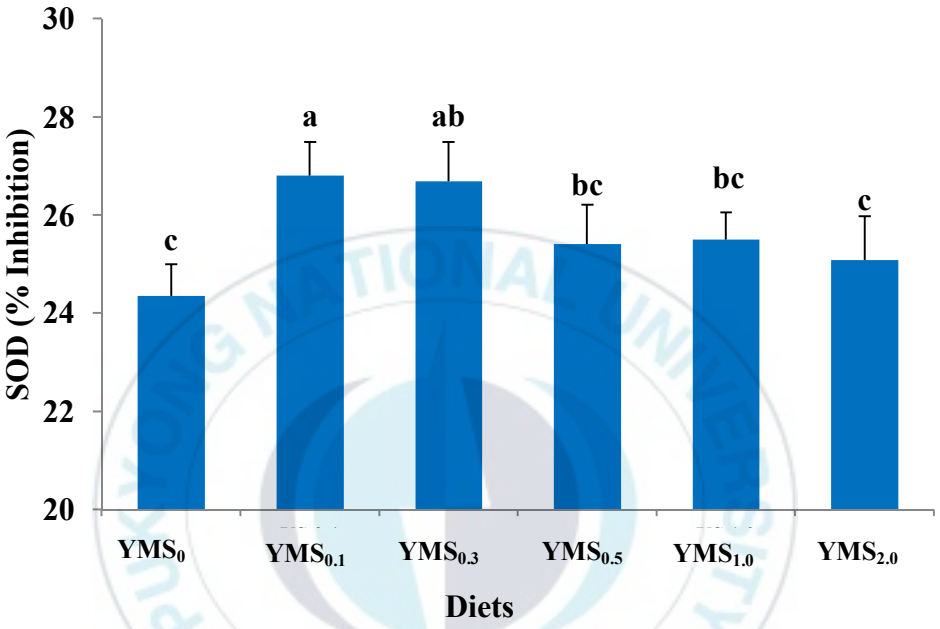

Fig. 19. Superoxide dismutase inhibition of juvenile Nile tilapia fed on the experimental diets for 10 weeks

YMS_0, $YMS_{0.1}$, $YMS_{0.3}$, $YMS_{0.5}$, $YMS_{1.0}$, $YMS_{2.0}$ represent diets with Yucca meal supplementation level of 0%, 0.1%, 0.3%, 0.5%, 1.0% and 2.0% in the diet, respectively

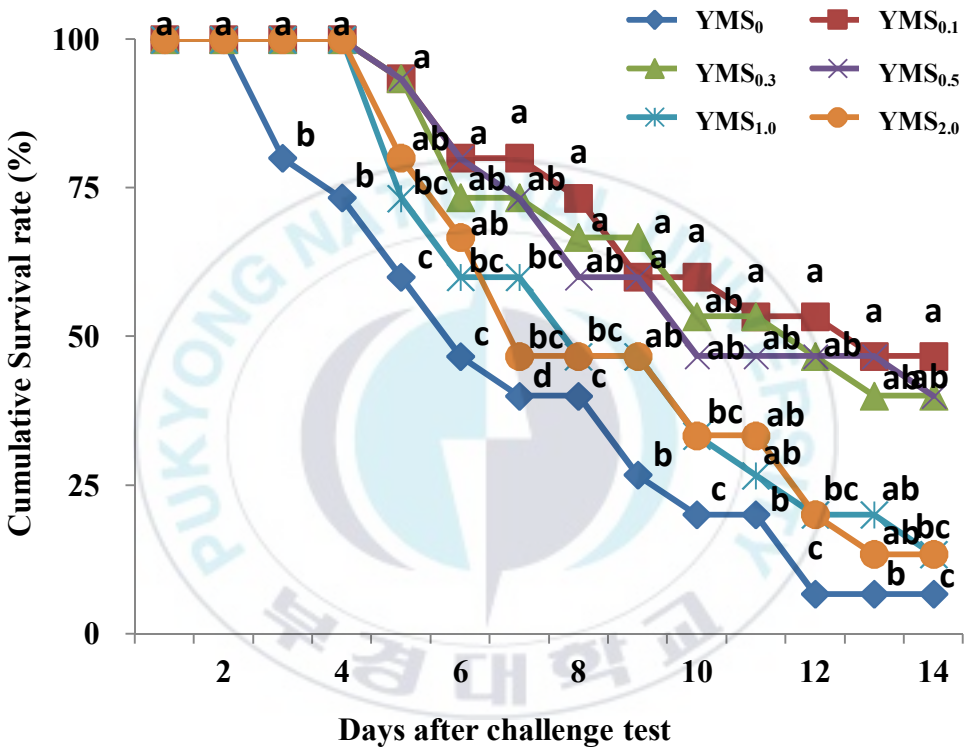

Fig. 20. Cumulative survival rate due to *Aeromonas hydrophila* of juvenile Nile tilapia fed on the experimental diets for 10 weeks as observed for 14 day

IV. Discussion and conclusion

Increasing research is being conducted to evaluate the efficacy of plant extracts as feed additives in aquaculture for promoting growth performance and non-specific immune responses. This has largely been attributed to their growth promoting factors and for replacement of chemotherapy due to their immunostimulating factors. The effects of *Yucca schidigera* extract on the growth of other finfish species have been well studied (Kohler, 2003; Gaber, 2006)

The results of the current study indicate an improved growth performance in juvenile Nile tilapia fed on a diet supplemented with 0.1% Yucca meal (23.9 mg kg^{-1} saponin) in the diet. Fish fed on diet $YMS_{0.1}$ had significantly higher specific growth rate and weight gain than did fish fed on the control diet YMS_0. Stimulation of growth has previously been reported in Nile tilapia (Francis et al., 2001) in which fish fed on a diet containing Quillaja saponin supplemented at 150 mg kg^{-1} had improved growth performance compared to the fish fed on the control diet. Striped catfish (*Pangasianodon hypophthalmus*) fed on a diet containing 0.15% *Yucca Schidigera* extract

had improved growth performance as compared to the fish fed on the control diet. (Güroy et al., 2014). Consistent with these results are the findings that supplementation with Quillaja saponaria at a level of 150 mg kg^{-1} caused an 18% increase in the average weight of common carp, *Cyprinus carpio* compared with the control diet (Francis et al., 2002b). Thus, present findings are in agreement with previous reports and suggest that Yucca meal could be used as a natural feed additive in Nile tilapia diets to improve growth performance. Yucca meal inclusion in the diets did not affect protein efficiency ratio (PER) nor survival rates. The effect of Yucca meal on feed utilization in fish may vary among finfish species, age, environmental conditions, Yucca meal inclusion level and saponin contents.

It was revealed that plant -protein-based diets supplemented with Yucca had a higher apparent protein digestibility coefficient and increased whole body protein content in Nile tilapia (Gaber, 2006). This is consistent with the current findings in which fish fed on the diet containing 0.1 % Yucca meal had significantly higher whole body protein content than did fish the other diets in the experiment. It is suggested that several medicinal herbs promote lipid metabolism that catabolizes body fatty acids as the main energy

expenditure, resulting in an efficient protein accumulation and growth performance (Ji S.C. et al., 2007).

Blood is a pathophysiological reflector of the entire body and the counts of hematological parameters in blood give an indication to the health status of fish by determining any abnormality occurring owing to the use of immunostimulants. Plasma proteins include the humoral elements of the non-specific immune system, e.g. immunoglobulins, transferrin, agglutinins or precipitins (Magnadottir, 2006). Blood glucose concentration on the other hand is often used as an indicator of non-specific stress in fish rather than raised cortisol and adrenaline levels. In fact, during stressful situations, there is an abrupt increase in blood cortisol which causes a breakdown of glycogen from the liver through glycogenolysis and, consequently, a rise in blood glucose levels (Shalaby et al., 2006). In this experiment, total plasma protein and glucose levels were measured, but neither of the treatment groups was affected by Yucca meal inclusion.

Fish fed on the control diet without Yucca meal had significantly lower glutamatic pyruvic transaminase (GPT) activity than fish in all the other

treatment groups. The observed increase in levels of GOT and GPT in all diets with Yucca meal suggest that Yucca meal may cause liver stress to the fish and thus inclusion must be done with good adherence to dosage to avoid inducing clinically negative effects.

The inclusion of Yucca meal at 0.1% in the present study succeeded in improving the immunological parameters of Nile tilapia. Notably, the innate immune system is very crucial in the defence mechanisms of fish (Magnadottir, 2006). Lysozyme is a bactericidal enzyme that hydrolyses the β-1, 4 glycosidic linkages between N-acetyl glucosamine and N-acetyl muramic acid of bacterial cell wall peptidoglycan, thereby causing bacteriolysis and preventing the growth of bacteria. Lysozyme is also known to activate the complement system and phagocytes by acting as an opsonin, as well as to display anti-viral and anti-inflammatory properties (Saurabh and Sahoo, 2008). In the current study, it was observed that fish fed on diet $YMS_{0.1}$ had significantly higher lysozyme activity as compared to that of fish fed on the control diet, YMS_0. A similar immunostimulatory effect was observed in tilapia *O. niloticus* fed on diets supplemented with extract from Chinese herbs (Ardo et al., 2008; Yin et al., 2009).

Previous studies have revealed that saponins have the ability to form an immune stimulating complex (Saponin + cholesterol + phospholipid + amphipathic proteins) which leads to induced production of cytokines such as interleukins and interferon's that might mediate their immunostimulant effects (Piacente et al., 2005).

Nitro Blue Tetrazolium (NBT) test measures the production of oxygen radicals from phagocytes in the blood and can be a good indicator of the fish health status and immune system of the fish. The significantly higher NBT activity in fish fed on $YMS_{0.1}$ diet suggests that fish had a better immune system as compared to that of the fish fed on the control diet (YMS_0). Decreasing NBT activity observed with increasing Yucca meal inclusion levels suggest that high Yucca meal inclusion levels could have immune and growth suppressing effects.

SOD has been detected in a wide variety of mammalian cells and plays an important role in protecting the cell against the potentially toxic effects. This enzyme catalyzes the dismutation of the superoxide ion (O_2-) to hydrogen peroxide and molecular oxygen during oxidative energy processes.

The reaction diminishes the destructive oxidative processes in cell. In the current study, it was observed that fish fed on diet $YMS_{0.1}$ had significantly higher SOD inhibition as compared to that of fish fed on the control diet, YMS_0.

Better immune responses as a result of saponin inclusion have also been attributed to their antioxidant factors (Cheeke, 1996). Saponins have an antioxidant moiety that enables for scavenging of superoxide anions by forming hydroxide intermediates and thus preventing molecular damage (Sparg et al., 2004). After challenge with *A. hydrophila,* all treated groups showed a reduced mortality compared to the control group. The first mortality occurred on day two. The best survival rates were observed in the group fed on $YMS_{0.1}$ diet. Similar results were observed in Nile tilapia fed on diets containing a mixture of Chinese herbs (*Astragalus membranaceus and Lonicera japonica*) in which fish had enhanced resistance towards *A. hydrophila* challenge (Ardó et al., 2008). These results are consistent with the findings that Nile tilapia had enhanced resistance to *A. hydrophila* challenge when fed on diets containing American Ginseng (*Panax quinquefolium*) (Abdel-Tawwab, 2012).

Increased research on the use of plant-derived products for replacement of chemotherapy in farmed fish has been noted in the recent past (Bulfon et al., 2015). This is because they represent a promising tool, complementary to vaccination and traditional drugs, being able to improve growth, survival, health status, innate (lysozyme, complement, antiproteases phagocytosis and microbicidal capacity of phagocytic cells) and adaptive (specific Ig production) immune responses as well as disease resistance in various marine and freshwater fish species.

Some of the crucial factors that may influence the effectiveness of herbal products in fish are their dosage and the duration of administration. It has been pointed out that only an appropriate dosage can significantly induce a stimulation of immune responses, with subsequent increase in disease resistance, without being toxic to animals (Sakai, 1999). The results obtained in the current study supports the hypothesis that dietary Yucca meal supplementation is likely to improve growth, promote non-specific immune responses and enhance resistance against *A. hydrophila* bacterial challenge in juvenile Nile tilapia. Therefore, based on the results obtained, it can be concluded that Yucca meal can be used as a cost effective, safe and

biocompatible feed additive for supplementation in Nile tilapia diets to improve growth, promote non-specific immune responses and enhance disease resistance in cultured fish.

Based on polynomial regression analysis of weight gain, the optimum maximum Yucca meal inclusion levels for juvenile Nile tilapia diets was found to be 0.14% in the diet. The upper limit for Yucca meal inclusion level for juvenile Nile tilapia diets was found to be 0.58 % in the diet based on polynomial and broken line linear regression analysis. This suggests that Yucca meal inclusion levels greater than 0.58 % in the diets could have both growth and immune suppressing effects. The best results in the current study were obtained at Yucca meal inclusion levels of 0.1 % (23.9 mg kg^{-1} saponin) in diet $YMS_{0.1}$.

In conclusion, Yucca meal inclusion level of 0.1% ~ 0.58 (23.9 ~ 138.6 mg kg^{-1} saponin) in the diet can be recommended as a feed additive for use in Nile tilapia culture.

Acknowledgment

I am most grateful to the Almighty God for His mercies and favour upon my life and for seeing me through this course. I owe it all to Him.

My profound gratitude goes to my advisor, Prof. Bai Sungchul Charles whose candid and enthusiastic guidance made this work a success. His patience, advice, constructive criticism and direction have enormously contributed to my graduate capability.

I also extend my unflinching appreciation to the Korean Government, which through the Korea International Cooperation Agency (KOICA) took the pecuniary responsibility for my Master's study at Pukyong National University (PKNU). To the people of Korea, I am grateful for your hospitability.

My appreciation also goes to my thesis assessment committee members, Prof. Yang-Bong Lee and Prof. In-Soo Kong for their valuable comments and contributions which helped to refine the coherence of this study. I salute all professors at PKNU for their insightful lectures and thank KOICA administration fraternity, especially Prof. Kang Kyoungmi for their exemplary performance in coordinating our program. Special thanks to staff members at FFNRC lab and each of my lab mates, especially Yuri Jo for their assistance during my experiment and thesis writing.

To my fellow KOICA and PKNU students, it was great walking the journey together. To my fellow colleagues at Sagana, thank you for your kind support and prayers. I also wish to convey my sincere appreciation to my KCK family (Kenya Community in Korea) for their love, friendship, support and inspiration throughout my study duration.

My heartfelt appreciation goes to my lovely daughter Samantha, who gave me a reason to keep pushing on every day. To each and every one of my family members and friends, for your love and invaluable support and for being my pillar through it all, I thank you immensely and may God bless you abundantly.

References

ABDEL-TAWWAB, M. 2012. The use of American Ginseng (Panax quinquefolium) in practical diets for Nile Tilapia (Oreochromis niloticus): Growth performance and challenge with *Aeromonas hydrophila*. *Journal of Applied Aquaculture,* 24**,** 366-376.

ABDEL, H. E. & MOHAMED, K. A. 2008. Effect of using probiotic as growth promoters in commercial diets for monosex nile tilapia (Oreochromis niloticus) fingerlings. *8th International Symposium on Tilapia in Aquaculture 2008.* Cairo International Convention Center (CICC). Egypt.

ANDERSON, D. P. & SIWICKI, A. K. 1995. Basic haematology and serology for fish health programs. *In:* SHARIFF, M., J.R.AUTHUR & SUBASINGHE, R. P. (eds.) *Diseases in Asian Aquaculture II, Fish Health Section. Asian Fisheries Society.* Manila,Philippines.

AOAC, ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS 2000. Official Methods of Analysis, 17th Edition. Arlington, Virginia.: AOAC International.

ARDÓ, L., YIN, G., XU, P., VÁRADI, L., SZIGETI, G., JENEY, Z. & JENEY, G. 2008. Chinese herbs (Astragalus membranaceus and Lonicera japonica) and boron enhance the non-specific immune response of Nile tilapia (Oreochromis niloticus) and resistance against Aeromonas hydrophila. *Aquaculture,* 275**,** 26-33.

AREGHEORE, E. M. 2005. Effect of Yucca schidigera saponin on the nutritive value of urea-ammoniated maize stover and its feeding value when supplemented with forage legume (Calliandra calothyrsus) for goats. *Small Ruminant Research,* 56**,** 95-102.

BAI, S. C. & KIM, K. W. 1997. Effects of dietary animal protein sources on growth and body composition in Korean rockfish, Sebastes schlegeli. *Journal of Aquaculture,* 10**,** 77-85.

BONDAD-REANTASO, M. G., SUBASINGHE, R. P., ARTHUR, J. R., OGAWA, K., CHINABUT, S., ADLARD, R., TAN, Z. & SHARIFF, M. 2005. Disease and health management in Asian aquaculture. *Vet Parasitol,* 132**,** 249-72.

BRANDER, K. 2010. Impacts of climate change on fisheries. *Journal of Marine Systems,* 79**,** 389-402.

BRICKNELL, I. & DALMO, R. A. 2005. The use of immunostimulants in fish larval aquaculture. *Fish Shellfish Immunol,* 19**,** 457-72.

BULFON, C., VOLPATTI, D. & GALEOTTI, M. 2015. Current research on the use of plant-derived products in farmed fish. *Aquaculture Research,* 46**,** 513-551.

CABELLO, F. C. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environ Microbiol,* 8**,** 1137-44.

CABELLO, F. C., GODFREY, H. P., TOMOVA, A., IVANOVA, L., DOLZ, H., MILLANAO, A. & BUSCHMANN, A. H. 2013. Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. *Environ Microbiol,* 15**,** 1917-42.

CARNEVALI, O., DE VIVO, L., SULPIZIO, R., GIOACCHINI, G., OLIVOTTO, I., SILVI, S. & CRESCI, A. 2006. Growth improvement by probiotic in European sea bass juveniles (Dicentrarchus labrax, L.), with particular attention to IGF-1, myostatin and cortisol gene expression. *Aquaculture,* 258**,** 430-438.

CHEEKE, P. R. 1996. Biological Effects of Feed and Forage Saponins and Their Impacts on Animal Production. *In:* WALLER, G. & YAMASAKI, K. (eds.) *Saponins Used in Food and Agriculture.* Springer US.

CHEOK, C. Y., SALMAN, H. A. K. & SULAIMAN, R. 2014. Extraction and quantification of saponins: A review. *Food Research International,* 59**,** 16-40.

CHO, C. Y. & SLINGER, S. J. Apparent digestibility measurement in feedstuffs for rainbow trout. *In:* HALVER, J. E. & TIEWS, K., eds. Proceedings of the World Symposium of Finfish Nutrition and Fishfeed Technology, 1979 Berlin, Germany.

DEFOIRDT, T., BOON, N., SORGELOOS, P., VERSTRAETE, W. & BOSSIER, P. 2007. Alternatives to antibiotics to control bacterial infections: luminescent vibriosis in aquaculture as an example. *Trends in Biotechnology,* 25**,** 472-479.

EL-SAYED, A.-F. M. 2006. *Tilapia Culture* Wallingford Oxfordshire,OX10 8DE, UK, CABI Publishing.

ERYAVUZ, A. & DEHORITY, B. A. 2004. Effect of Yucca schidigera extract on the concentration of rumen microorganisms in sheep. *Animal Feed Science and Technology,* 117**,** 215-222.

FAO 2009. *Food and Agriculture Organization of the UN, High-level expert forum on 'How to Feed the World in 2050'* . Rome.

FAO 2014. Food and Agriculture Organization of the United Nations.The State of World Fisheries and Aquaculture. Rome,Italy.

FRANCIS, G., LEVAVI-SIVAN, B., AVITAN, A. & BECKER, K. 2002a. Effects of long term feeding of Quillaja saponins on sex ratio, muscle and serum cholesterol and LH levels in Nile tilapia (Oreochromis niloticus (L.)). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 133**,** 593-603.

FRANCIS, G., MAKKAR, H. P. S. & BECKER, K. 2001. Effects of Quillaja saponins on growth, metabolism, egg production and muscle cholesterol in individually reared Nile tilapia (Oreochromis niloticus). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 129**,** 105-114.

FRANCIS, G., MAKKAR, H. P. S. & BECKER, K. 2002b. Dietary supplementation with a Quillaja saponin mixture improves growth

performance and metabolic efficiency in common carp. *Aquaculture,* 203**,** 311-320.

FRANCIS, G., MAKKAR, H. P. S. & BECKER, K. 2005. Quillaja saponins—a natural growth promoter for fish. *Animal Feed Science and Technology,* 121**,** 147-157.

GABER, M. M. 2006. The effects of plant-protein-based diets supplemented with yucca on growth, digestibility and chemical composition of Nile tilapia (Oreochromis niloticus, L) fingerlings. *Journal of the World Aquaculture Society,* 37**,** 74–81.

GÜROY, B., MANTOĞLU, S., KAYALı, S. & ŞAHIN, İ. 2014. Effect of dietaryYucca schidigera extract on growth, total ammonia-nitrogen excretion and haematological parameters of juvenile striped catfishPangasianodon hypophthalmus. *Aquaculture Research,* 45**,** 647-654.

HARIKRISHNAN, R., BALASUNDARAM, C. & HEO, M.-S. 2011. Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. *Aquaculture,* 317**,** 1-15.

HULTMARK, D., STEINER, H., RASMUSON, T. & BOMAN, H. G. 1980. Insect immunity. Purification and properties of three inducible

bactericidal proteins from hemolymph of immunized pupae of Hyalophora cecropia. *European Journal of Biochemistry,* 106**,** 7-16.

JI S.C., JEONG G.S., IM G.S., LEE S.W., YOO J.H. & K., T. 2007. Dietary medicinal herbs improve growth performance, fatty acid utilization, and stress recovery of Japanese flounder. *Fisheries Science,* 73**,** 70-76.

JOHNSON, R. 2011. Potential Trade Implications of Restrictions on Antimicrobial Use in Animal Production. *FAS-Federation of American Scientists,* CRS Report for Congress-R41047.

KIM, W.-S., KIM, S.-R., KIM, D., KIM, J.-O., PARK, M.-A., KITAMURA, S.-I., KIM, H.-Y., KIM, D.-H., HAN, H.-J., JUNG, S.-J. & OH, M.-J. 2009. An outbreak of VHSV (viral hemorrhagic septicemia virus) infection in farmed olive flounder Paralichthys olivaceus in Korea. *Aquaculture,* 296**,** 165-168.

KOHLER, A. M. K. A. C. C. 2003. Effects of Yucca shidigera Extract on Growth, Nitrogen Retention, Ammonia Excretion, and Toxicity in Channel Catfish Ictalurus punctatus and Hybrid Tilapia Oreochromis rnossambicus X 0. niloticus. *Journal of the world Aqauculture Society,* 34.

KUMAR, B. K., DEEKSHIT, V. K., RAJ, J. R. M., RAI, P., SHIVANAGOWDA, B. M., KARUNASAGAR, I. & KARUNASAGAR, I. 2014. Diversity of Vibrio parahaemolyticus associated with disease outbreak among cultured Litopenaeus vannamei (Pacific white shrimp) in India. *Aquaculture,* 433**,** 247-251.

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.-H. & 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.

MAGNADOTTIR, B. 2006. Innate immunity of fish (overview). *Fish Shellfish Immunol,* 20**,** 137-51.

MURRAY, A. G., MARCOS-LOPEZ, M., COLLET, B. & MUNRO, L. A. 2012. A review of the risk posed to Scottish mollusc aquaculture from Bonamia, Marteilia and oyster herpesvirus. *Aquaculture,* 370-371**,** 7-13.

MURRAY, A. G. & PEELER, E. J. 2005. A framework for understanding the potential for emerging diseases in aquaculture. *Prev Vet Med,* 67**,** 223-35.

NATALE, F., HOFHERR, J., FIORE, G. & VIRTANEN, J. 2013. Interactions between aquaculture and fisheries. *Marine Policy,* 38**,** 205-213.

NRC, NATIONAL RESEARCH COUNCIL (U.S.). COMMITTEE ON THE NUTRIENT REQUIREMENTS OF FISH AND SHRIMP. 2011. *Nutrient Requirements of Fish and Shrimp*, National Academies Press.

PIACENTE, S., PIZZA, C. & OLESZEK, W. 2005. Saponins and Phenolics of Yucca schidigera Roezl: Chemistry and Bioactivity. *Phytochemistry Reviews,* 4**,** 177-190.

REVERTER, M., BONTEMPS, N., LECCHINI, D., BANAIGS, B. & SASAL, P. 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. *Aquaculture,* 433**,** 50-61.

RICO, A., PHU, T. M., SATAPORNVANIT, K., MIN, J., SHAHABUDDIN, A. M., HENRIKSSON, P. J. G., MURRAY, F. J.,

LITTLE, D. C., DALSGAARD, A. & VAN DEN BRINK, P. J. 2013. Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. *Aquaculture,* 412-413**,** 231-243.

ROBERTS, R. J. (ed.) 2012. *Fish Pathology, 4th Edition,pg 359. ,* A John Wiley and Sons Ltd. Publication.: Blackwell Publishing Ltd.

SAKAI, M. 1999. Current research status of fish immunostimulant. *Aquaculture Journal,* 172**,** 63-92.

SANTACRUZ-REYES, R. A. & CHIEN, Y.-H. 2009. Efficacy of Yucca schidigera extract for ammonia reduction in freshwater: Effectiveness analysis and empirical modeling approach. *Aquaculture,* 297**,** 106-111.

SANTACRUZ-REYES, R. A. & CHIEN, Y. H. 2012. The potential of Yucca schidigera extract to reduce the ammonia pollution from shrimp farming. *Bioresour Technol,* 113**,** 311-4.

SAPKOTA, A., SAPKOTA, A. R., KUCHARSKI, M., BURKE, J., MCKENZIE, S., WALKER, P. & LAWRENCE, R. 2008. Aquaculture practices and potential human health risks: current knowledge and future priorities. *Environ Int,* 34**,** 1215-26.

SAURABH, S. & SAHOO, P. K. 2008. Lysozyme: an important defence molecule of fish innate immune system. *Aquaculture Research,* 39**,** 223-239.

SHALABY, A. M., KHATTAB, Y. A. & A.M., A. R. 2006. Effects of garlic (Allium sativum) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (Oreochromis niloticus). *Journal of Venomous Animals and Toxins Including Tropical Diseases,* 12**,** 172-201.

SHANE, D. S. M. 2014. *Antibiotics may Impede Trade with Korea* [Online]. http://www.alltech.com/blog/posts/antibiotics-may-impede-trade-korea.

SPARG, S. G., LIGHT, M. E. & VAN STADEN, J. 2004. Biological activities and distribution of plant saponins. *J Ethnopharmacol,* 94**,** 219-43.

STENTIFORD, G. D., NEIL, D. M., PEELER, E. J., SHIELDS, J. D., SMALL, H. J., FLEGEL, T. W., VLAK, J. M., JONES, B., MORADO, F., MOSS, S., LOTZ, J., BARTHOLOMAY, L., BEHRINGER, D. C., HAUTON, C. & LIGHTNER, D. V. 2012.

Disease will limit future food supply from the global crustacean fishery and aquaculture sectors. *J Invertebr Pathol,* 110**,** 141-57.

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.

TANG, J., CAI, J., LIU, R., WANG, J., LU, Y., WU, Z. & JIAN, J. 2014. Immunostimulatory effects of artificial feed supplemented with a Chinese herbal mixture on Oreochromis niloticus against Aeromonas hydrophila. *Fish Shellfish Immunol,* 39**,** 401-6.

UN 2010. United Nations, World Population Prospects,The 2010 Revisison. Department of Economic and Social Affairs. Population Division, New York

VENKAT, H. K., SAHU, N. P. & JAIN, K. K. 2004. Effect of feeding lactobacillus-based probiotics on the gut microflora, growth and survival of Myobacterium rosenbergii (de man). *Aquaculture Research,* 35**,** 501-507.

WANG, J.-L., MENG, X.-L., LU, R.-H., WU, C., LUO, Y.-T., YAN, X., LI, X.-J., KONG, X.-H. & NIE, G.-X. 2015. Effects of Rehmannia

glutinosa on growth performance, immunological parameters and disease resistance to Aeromonas hydrophila in common carp (Cyprinus carpio L.). *Aquaculture,* 435**,** 293-300.

YEH, S.-P., LIU, C.-H., SUNG, T.-G., LEE, P.-P. & CHENG, W. 2006. Effect of saponin on hematological and immunological parameters of the giant freshwater prawn, Macrobrachium rosenbergii. *Aquaculture,* 261**,** 1432-1439.

YIN, G., ARDO, L., THOMPSON, K. D., ADAMS, A., JENEY, Z. & JENEY, G. 2009. Chinese herbs (Astragalus radix and Ganoderma lucidum) enhance immune response of carp, Cyprinus carpio, and protection against Aeromonas hydrophila. *Fish Shellfish Immunol,* 26**,** 140-5.

YIN, G., JENEY, G., RACZ, T., XU, P., JUN, X. & JENEY, Z. 2006. Effect of two Chinese herbs (Astragalus radix and Scutellaria radix) on non-specific immune response of tilapia, Oreochromis niloticus. *Aquaculture,* 253**,** 39-47.

ZAKI, M. A., LABIB, E. M., NOUR, A. M., TONSY, H. D. & MAHMOUD, S. H. 2012. Effect Some Medicinal Plants Diets on Mono Sex Nile Tilapia (Oreochromis niloticus), Growth

Performance, Feed Utilization and Physiological Parameters. *APCBEE Procedia,* 4**,** 220-227.

ZHANG, G., GONG, S., YU, D. & YUAN, H. 2009. Propolis and Herba Epimedii extracts enhance the non-specific immune response and disease resistance of Chinese sucker, Myxocyprinus asiaticus. *Fish Shellfish Immunol,* 26**,** 467-72.