Fish and Shellfish Immunology 93 (2019) 328–335

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Effects of elephant's foot (*Elephantopus scaber*) extract on growth performance, immune response, and disease resistance of nile tilapia (*Oreochromis niloticus*) fingerlings

Hien Van Doan[a,b,*], Seyed Hossein Hoseinifar[c], Korawan Sringarm[a], Sanchai Jaturasitha[a,b], Trisadee Khamlor[a], Mahmoud A.O. Dawood[d], Maria Ángeles Esteban[e], Mehdi Soltani[f], Mohamed Saiyad Musthafa[g]

[a] Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai, 50200, Thailand
[b] Science and Technology Research Institute, Chiang Mai University, 239 Huay Keaw Rd, Suthep, Muang, Chiang Mai, 50200, Thailand
[c] Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[d] Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, 33516, Kafrelsheikh, Egypt
[e] Fish Innate Immune System Group, Department of Cell Biology & Histology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Spain
[f] Fresh water and Fish Health Group, Centre for Sustainable Aquatic Ecosystems, Harry Butler Institute, Murdoch University, Australia
[g] P.G. & Research Department of Zoology, The New College, Chennai, 600014, Tamil Nadu, India

ARTICLE INFO

Keywords:
Elephantopus scaber
Growth performance
Mucosal immunity
Humoral immunity
Disease resistance
Nile tilapia
Streptococcus agalactiae

ABSTRACT

Medicinal plant has been applied as an alternative strategy for antibiotics and chemotherapeutics for controlling the outbreak of diseases in tilapia farming. In this study, five doses of *Elephantopus scaber* extract (ESE) were added to the basal diet at 0, 2.5, 5, 10, and 20 g kg^{-1} feed of Nile tilapia fingerlings (13.92 ± 0.06 g initial weight) in triplicate. After 4- and 8- weeks post-feeding, fish were sampled to determine the effects of the ESE supplemented on fish's growth performance, humoral, and skin mucus immune response. After 8 weeks post-feeding, a challenge test against *Streptococcus agalactiae* was carried out using 10 fish from each tank. Fish fed ESE showed significantly increased serum lysozyme (SL), serum peroxidase (SP), alternative complement (ACH50), phagocytosis (PI), and respiratory burst (RB) compared to the control group ($P < 0.05$). The skin mucus lysozyme (SMLA) and skin peroxidase (SMPA) were stimulated in fish fed ESE diets. Dietary inclusion of ESE significantly ($P < 0.05$) promoted final body weight (FW), weight gain (WG), and specific growth rate (SGR); while a reduction in feed conversion ratio (FCR) was observed in fish fed 5 g kg^{-1} ESE, after 8 weeks post-feeding. The challenge study indicated that the relative percent survival (RSP) was 38.10%, 76.19%, 66.67%, and 47.62% in Diet 2, Diet 3, Diet 4, and Diet 5, respectively. Among the supplemented groups, dietary of 5 g kg^{-1} ESE showed significantly higher RPS and the highest resistance to *S. agalactiae* in comparison with other groups. In conclusion, supplementation of ESE (5 g kg^{-1}) enhanced the humoral and mucosal immunity, promoted growth performance, and improved disease resistance of Nile tilapia against *Streptococcus agalactiae*.

1. Introduction

Nile tilapia (*Oreochromis niloticus*) is a widely preferred fish and is also one of the commonly farmed fish worldwide [1]. The benefits of rearing this species for food are numerous, such as tasting good, tolerate to a wide range of environments and diseases. Its global production was estimated at an approximately 6.3 million tons in 2018 [2]. Subsequently, the demand had increased worldwide, owing to immense infection in fingerlings of this species through unprotected and bulk production [3]. Moreover, growth performance and survival percentage of *O. niloticus* adversely declined due to microbial infection and indecorous maintenance of water quality in cram-full biomass producing fish farms [4].

Recently, Delannoy et al. [5] reported that the pivotal role of pathogen *Streptococcus agalactiae* on the tremendous economic loss for fish industry particularly tilapia species owing to intensification and also main obstacles in the sustainable development. *S. agalactiae* is a gram-positive bacterium, the causal pathogen of streptococcosis in Nile

* Corresponding author.Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai, 50200, Thailand.(;)
E-mail addresses: hienqbuni@gmail.com, hien.d@cmu.ac.th (H.V. Doan).

https://doi.org/10.1016/j.fsi.2019.07.061
Received 28 April 2019; Received in revised form 24 June 2019; Accepted 22 July 2019
Available online 22 July 2019

tilapia [6,7]. Outbreaks of *S. agalactiae* infection in tilapia have been reported in several countries around the world [8–13]. More than three decades, synthetic chemotherapeutics were regularly administered for control of microbial infection and their outbreaks in commercial aquaculture [14]. However, as the abuse of chemotherapeutics lead to emergence antibiotic resistance and immune depression, necessitating the researchers to explore for alternative control strategies such as administration of supplements to substitute the therapeutic use of antimicrobials [15]. These reports were accords with the earlier studies that, the synthetic antibiotics, such as oxytetracycline, erythromycin, doxycycline, kitas amycin, and lincomycin have been used to control streptococcosis by *S. iniae* in blue tilapia [16]. It should be noted that albeit the studies where fish with the pathogenesis of Streptococcus invasion to re-cover by antibiotic therapy, the disease cannot be controlled but reversely, Streptococcus strains develop antibiotic resistance [17]. In this situation, an alternatives potential traditional herbal with multifunctional active and its administration has been suggested as promising in eco-friendly aquaculture.

The medicinal plants have been used for health-promoting of human and animals for a long time. This is one of the most promising industries in the future [18,19]. The global herbal medicine market is anticipated to obtain a compound annual growth rate (CAGR) of 5.88% with a value of USD 1,29,689.3 million by 2023 [20]. In aquaculture, there has been growing attention in using medicinal plants as alternatives for antibiotics and chemotherapeutics in diseases prevention and treatment [21]. The medicinal plant products application in aquaculture practice normally have growth promoting and immunostimulating properties, as well as act as appetizers [21]. These products possess a wide range of bioactive compounds, such as phenolic, polyphenolic, alkaloid, quinone, terpenoid, lectine, and polypeptide compounds, which are effective alternatives to antibiotics, chemicals, vaccines, and other synthetic compounds [22–24]. Additionally, medicinal plants are rich sources of nutrients [25]. They can be applied as whole plant, parts or extract forms, via water route or supplemented to the diet, either singular or combined extract compounds, or even as a mixture with other bioactive compounds [26,27]. In the present work, the traditional herb *Elephantopus scaber* was selected as an additive in the basal diet to evaluate its protective efficacy against *S. agalactiae* infection in *O. niloticus* fingerlings under laboratory conditions. This is an attempt in aquaculture to explore its bio-medical utilization as a multi-potential herb. *E. scaber* belongs to Asteraceae family of 32 species. The plant has been extensively screened for anticancer activity. Previously, many researchers reported that bioactive components of *E. scaber,* which includes deoxyelephantopin, isodeoxyelephantopin, scabertopin, and isoscabertopin [28,29]. These compounds have been proven to be prominent anticancer constituents and other biological activities, such as antimicrobial, hepatoprotective, antioxidant, antidiabetic, anti-inflammatory, analgesic, antiasthamatic, antiplatelet, and wound healing properties [28]. Jenny et al. [30] reported that methanolic extract of *E. scaber* exhibits most potent antibacterial activity in the test organisms against *Staphylococcus aureus*, *Salmonella paratyphi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Shigella sonnei*, *Escherichia coli*, and *Salmonella typhimurium*. However, as far as we know, there is no available data regarding effect of *Elephantopus scaber* on fish and shellfish. Therefore, present work was performed to evaluate the potential effects of *E. scaber* extract on growth performance, immune response, and disease resistance of Nile tilapia, *O. niloticus*.

2. Materials and methods

2.1. Preparation of medicinal plants

The *Elephantopus scaber* leaves were collected from Chiang Dao District, Chiang Mai, Thailand. The leaves were then oven dried at 60 °C for 48 h, and then ground into fine particles (0.2-mm) for further extraction. Five hundred gram of ground sample was thoroughly mixed with 5 L of ethanol (AR grade; RCI Lab-Scan), and incubated in the dark condition, at room temperature for 72 h. Thereafter, the supernatant was filtered using a Whatman No. 41 filter paper. The supernatant was then evaporated to dried under reduced pressure condition (40 °C), via a rotary evaporator (Büchi, Flawil, Switzerland). The obtained extraction was stored at (−20 °C) until use.

2.2. Fish

Healthy Nile tilapia (*Oreochromis niloticus*) fingerlings were obtained from a commercial hatchery in Chiang Mai, Thailand and transported to the laboratory in oxygenated bags and released into 5 × 5 × 2 cages. Healthy status of fish was examined immediately upon arrival, ten fish were randomly selected for health status checking through observation of gills and internal organs under a microscope. They were then acclimatized for 15 days under laboratory conditions. The water quality parameters were analyzed and maintained within the normal range (pH – 7.5, dissolved oxygen – 5.2 mg/L, and temperature – 28 ± 1 °C). During the cultivation and acclimatization period, they were fed daily to satiation with commercial feed (CP 9950) and a subsample was cultured and determined to be free of pathogenic bacteria.

2.3. Experimental diets and feeding regime

The basal diet contained fish meal and soybean meal as the protein sources. The cornmeal, wheat flour, and rice bran were used as carbohydrate source; while soybean oil was used as a lipid source; in combination with vitamins and mineral premix. The *E. scaber* extract was supplemented to the basal diet at concentration of 0 g kg^{-1} (Control – Diet 1), Diet 2 (2.5 g kg^{-1}), Diet 3 (5 g kg^{-1}), Diet 4 (10 g kg^{-1}), and 20 g kg^{-1} Diet 5 by evenly mixing with the basal diet thoroughly (Table 1). The ingredients were grounded and extruded by passing through 4 mm mesh sieve. The obtained diets were stored at −20 °C until used. Proximate analysis of the experimental diets was measured according to the method of the AOAC [31] method comprised the percentage of crude protein, crude lipid, crude ash, and crude fibre (Table 1).

All groups of fish were fed daily *ad libitum* in two instalments at 08:00 and 16:30 h. Fishes were exposed to the diet for 2 h during each

Table 1
The formulation and proximate composition of the *Elephantopus scaber* extract experiment (g kg^{-1}).

Ingredients	Diets (g kg^{-1})
Fish meal	270
Corn meal	200
Soybean meal	270
Wheat flour	60
Rice bran	150
Cellulose	30
Soybean oil	5
Premix[a]	10
Vitamin C[b]	5
Proximate composition of the experimental diets (g kg^{-1} dry matter basis)	
Crude protein	322.06
Crude lipid	74.75
Fibre	52.48
Ash	106.68
Dry matter	817.80
GE (cal/g)[c]	4105

[a] Vitamin and trace mineral mix supplemented as follows (IU kg^{-1} or g kg^{-1} diet): retinyl acetate 1,085,000 IU; cholecalciferol 217,000 IU; D, L-a-tocopherol acetate 0.5 g; thiamin nitrate 0.5 g; pyridoxine hydrochloride 0.5 g; niacin 3 g; folic 0.05 g; cyanocobalamin 10 g; Ca pantothenate 1 g kg^{-1}; inositol 0.5 g; zinc 1 g; copper 0.25 g; manganese 1.32 g; iodine 0.05 g; sodium 7.85 g.
[b] Vitamin C 98% 8 g.
[c] GE = gross energy.

Table 2
Skin mucus lysozyme and peroxidase activities of *O. niloticus* after 4 and 8 weeks feeding with experimental diets: Diet 1 (0 - control), Diet 2 (2.5 g kg^{-1} ESE), Diet 3 (5 g kg^{-1} ESE), Diet 4 (10 g kg^{-1} ESE), and Diet 5 (20 g kg^{-1} ESE). Different letter in a row denote significant difference ($P < 0.05$).

		Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
4 weeks	SMLA	1.68 ± 0.08^{c}	1.96 ± 0.10^{c}	2.80 ± 0.11^{a}	2.33 ± 0.08^{b}	2.33 ± 0.09^{b}
	SMPA	0.05 ± 0.008^{c}	0.08 ± 0.006^{b}	0.13 ± 0.005^{a}	0.09 ± 0.005^{b}	0.07 ± 0.005^{b}
8 weeks	SMLA	3.09 ± 0.24^{c}	4.79 ± 0.13^{b}	5.90 ± 0.08^{a}	4.99 ± 0.10^{b}	4.53 ± 0.29^{b}
	SMPA	0.11 ± 0.006^{c}	0.15 ± 0.005^{b}	0.18 ± 0.003^{a}	0.15 ± 0.008^{b}	0.14 ± 0.005^{b}

SMLA (μg ml^{-1}) = Skin mucus lysozyme activity.
SMPA (μg ml^{-1}) = Skin mucus peroxidase activity.

ration thereafter the uneaten feed was siphoned out, stored, dried and weighed for calculating feed conversion ratio (FCR).

2.4. Fish and experimental design

Three hundred Nile tilapia (*O. niloticus*) fingerlings (average weight of 13.92 ± 0.06 g) were divided into five experimental groups of 20 each in triplicate (5 x 20 x 3 = 300 fish). Growth rates, as well as immune responses to *O. niloticus* were computed every four weeks. Eight weeks after feeding, ten fish were randomly retrieved from each replication and presented with the *S. agalactiae* challenge.

2.5. Immune response analysis

2.5.1. Samples collection

Four fish from each replication were collected for immune response analysis at week 4 and 8 post-feeding. Skin mucus preparation was followed the protocol as described in our previous study [32].

Blood collection and serum separation were conducted as described in previous studies [33,34]. Briefly, collected bloods from caudal vein were immediately transferred into the sterile Eppendorf tubes without anticoagulant. The samples were left to clot at room temperature for 1 h and at 4 °C for 4 h. They were then centrifuged at 4 °C, 1500 × *g* for 5 min. After centrifugation, serum was collected and stored at −20 °C until assay.

Blood collection and leukocytes isolation were measured following the method of Chung and Secombes [35] with some modifications as detailed in previous studies [33,34].

2.5.2. Immunological measurements

Lysozyme activity: Serum and skin mucus lysozyme activities were performed based on the method described by Parry, Chandan and Shahani [36].

Peroxidase activity: Serum and skin mucus peroxidase activities were analysed following the method of Quade and Roth [37] and Cordero et al. [38].

Phagocytosis activity: Leukocytes phagocytosis activity was measured as the protocol described by Yoshida and Kitao [39], with some modification described in previous studies of [33,34].

Respiratory burst activity: The activity was measured as the previous method of Secombes and Fletcher [40].

Alternative complement pathway activity: Measurement of this activity was conducted following the method of Yanno [41].

2.6. Challenge study

Challenge test using *Streptococcus agalactiae* as pathogen was conducted after 8 weeks post-feeding. The details in *S. agalactiae* preparation and concentration were previously described in the study of Van Doan et al. [42]. Ten fish from each replication was injected intraperitoneally with 0.1 ml of 0.85% saline solution (NSS) containing 10^{7} CFU ml^{-1} of *S. agalactiae* [43]. Fish syndromes were observed daily and dead fish were moved out and recorded. At day 15th post-challenge, mortality (%) of fish in each replication was computed, and the relative percentage of survival (RPS) calculated following the equation of [44]: RPS = 100 - (test mortality/control mortality) *100.

2.7. Growth performance

Growth performance of the fish in each replication at week 4 and 8 was determined as the previously described in Ref. [42].

2.8. Statistical analysis

The obtained data were analyzed using a SAS Computer Program [45] for the least significant differences among the treatments where the Duncan's Multiple Range Test was used. Mean values were considered significantly different when $P < 0.05$. Data are presented as means ± standard error (SE).

3. Results

3.1. Skin mucus immune response

Dietary inclusion of *Elephantopus scaber* extract (ESE) was significantly increased skin mucus lysozyme (SMLA) and peroxidase (SMPA) activities in comparison to the control diet after 4- and 8-weeks post-feeding ($P < 0.05$; Table 2). The highest skin mucus parameters were observed in fish fed Diet 3 (5 g kg^{-1} ESE). Nevertheless, no significant difference was found among Diet 2, Diet 4 and Diet 5, except for SMLA at week 4 post-feeding ($P > 0.05$; Table 2).

3.2. Serum immune responses

Dietary administration of ESE resulted in improvement of SL, SP, ACH50, PI, and RB after 4- and 8- weeks post-feeding ($P < 0.05$; Table 3). The highest values were observed in fish Diet 3 (5 g kg^{-1} ESE) compared to the control and other inclusion diets. However, no significant difference in SP, ACH50, PI, and RB were recorded in fish fed Diet 3 and Diet 4 (10 g kg^{-1} ESE) Similarly, no significant difference in serum immunity parameters was observed between fish fed Diet 2 (2.5 g kg^{-1} ESE) and Diet 5 (20 g kg^{-1} ESE) ($P > 0.05$; Table 3).

3.3. Challenge test

Challenge test against *S. agalactiae* was conducted after 8 weeks post-feeding for a period of 15 days. The results showed that compared to the control treatment (30%), the survival rate in ESE supplemented groups diets were significantly higher ($P < 0.05$); by 56.68% (Diet 2), 83.33% (Diet 3), 76.68 (Diet 4), and 63.33% (Diet 5) (Fig. 1). Dead fish appearance including loss of appetite, darkness, exophthalmia, pair-fins basal haemorrhage and pale liver, which are typical symptoms of *Streptococcus* infection. Therefore, the relative percent survival (RSP) was 38.10%, 76.19%, 66.67%, and 47.62% in Diet 2, Diet 3, Diet 4, and Diet 5, respectively (Fig. 1). Among the supplemented groups, dietary of 5 g kg^{-1} ESE showed significantly higher RPS and the highest resistance to *S. agalactiae* in comparison with other groups (Fig. 1).

Table 3
Serum immunity of *O. niloticus* after 4 and 8 weeks feeding with experimental diets: Diet 1 (0 - control), Diet 2 (2.5 g kg^{-1} ESE), Diet 3 (5 g kg^{-1} ESE), Diet 4 (10 g kg^{-1} ESE), and Diet 5 (20 g kg^{-1} ESE). Different letter in a row denote significant difference ($P < 0.05$). Different letter in a row denote significant difference ($P < 0.05$).

		Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
4 weeks	SL	4.76 ± 0.25^{c}	6.32 ± 0.18^{b}	8.06 ± 0.14^{a}	6.72 ± 0.41^{b}	6.26 ± 0.44^{b}
	SP	0.12 ± 0.005^{c}	0.16 ± 0.008^{b}	0.20 ± 0.006^{a}	0.19 ± 0.012^{a}	0.18 ± 0.05^{ab}
	ACH50	144.58 ± 4.87^{c}	158.54 ± 1.29^{bc}	185.15 ± 6.75^{a}	179.97 ± 3.30^{a}	162.40 ± 3.95^{b}
	PI	1.63 ± 0.04^{c}	2.31 ± 0.08^{b}	2.62 ± 0.04^{a}	2.42 ± 0.03^{b}	2.29 ± 0.07^{b}
	RB	0.05 ± 0.003^{c}	0.08 ± 0.003^{b}	0.10 ± 0.006^{a}	0.10 ± 0.003^{a}	0.07 ± 0.005^{b}
8 weeks	SL	7.03 ± 0.31^{c}	8.55 ± 0.21^{b}	10.77 ± 0.18^{a}	8.80 ± 0.38^{b}	8.80 ± 0.25^{b}
	SP	0.17 ± 0.008^{c}	0.23 ± 0.02^{b}	0.28 ± 0.06^{a}	0.27 ± 0.003^{a}	0.2 ± 0.02^{ab}
	ACH50	181.25 ± 8.01^{c}	224.73 ± 4.99^{b}	282.46 ± 9.25^{a}	268.87 ± 13.69^{a}	226.88 ± 10.26^{b}
	PI	2.26 ± 0.09^{c}	2.72 ± 0.08^{b}	3.21 ± 0.10^{a}	2.74 ± 0.06^{b}	2.73 ± 0.07^{b}
	RB	0.11 ± 0.02^{c}	0.15 ± 0.01^{b}	0.19 ± 0.01^{a}	0.18 ± 0.01^{a}	0.15 ± 0.01^{b}

SL = Serum lysozyme activity (μg ml^{-1}); SP = Serum peroxidase activity (μg ml^{-1}); ACH50 = Alternative complement activity (units ml^{-1}); PI = Phagocytosis activity (bead cell^{-1}); RB = Respiratory burst activity (OD655).

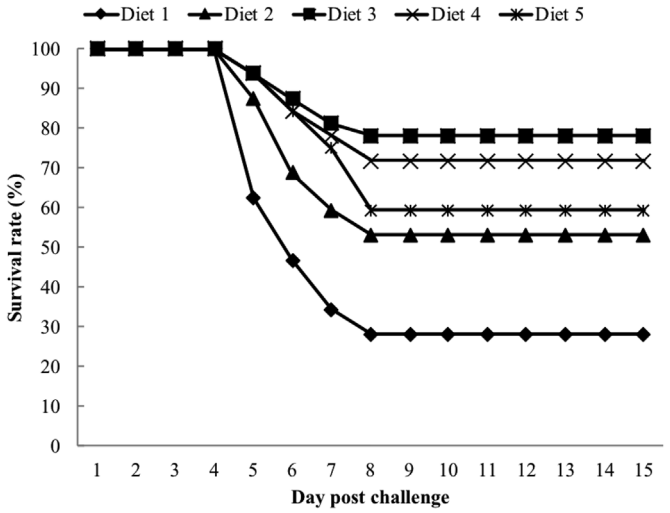

Fig. 1. Survival rate of *O. niloticus* after 4 and 8 weeks feeding with experimental diets (n = 10, mean ± SD): Diet 1 (0 - control), Diet 2 (2.5 g kg^{-1} ESE), Diet 3 (5 g kg^{-1} ESE), Diet 4 (10 g kg^{-1} ESE), and Diet 5 (20 g kg^{-1} ESE).

3.4. Growth performance

After 4- and 8-weeks post-feeding, fish fed dietary inclusion of ESE was statistically significantly increased the specific growth rate (SGR), weight gain (WG), and final weight (FW) compared to the control group ($P < 0.05$; Table 4). The highest SGR, FW, and WG were observed in fish fed 5 g kg^{-1} ESE (Table 4). However, no significant differences in these values were observed in fish fed Diet 3 (5 g kg^{-1} ESE) and Diet 4 (10 g kg^{-1} ESE) ($P > 0.05$; Table 4). The FCR was significantly lower in fish fed 5 g kg^{-1} ESE than in other groups, while the control group presented the highest values ($P < 0.05$). However, no significant in FCR was observed between Diet 3 and Diet 4, and between Diet 5 and Diet 2. Similarly, the survival rate and feed intake (FI) of the fish showed no significant differences between the experimental groups (Table 4).

4. Discussion

Nowadays, several researchers are interested in searching suitable dietary additives to apply in aquaculture instead of synthetic antibiotics owing its extreme down-regulation of metabolic enzymes, muscular development, and a resurgence of virulent pathogens in aquatic biota [46,47]. For these factors, several countries completely prohibited the application of chemical drugs in aquaculture practices. Medicinal plants have been proven to have a positive effect on growth performance, immune systems, and diseases resistance of fish and shellfish [48–50]. The scientific community has therefore been searching for suitable feed additives that can improve both the immune systems and general wellbeing of fish. For the first time, the current study has been carried to evaluate the possibility of supplementing *E. scaber* on the performances of aquatic animals especially Nile tilapia (*O. niloticus*). *E. scaber* has been found to possess immunostimulant, antioxidative and antibacterial properties, which have been attributed to its content from terpenoids, steroids, flavonoids, phenols, sesquiterpene lactones, triterpenes, stigmasterol, epofriedelinol, and lupeol [28,51–53].

The studied immune parameters in this study exhibited significant enhancements in Nile tilapia after 4 and 8 weeks on feed supplemented with *E. scaber*. Lysozyme represents an important defense component which is responsible for the lysis of pathogenic bacteria [54]. In this study, fish fed with *E. scaber* demonstrated significantly enhanced lysozyme activity. The result was similar to previous studies reported in grouper, *Epinephelus bruneus* [55]; snakehead fish, *Channa argus* [56]; bulatmai barbel, *Luciobarbus capito* [57]; rainbow trout, *Oncorhynchus mykiss* [58,59], and common carp, *Cyprinus carpio* [60]. This type of natural complement activity has been proven to be one of the most significant methods of moving pathogenic bacteria from fish. Furthermore, its activation as an independent alternative complement pathway can be achieved through immunostimulants [61–63]. The present study has shown that alternative complement activity could be stimulated in both weeks four and eight, through the recommended *E. scaber* supplementary diets. This result is inconsistent with previous works [55,58,59]; in which the oral administration of medicinal herbs in grouper enhanced the alternate complement activity. Fish neutrophils contain various phagocytic, bactericidal, respiratory burst, and peroxidase activities [64–67]. Evaluation of the neutrophil function is necessary for the assessment of the general health of fish [68,69]. It was determined, herein, that the administration of all *E. scaber* doses appreciably enhanced serum peroxidase activity and respiratory burst activity after 4 and 8 weeks. Similarly, in grouper fed with a tea as a medicinal herb, peroxidase activity also rose after 4 weeks of feeding [55]. Respiratory burst, through stimulation by foreign agents, has been found to increase the oxidation levels in phagocytes, and are considered to be a crucial factor in the general defense mechanisms in fish [70,71]. The creation of respiratory burst activities and reactive oxygen metabolites by phagocytes are vital factors in limiting the spread of diseases in fish [65]. The present study recorded a significantly improved respiratory burst activity of Nile tilapia, *O. niloticus*; which was similar to the previous study in rainbow trout, *O. mykiss* [58]. Phagocytosis is an important cellular immune system component in fish [72–74]. Its role is to assist fish in avoiding pathogen attacks more efficiently by recognizing the existing pathogens and to limit their spread and progress [55]. Through the increase of phagocytosis, the present study has revealed that *E. scaber* promotes heightened immune responses and

Table 4
Growth performances and feed utilization (mean ± SE) of *O. niloticus* after 4 and 8 weeks feeding with experimental diets: Diet 1 (0 - control), Diet 2 (2.5 g kg^{-1} ESE), Diet 3 (5 g kg^{-1} ESE), Diet 4 (10 g kg^{-1} ESE), and Diet 5 (20 g kg^{-1} ESE). Different letter in a row denote significant difference ($P < 0.05$). Different letter in a row denote significant difference ($P < 0.05$).

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
IW (g)	13.98 ± 0.01	13.88 ± 0.04	13.88 ± 0.04	13.95 ± 0.02	13.88 ± 0.04
FW (g)					
4 weeks	42.53 ± 0.96^{c}	44.99 ± 0.27^{ab}	**46.49 ± 0.30^{a}**	45.85 ± 0.20^{ab}	44.18 ± 42^{b}
8 weeks	90.73 ± 0.42^{b}	90.47 ± 0.44^{b}	**97.87 ± 0.57^{a}**	96.17 ± 0.42^{a}	96.06 ± 2.63^{a}
WG (g)					
4 weeks	27.54 ± 0.95^{c}	31.11 ± 0.31^{ab}	**32.61 ± 0.29^{a}**	31.90 ± 0.22^{ab}	30.29 ± 0.41^{b}
8 weeks	76.74 ± 0.41^{b}	76.59 ± 0.41^{b}	**83.99 ± 0.56^{a}**	82.22 ± 0.43^{a}	82.18 ± 2.63^{a}
SGR (%)					
4 weeks	3.63 ± 0.07^{c}	3.92 ± 0.03^{ab}	**4.03 ± 0.02^{a}**	3.97 ± 0.02^{ab}	3.86 ± 0.03^{b}
8 weeks	3.11 ± 0.007^{b}	3.12 ± 0.006^{b}	**3.26 ± 0.010^{a}**	3.21 ± 0.009^{a}	3.22 ± 0.047^{a}
FCR					
4 weeks	1.50 ± 0.01^{a}	1.45 ± 0.01^{b}	**1.41 ± 0.02^{c}**	1.44 ± 0.02^{bc}	1.46 ± 0.01^{b}
8 weeks	1.57 ± 0.01^{a}	1.55 ± 0.01^{a}	**1.49 ± 0.01^{b}**	1.49 ± 0.02^{b}	1.50 ± 0.01^{b}
SR (%)					
4 weeks	100	100	**100**	100	100
8 weeks	97.33 ± 0.20	98.00 ± 0.13	**99.00 ± 0.13**	97.67 ± 0.07	98.00 ± 0.13
FI (g)					
4 weeks	108.55 ± 0.93	111.58 ± 0.78	115.25 ± 0.45	114.25 ± 0.67	114.25 ± 0.47
8 weeks	126.28 ± 1.09	132.69 ± 0.86	135.39 ± 0.88	132.85 ± 0.85	131.02 ± 0.40

IW = Initial weight; FW = Final weight; WG = Weight gain; SGR = Specific growth rate; FCR = Feed conversion ratio; SR = Survival rate; FI = Feed intake.

provides greater tolerance against infectious pathogens. Similarly, significant increase in phagocytosis activity by using medicinal plants have been recorded in gilthead seabream, *Sparus aurata* [75–77] and sea cucumber, *A. japonicus* [78]. Although the exact mechanism in which *E. scaber* enhanced serum immunity in fish is not clarified yet, it may be attributable to the presence of biological compounds in *E. scaber*. It has been demonstrated that flavonoid and saponin extracted from *E. scaber* could regulate the immune system, particularly the proliferation of immune cells [79]. High concentrations of flavonoids and alkaloids in *E. scaber* allow this plant to act as an anti-oxidant that is able to control the nitrix oxide activity [80]. In addition, flavonoids have the capability of initiating mitogen-activated protein kinase activity that can enhance IL-2 production [81]. Nonetheless, further studies are needed to clarify the mechanism in which *E. scaber* stimulate serum immunity in fish.

Skin mucus is an important element of innate immunity, and represents the first defensive stand against invading microorganisms, as it contains a diverse range of non-specific and specific immune factor which create a physio-chemical barrier that protects fish against infectious pathogens [82–84]. We found that the administration of supplementary *E. scaber* created remarkable boosts of mucus lysozyme and peroxidase activities. Similarly, beneficial effects of medicinal plants on mucus lysozyme and peroxidase activities have reported in Nile tilapia, *O. niloticus* fed *Cordyceps militaris* mushroom substrate [85]; common carp, *C. carpio* fed *Psidium guajava* and *Mespilus germanica* [60,86]; rainbow trout, *O. mykiss* fed *Myrtus communis* [87], and Indian major carp, *Labeo rohita* fed aloin [88]. This elevated mucus lysozyme and peroxidase activities may suggest the more idiosyncratic immunomodulatory character of *E. scaber*; as observed above, in the case of the humoral and mucosal immune parameters. It is well-established that fish's mucosal immune system could be triggered via the use of prebiotics, probiotics, and medicinal plants [89]. Skin-associated lymphoid tissues, (SALT) and gill-associated lymphoid tissues (GIALT), and gut-associated lymphoid tissues (GALT) are considering as immunological sites, which can boost a robust immune response against pathogens [89–92]. At an immunologically level, GALT is assembled of granulocytes, macrophages, lymphocytes, and plasma cells, as well as T and B cells. These cells along with epithelial cells, goblet cells, and neuroendocrine cells can generate and control gut immune responses [67,93]. Nonetheless, confirmation of this hypothesis would require future investigations serum and skin mucus immunities, as affected by the inclusion of dietary *E. scaber*.

The application of antibiotics and chemotherapeutics to monitor infectious diseases in aquaculture not only eradicates favourable microflora but also emerges the antimicrobial bacteria, as well as accumulates as residues in the human body [94]. These harmful effects of antibiotics and chemotherapeutics have attracted global attention to search for environmentally friendly manners as an alternative strategy in aquafeed [95]. In this sense, immunostimulants, prebiotics, probiotics, synbiotics, and unclassified dietary feed additives have been supplemented to the fish's diets. Similar to the growth-promoting drugs and synthetic antibiotics, these feed-additives mostly enhance growth performance and stimulate the immune system to combat pathogens. In this study, it is clear that *E. scaber* can be used as an immunostimulant in tilapia aquaculture. Tilapia mortality from *S. agalactiae* was decreased by dietary inclusion of *E. scaber*. Abdel-Tawwab et al. [96] also observed that inclusion of green tea in Nile tilapia diet presented commensurate decreases in fish mortality. Abdel-Tawwab et al. [96] and Sheikhzadeh et al. [97] indicated that Nile tilapia and rainbow trout exhibited enhanced resistance against bacterial infection by herbs feeding. It is probable that the addition of *E. scaber* prevents the assault of *S. agalactiae* infection by increasing the bactericidal activity, and the humoral and mucosal immune functions of Nile tilapia. It has been demonstrated that flavonoids from *E. scaber* could increase IL-2 activity and lymphocytes proliferation [98]. This IL-2 in turns can trigger the $CD8^{+}$ activation to $CD8^{+}$ produce perforin and granzyme that will destroy infected cells [99]. Moreover, previous studies showed that bioactive compounds isolated *E. scaber* possessed antibacterial activity against several bacteria. Daisy and Priya [100] reported that the novel terpenoid extracted from *E. scaber* showed strong antibacterial activity against several multi drug-resistance extended-spectrum beta-lactamases producing clinical isolates. Molecular docking investigations showed that lupeol from *E. scaber* can prohibit autolysin activity by forming a firm interaction with active site residues for treating *Staphylococcus aureus* [101]. Similarly, the methanolic extract from *E. scaber* showed strong antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella sonnei*, *Escherichia coli*, *Enterococcus faecalis*, and *Salmonella typhimurium* [30,102,103].

Growth performance and feed conversion ratio are essential parameters need to judge the potential use of feed additives in aqua-feed [104,105]. The present study determined that the dietary supplement of 5 g kg^{-1} *E. scaber* significantly improved the WG and SGR of Nile

tilapia, while concurrently reducing FCR. It has been demonstrated that the dietary inclusion of medicinal herbs improves WG and FCR by dietary tea is related to improved metabolic parameters or utilization of nutrients, and the activation of the functionality of intestinal flora [106–108]. Significant decreases in growth rates and feed utilization were present in the higher doses of tea within this study. Herbs have a high fiber content which may negatively affect the feed efficiency of fish, and growth performance accordingly [109]. Li, Tse and Li [106] reported that fish can consume up to 23% total dietary fibre before showing a decline in growth rate. Polyphenols have been found to exert their influence upon the emulsion interface, interacting with digestive enzymes, in order to decrease feed utilization and WG [110]. In addition, it is believe that medicinal herbs possess a special taste and smell, which play a vital r smell and taste, which play a crucial role in enhancing feed intake [111]. Also, herbal plants can motivate the release of digestive fluids and promote intestinal motility, which may consider as a reason for the increase of feed intake from their use [112]. However, the exact nature of these compounds remains unclear and requires further study.

In conclusion, the present study concluded that *E. scaber* supplementation may potentially activate the humoral, mucosal, and cellular immune mechanisms; generate disease resistance to *S. agalactiae* and improve growth rate and feed utilization.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

The study was performed in accordance with the guidelines on the use of animals for scientific purposes (Chiang Mai University).

Acknowledgments

The authors wish to the thank National Research Council of Thailand and the Functional Food Research Center for well-being, Chiang Mai University, Chiang Mai, Thailand for their financial assistance; as well as the staffs at Central and Biotechnology Laboratories, Faculty of Agriculture, Chiang Mai University for their kind support with the data analysis process.

References

[1] C. Chen, W. Guan, Q. Xie, G. Chen, X. He, H. Zhang, W. Guo, F. Chen, Y. Tan, Q. Pan, n-3 essential fatty acids in Nile tilapia, *Oreochromis niloticus*: bioconverting LNA to DHA is relatively efficient and the LC-PUFA biosynthetic pathway is substrate limited in juvenile fish, Aquaculture 495 (2018) 513–522.
[2] FAO, Globefish Highlights, F.a.A.O.o.t.U. Nations (Ed.), A Quarterly Update on World Seafood Markets, 2019.
[3] M. Chen, R. Wang, L.-P. Li, W.-W. Liang, J. Li, Y. Huang, A.-Y. Lei, W.-Y. Huang, X. Gan, Screening vaccine candidate strains against *Streptococcus agalactiae* of tilapia based on PFGE genotype, Vaccine 30 (42) (2012) 6088–6092.
[4] P. De Schryver, W. Verstraete, Nitrogen removal from aquaculture pond water by heterotrophic nitrogen assimilation in lab-scale sequencing batch reactors, Bioresour. Technol. 100 (3) (2009) 1162–1167.
[5] C.M.J. Delannoy, R.N. Zadoks, F.A. Lainson, H.W. Ferguson, M. Crumlish, J.F. Turnbull, M.C. Fontaine, Draft genome sequence of a nonhemolytic fish-pathogenic *streptococcus agalactiae* strain, J. Bacteriol. 194 (22) (2012) 6341–6342.
[6] E. Hardi, E. Harris, A. Lusiastuti, Characterization and phatogenicity of β and non haemolytic *Streptococcus agalactiae* in cultured Nila tilapia, J. Vet. 12 (2) (2011) 152–164.
[7] N.B. Buller, Bacteria and Fungi from Fish and Other Aquatic Animals: a Practical Identification Manual, Cabi2014.
[8] M.N.A. Amal, M. Zamri-Saad, A. Siti-Zahrah, A.R. Zulkafli, Transmission of *Streptococcus agalactiae* from a hatchery into a newly established red hybrid tilapia, *Oreochromis niloticus* (L.) × *Oreochromis mossambicus* (Peters), farm, J. Fish Dis. 36 (8) (2013) 735–739.
[9] D. Zhang, A. Li, Y. Guo, Q. Zhang, X. Chen, X. Gong, Molecular characterization of *Streptococcus agalactiae* in diseased farmed tilapia in China, Aquaculture 412– 413 (0) (2013) 64–69.
[10] A.M. Lusiastuti, M. Textor, H. Seeger, Ö. Akineden, M. Zschöck, The occurrence of *Streptococcus agalactiae* sequence type 261 from fish disease outbreaks of tilapia *Oreochromis niloticus* in Indonesia, Aquacult. Res. 45 (7) (2014) 1260–1263.
[11] P. Barato, E.R. Martins, J. Melo-Cristino, C.A. Iregui, M. Ramirez, Persistence of a single clone of *Streptococcus agalactiae* causing disease in tilapia (*Oreochromis* sp.) cultured in Colombia over 8 years, J. Fish Dis. 38 (12) (2015) 1083–1087.
[12] A.H. Al-Harbi, Phenotypic and genotypic characterization of *Streptococcus agalactiae* isolated from hybrid tilapia (*Oreochromis niloticus* × *O. aureus*), Aquaculture 464 (Supplement C) (2016) 515–520.
[13] Y. Ortega Asencios, F. Barreiro Sánchez, H. Bueno Mendizábal, K. Huancaré Pusari, H. Ostos Alfonso, A. Manchego Sayán, M.A. Pereira Figueiredo, W. Gómez Manrique, M.A. de Andrade Belo, N. Sandoval Chaupe, First report of *Streptococcus agalactiae* isolated from *Oreochromis niloticus* in Piura, Peru: molecular identification and histopathological lesions, Aquaculture Reports 4 (2016) 74–79.
[14] C. Faggio, M. Morabito, S.A. Minicante, G.L. Piano, M. Pagano, G. Genovese, Potential use of polysaccharides from the Brown alga *Undaria pinnatifida* as anticoagulants, Braz. Arch. Biol. Technol. 58 (2015) 798–804.
[15] F. Prestinaci, P. Pezzotti, A. Pantosti, Antimicrobial resistance: a global multifaceted phenomenon, Pathog. Glob. Health 109 (7) (2015) 309–318.
[16] A. Darwish, B. Griffin, Study shows oxyetracycline controls Streptococcus in tilapia, Global Aquaculture Advocate 5 (6) (2002) 34–35.
[17] A.M. Darwish, M.S. Hobbs, Laboratory efficacy of amoxicillin for the control of *Streptococcus iniae* infection in blue tilapia, J. Aquat. Anim. Health 17 (2) (2005) 197–202.
[18] H. Yuan, Q. Ma, L. Ye, G. Piao, The traditional medicine and modern medicine from natural products, Molecules 21 (5) (2016) 559.
[19] M. Ekor, The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety, Front. Pharmacol. 4 (2014) 177.
[20] MRFR, Herbal Medicine Market Research Report - Forecast to 2023, (2018).
[21] E. Awad, A. Awaad, Role of medicinal plants on growth performance and immune status in fish, Fish Shellfish Immunol. 67 (2017) 40–54.
[22] M. Wink, Modes of action of herbal medicines and plant secondary metabolites, Medicine 2 (3) (2015) 36.
[23] P.D. Gupta, T.J. Birdi, Development of botanicals to combat antibiotic resistance, J. Ayurveda Integr. Med. 8 (4) (2017) 266–275.
[24] C.I. Abuajah, A.C. Ogbonna, C.M. Osuji, Functional components and medicinal properties of food: a review, J. Food Sci. Technol. 52 (5) (2015) 2522–2529.
[25] K.B. Pandey, S.I. Rizvi, Plant polyphenols as dietary antioxidants in human health and disease, Oxidative Medicine and Cellular Longevity 2 (5) (2009).
[26] T. Citarasu, Herbal biomedicines: a new opportunity for aquaculture industry, Aquacult. Int. 18 (3) (2010) 403–414.
[27] R. Harikrishnan, C. Balasundaram, M.-S. Heo, Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish, Aquaculture 317 (1–4) (2011) 1–15.
[28] S.M. Hiradeve, V.D. Rangari, *Elephantopus scaber* Linn.: a review on its ethnomedical, phytochemical and pharmacological profile, J. Appl. Biomed. 12 (2) (2014) 49–61.
[29] C.-K. Chan, L.T.-H. Tan, S.N. Andy, M.N.A. Kamarudin, B.-H. Goh, H.A. Kadir, Anti-neuroinflammatory activity of *Elephantopus scaber* L. Via activation of Nrf2/HO-1 signaling and inhibition of p38 MAPK pathway in LPS-induced microglia BV-2 cells, Front. Pharmacol. 8 (397) (2017).
[30] A. Jenny, D. Saha, S. Paul, M. Dutta, M.Z. Uddin, A.K. Nath, Antibacterial activity of aerial part of extract of *Elephantopus scaber* Linn, Bull. Pharmaceut. Res. 2 (1) (2012) 38–41.
[31] AOAC, sixteenth ed., Official Methods of Analysis of AOAC International vol. 1, AOAC International, Arlington, 1995.
[32] H. Van Doan, S.H. Hoseinifar, C. Faggio, C. Chitmanat, N.T. Mai, S. Jaturasitha, E. Ringø, Effects of corncob derived xylooligosaccharide on innate immune response, disease resistance, and growth performance in Nile tilapia (*Oreochromis niloticus*) fingerlings, Aquaculture 495 (2018) 786–793.
[33] H. Van Doan, S.H. Hoseinifar, W. Tapingkae, S. Tongsiri, P. Khamtavee, Combined administration of low molecular weight sodium alginate boosted immunomodulatory, disease resistance and growth enhancing effects of *Lactobacillus plantarum* in Nile tilapia (*Oreochromis niloticus*), Fish Shellfish Immunol. 58 (2016) 678–685.
[34] H. Van Doan, W. Tapingkae, T. Moonmanee, A. Seepai, Effects of low molecular weight sodium alginate on growth performance, immunity, and disease resistance of tilapia, *Oreochromis niloticus*, Fish Shellfish Immunol. 55 (2016) 186–194.
[35] S. Chung, C.J. Secombes, Analysis of events occurring within teleost macrophages during the respiratory burst, Comp. Biochem. Physiol. Part B: Comparative Biochemistry 89 (3) (1988) 539–544.
[36] R.M. Parry Jr., R.C. Chandan, K.M. Shahani, A rapid and sensitive assay of muramidase, PSEBM (Proc. Soc. Exp. Biol. Med.) 119 (1965) 384–386 Society for Experimental Biology and Medicine (New York, N.Y.).
[37] M.J. Quade, J.A. Roth, A rapid, direct assay to measure degranulation of bovine neutrophil primary granules, Vet. Immunol. Immunopathol. 58 (3–4) (1997) 239–248.
[38] H. Cordero, A. Cuesta, J. Meseguer, M.A. Esteban, Changes in the levels of humoral immune activities after storage of gilthead seabream (*Sparus aurata*) skin mucus, Fish Shellfish Immunol. 58 (2016) 500–507.
[39] T. Yoshida, T. Kitao, The opsonic effect of specific immune serum on the phagocytic and chemiluminescent response in rainbow trout, *Oncorhynchus mykiss* phagocytes, Fish Pathol. 26 (1) (1991) 29–33.
[40] C.J. Secombes, T.C. Fletcher, The role of phagocytes in the protective mechanisms of fish, Annu. Rev. Fish Dis. 2 (1992) 53–71.
[41] T. Yanno, Assays of hemolitic complement activity, in: J.S. Stolen, T.C. Fletcher, D.P. Anderson, S.L. Kaatari, A.F. Roley (Eds.), Techniques in Fish Immunology,

SOS Publications, Fair Haven, NJ, 1992, pp. 131–141.
[42] H. Van Doan, S.H. Hoseinifar, C. Khanongnuch, A. Kanpiengjai, K. Unban, V. Van Kim, S. Srichaiyo, Host-associated probiotics boosted mucosal and serum immunity, disease resistance and growth performance of Nile tilapia (*Oreochromis niloticus*), Aquaculture 491 (2018) 94–100.
[43] B. Wang, Z. Gan, S. Cai, Z. Wang, D. Yu, Z. Lin, Y. Lu, Z. Wu, J. Jian, Comprehensive identification and profiling of Nile tilapia (*Oreochromis niloticus*) microRNAs response to *Streptococcus agalactiae* infection through high-throughput sequencing, Fish Shellfish Immunol. 54 (2016) 93–106.
[44] D.F. Amend, Potency testing of fish vaccines, Dev. Biol. Stand. 49 (1981) 8.
[45] SAS, SAS Institute Inc, SAS Campus Drive, Cary, NC USA, (2003) 27513-2414.
[46] M.A.O. Dawood, S. Koshio, M. Esteban, Beneficial roles of feed additives as immunostimulants in aquaculture: a review, Rev. Aquac. 0 (2017) 1–25 (n/a-n/a).
[47] M.A.O. Dawood, S. Koshio, M.M. Abdel-Daim, D.V. Hien, Probiotic application for sustainable aquaculture, Rev. Aquac. 0 (0) (2018).
[48] M. Adel, S. Yeganeh, M. Dadar, M. Sakai, M.A.O. Dawood, Effects of dietary *Spirulina platensis* on growth performance, humoral and mucosal immune responses and disease resistance in juvenile great sturgeon (*Huso huso* Linnaeus, 1754), Fish Shellfish Immunol. 56 (2016) 436–444.
[49] M.R. Saeidi asl, M. Adel, C.M.A. Caipang, M.A.O. Dawood, Immunological responses and disease resistance of rainbow trout (*Oncorhynchus mykiss*) juveniles following dietary administration of stinging nettle (*Urtica dioica*), Fish Shellfish Immunol. 71 (2017) 230–238.
[50] N. Van Hai, The use of medicinal plants as immunostimulants in aquaculture: a review, Aquaculture 446 (0) (2015) 88–96.
[51] C.K. Chan, H. Supriady, B.H. Goh, H.A. Kadir, *Elephantopus scaber* induces apoptosis through ROS-dependent mitochondrial signaling pathway in HCT116 human colorectal carcinoma cells, J. Ethnopharmacol. 168 (2015) 291–304.
[52] M.S. Djati, Implementing 'bio-Prospecting Reproductive Knowledge': an Effort Scenario to Conserved Indonesian Biodiversity and Endemicity toward a Post-modern Globalized World, AIP Conference Proceedings, AIP Publishing, 2017020002.
[53] J. Wang, P. Li, B. Li, Z. Guo, E.J. Kennelly, C. Long, Bioactivities of compounds from *Elephantopus scaber*, an ethnomedicinal plant from southwest China, Evid. Based Complement Altern. Med. 2014 (2014) 7.
[54] B. Magnadóttir, Innate immunity of fish (overview), Fish Shellfish Immunol. 20 (2) (2006) 137–151.
[55] R. Harikrishnan, J.-S. Kim, M.-C. Kim, C. Balasundaram, M.-S. Heo, *Prunella vulgaris* enhances the non-specific immune response and disease resistance of *Paralichthys olivaceus* against *Uronema marinum*, Aquaculture 318 (1–2) (2011) 61–66.
[56] M. Li, X. Zhu, J. Tian, M. Liu, G. Wang, Dietary flavonoids from *Allium mongolicum* Regel promotes growth, improves immune, antioxidant status, immune-related signaling molecules and disease resistance in juvenile northern snakehead fish (*Channa argus*), Aquaculture 501 (2019) 473–481.
[57] C. Wu, J. Shan, J. Feng, J. Wang, C. Qin, G. Nie, C. Ding, Effects of dietary *Radix Rehmanniae* Preparata polysaccharides on the growth performance, immune response and disease resistance of *Luciobarbus capito*, Fish Shellfish Immunol. 89 (2019) 641–646.
[58] Z. Mehrabi, F. Firouzbakhsh, G. Rahimi-Mianji, H. Paknejad, Immunostimulatory effect of *Aloe vera* (*Aloe barbadensis*) on non-specific immune response, immune gene expression, and experimental challenge with *Saprolegnia parasitica* in rainbow trout (*Oncorhynchus mykiss*), Aquaculture 503 (2019) 330–338.
[59] M. Naderi Farsani, S.H. Hoseinifar, G. Rashidian, H. Ghafari Farsani, G. Ashouri, H. Van Doan, Dietary effects of *Coriandrum sativum* extract on growth performance, physiological and innate immune responses and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Yersinia ruckeri*, Fish Shellfish Immunol. 91 (2019) 233–240.
[60] S.H. Hoseinifar, A. Sohrabi, H. Paknejad, V. Jafari, M. Paolucci, H. Van Doan, Enrichment of common carp (*Cyprinus carpio*) fingerlings diet with *Psidium guajava*: the effects on cutaneous mucosal and serum immune parameters and immune related genes expression, Fish Shellfish Immunol. 86 (2019) 688–694.
[61] A.E. Ellis, Immunity to bacteria in fish, Fish Shellfish Immunol. 9 (4) (1999) 291–308.
[62] R.E. Engstad, B. Robertsen, E. Frivold, Yeast glucan induces increase in lysozyme and complement-mediated haemolytic activity in Atlantic salmon blood, Fish Shellfish Immunol. 2 (4) (1992) 287–297.
[63] G. Jeney, D.P. Anderson, Glucan injection or bath exposure given alone or in combination with a bacterin enhance the non-specific defence mechanisms in rainbow trout (*Oncorhynchus mykiss*), Aquaculture 116 (4) (1993) 315–329.
[64] J. Lamas, A.E. Ellis, Atlantic salmon (*Salmo salar*) neutrophil responses to *Aeromonas salmonicida*, Fish Shellfish Immunol. 4 (3) (1994) 201–219.
[65] A. Rodríguez, M.Á. Esteban, J. Meseguer, Phagocytosis and peroxidase release by seabream (*Sparus aurata* L.) leucocytes in response to yeast cells, Anat. Rec. Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology 272A (1) (2003) 415–423.
[66] S.T. Solem, J.B. Jørgensen, B. Robertsen, Stimulation of respiratory burst and phagocytic activity in Atlantic salmon (*Salmo salar* L.) macrophages by lipopolysaccharide, Fish Shellfish Immunol. 5 (7) (1995) 475–491.
[67] E. Vallejos-Vidal, F. Reyes-López, M. Teles, S. MacKenzie, The response of fish to immunostimulant diets, Fish Shellfish Immunol. 56 (2016) 34–69.
[68] M. Abdel-Tawwab, M.N. Monier, S.H. Hoseinifar, C. Faggio, Fish Response to Hypoxia Stress: Growth, Physiological, and Immunological Biomarkers, Fish Physiology and Biochemistry, (2019).
[69] V. Aliko, M. Qirjo, E. Sula, V. Morina, C. Faggio, Antioxidant defense system, immune response and erythron profile modulation in gold fish, *Carassius auratus*, after acute manganese treatment, Fish Shellfish Immunol. 76 (2018) 101–109.
[70] T.-H. Lee, F. Qiu, G.R. Waller, C.-H. Chou, Three new flavonol galloylglycosides from leaves of *Acacia confusa*, J. Nat. Prod. 63 (5) (2000) 710–712.
[71] T. Miyazaki, A Simple Method to Evaluate Respiratory Burst Activity of Blood Phagocytes from Japanese Flounder, vol. 33, (1998), pp. 141–142 (3).
[72] M.A. Burgos-Aceves, A. Cohen, Y. Smith, C. Faggio, Estrogen regulation of gene expression in the teleost fish immune system, Fish Shellfish Immunol. 58 (2016) 42–49.
[73] E.R. Lauriano, S. Pergolizzi, G. Capillo, M. Kuciel, A. Alesci, C. Faggio, Immunohistochemical characterization of Toll-like receptor 2 in gut epithelial cells and macrophages of goldfish *Carassius auratus* fed with a high-cholesterol diet, Fish Shellfish Immunol. 59 (2016) 250–255.
[74] X. Zhang, H. Fan, Q. Zhong, Y.-c. Zhuo, Y. Lin, Z.-z. ZENG, Isolation, identification and pathogenicity of *Streptococcus agalactiae* from tilapia 5 (2008) 772–779.
[75] A. Fazio, R. Cerezuela, M.R. Panuccio, A. Cuesta, M.Á. Esteban, *In vitro* effects of Italian *Lavandula multifida* L. leaf extracts on gilthead seabream (*Sparus aurata*) leucocytes and SAF-1 cells, Fish Shellfish Immunol. 66 (2017) 334–344.
[76] J.M.G. Beltrán, C. Espinosa, F.A. Guardiola, M.Á. Esteban, In vitro effects of *Origanum vulgare* leaf extracts on gilthead seabream (*Sparus aurata* L.) leucocytes, cytotoxic, bactericidal and antioxidant activities, Fish Shellfish Immunol. 79 (2018) 1–10.
[77] F.A. Guardiola, A. Bahi, M.A. Esteban, Effects of dietary administration of fenugreek seeds on metabolic parameters and immune status of gilthead seabream (*Sparus aurata* L.), Fish Shellfish Immunol. 74 (2018) 372–379.
[78] H. Dang, T. Zhang, F. Yi, S. Ye, J. Liu, Q. Li, H. Li, R. Li, Enhancing the immune response in the sea cucumber *Apostichopus japonicus* by addition of Chinese herbs *Houttuynia cordata* Thunb as a food supplement, Aquaculture and Fisheries 4 (3) (2019) 114–121.
[79] D.R. Dwijayanti, M. Rifa'i, *Gynura procumbens* ethanolic extract promotes lymphocyte activation and regulatory T cell generation i*n vitro*, J. Tropical life Sci. 5 (1) (2015) 14–19.
[80] A. Subramanian, S.S. Kumar, S. Suja, M. Sudarshanand, A. Chakraborty, Antioxidant and free radical scavenging activity of annular and seasonal ayurvedic medicinal plants *Elephantopus scaber* L. and *Biophytum sensitivum* DC, Int. J. Pharm. Bio. Chem. Sci 1 (2014) 06–17.
[81] E. Middleton, C. Kandaswami, T.C. Theoharides, The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer, Pharmacol. Rev. 52 (4) (2000) 673–751.
[82] M.A.O. Dawood, S. Koshio, M. El-Sabagh, M.M. Billah, A.I. Zaineldin, M.M. Zayed, A.A.E.-D. Omar, Changes in the growth, humoral and mucosal immune responses following β-glucan and vitamin C administration in red sea bream, *Pagrus major*, Aquaculture 470 (2017) 214–222.
[83] F.A. Guardiola, A. Cuesta, M. Arizcun, J. Meseguer, M.A. Esteban, Comparative skin mucus and serum humoral defence mechanisms in the teleost gilthead seabream (*Sparus aurata*), Fish Shellfish Immunol. 36 (2) (2014) 545–551.
[84] F.A. Guardiola, C. Porcino, R. Cerezuela, A. Cuesta, C. Faggio, M.A. Esteban, Impact of date palm fruits extracts and probiotic enriched diet on antioxidant status, innate immune response and immune-related gene expression of European seabass (*Dicentrarchus labrax*), Fish Shellfish Immunol. 52 (2016) 298–308.
[85] H.V. Doan, S.H. Hoseinifar, W. Tapingkae, C. Chitmanat, S. Mekchay, Effects of *Cordyceps militaris* spent mushroom substrate on mucosal and serum immune parameters, disease resistance and growth performance of Nile tilapia, (*Oreochromis niloticus*), Fish Shellfish Immunol. 67 (Supplement C) (2017) 78–85.
[86] S.H. Hoseinifar, H. Khodadadian Zou, H. Kolangi Miandare, H. Van Doan, N. Romano, M. Dadar, Enrichment of common carp (*Cyprinus carpio*) diet with medlar (*Mespilus germanica*) leaf extract: effects on skin mucosal immunity and growth performance, Fish Shellfish Immunol. 67 (2017) 346–352.
[87] H. Mansouri Taee, A. Hajimoradloo, S.H. Hoseinifar, H. Ahmadvand, Dietary Myrtle (*Myrtus communis* L.) improved non-specific immune parameters and bactericidal activity of skin mucus in rainbow trout (*Oncorhynchus mykiss*) fingerlings, Fish Shellfish Immunol. 64 (2017) 320–324.
[88] A. Srivastava, A.K. Nigam, S. Mittal, A.K. Mittal, Role of aloin in the modulation of certain immune parameters in skin mucus of an Indian major carp, *Labeo rohita*, Fish Shellfish Immunol. 73 (2018) 252–261.
[89] C.M.A. Caipang, Nutritional impacts on fish mucosa: immunostimulants, pre- and probiotics, in: E.P. Benjamin, H. Beck (Eds.), Mucosal Health in Aquaculture, Academic Press, London, 2015.
[90] C. Sahlmann, B.J.G. Sutherland, T.M. Kortner, B.F. Koop, Å. Krogdahl, A.M. Bakke, Early response of gene expression in the distal intestine of Atlantic salmon (*Salmo salar* L.) during the development of soybean meal induced enteritis, Fish Shellfish Immunol. 34 (2) (2013) 599–609.
[91] H.K. Strand, R.A. Dalmo, Absorption of immunomodulating β(1,3)-glucan in yolk sac larvae of Atlantic halibut, *Hippoglossus hippoglossus* (L.), J. Fish Dis. 20 (1) (1997) 41–49.
[92] M. Bagni, N. Romano, M.G. Finoia, L. Abelli, G. Scapigliati, P.G. Tiscar, M. Sarti, G. Marino, Short- and long-term effects of a dietary yeast β-glucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass (*Dicentrarchus labrax*), Fish Shellfish Immunol. 18 (4) (2005) 311–325.
[93] D. Parra, F.E. Reyes-Lopez, L. Tort, Mucosal immunity and B cells in teleosts: effect of vaccination and stress, Front. Immunol. 6 (2015) 354.
[94] WHO, Report of a Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance, (2006) Seoul, Republic of Korea.
[95] M.A.O. Dawood, S. Koshio, Recent advances in the role of probiotics and prebiotics in carp aquaculture: a review, Aquaculture 454 (2016) 243–251.
[96] M. Abdel-Tawwab, M.H. Ahmad, M.E.A. Seden, S.F.M. Sakr, Use of green tea, *Camellia sinensis* L., in practical diet for growth and protection of Nile tilapia,

Oreochromis niloticus (L.), against *Aeromonas hydrophila* infection, J. World Aquac. Soc. 41 (SUPPL. 2) (2010) 203–213.
[97] N. Sheikhzadeh, K. Nofouzi, A. Delazar, A.K. Oushani, Immunomodulatory effects of decaffeinated green tea (*Camellia sinensis*) on the immune system of rainbow trout (*Oncorhynchus mykiss*), Fish Shellfish Immunol. 31 (6) (2011) 1268–1269.
[98] Q.-L. Liang, Z.-D. Min, Y.-P. Tang, A new elemanolide sesquiterpene lactone from *Elephantopus scaber*, J. Asian Nat. Prod. Res. 10 (5) (2008) 403–407.
[99] A.K. Abbas, A.H. Lichtman, S. Pillai, Cellular and Molecular Immunology, Saunders Elsevier, 2007.
[100] P. Daisy, C.E. Priya, Hypolipidemic and renal functionality potentials of the hexane extract fractions of *Elephantopus scaber* Linn, Int. J. Biomed. Sci.: IJBS 6 (3) (2010) 241.
[101] P. Daisy, S. Suveena, V. Lilly Sr., Molecular docking of medicinal compound Lupeol with autolysin and potential drug target of UTI, J. Chem. Pharm. Res. 3 (2011) 557–562.
[102] S. Koppula, K. Ammani, V. Bobbarala, Y. Usha, In vitro antibacterial screening of five local medicinal plants of araku environment, Drug Invent. Today 3 (3) (2011).
[103] V.T. Anitha, J. Marimuthu, S. Jeeva, Anti–bacterial studies on *Hemigraphis colorata* (blume) HG hallier and *Elephantopus scaber* L, Asian Pacific journal of tropical medicine 5 (1) (2012) 52–57.
[104] S.H. Hoseinifar, S. Yousefi, G. Capillo, H. Paknejad, M. Khalili, A. Tabarraei, H. Van Doan, N. Spanò, C. Faggio, Mucosal immune parameters, immune and antioxidant defence related genes expression and growth performance of zebrafish (*Danio rerio*) fed on *Gracilaria gracilis* powder, Fish Shellfish Immunol. 83 (2018) 232–237.
[105] G. Rashidian, S. Bahrami Gorji, M.N. Farsani, M.D. Prokić, C. Faggio, The Oak (*Quercus Brantii*) Acorn as a Growth Promotor for Rainbow Trout (*Oncorhynchus mykiss*): Growth Performance, Body Composition, Liver Enzymes Activity and Blood Biochemical Parameters, (2018), pp. 1–11.
[106] S. Li, I.M.Y. Tse, E.T.S. Li, Maternal green tea extract supplementation to rats fed a high-fat diet ameliorates insulin resistance in adult male offspring, J. Nutr. Biochem. 23 (12) (2012) 1655–1660.
[107] M.H. Li, D.F. Oberle, P.M. Lucas, Effects of dietary fiber concentrations supplied by corn bran on feed intake, growth, and feed efficiency of channel catfish, N. Am. J. Aquacult. 74 (2) (2012) 148–153.
[108] J.H. Weisburger, F.-L. Chung, Mechanisms of chronic disease causation by nutritional factors and tobacco products and their prevention by tea polyphenols, Food Chem. Toxicol. 40 (8) (2002) 1145–1154.
[109] S.H. Cho, S.-M. Lee, B.H. Park, S.-C. Ji, J. Lee, J. Bae, S.-Y. Oh, Effect of dietary inclusion of various sources of green tea on growth, body composition and blood chemistry of the juvenile olive flounder, *Paralichthys olivaceus*, Fish Physiol. Biochem. 33 (1) (2007) 49–57.
[110] P. Bandyopadhyay, A.K. Ghosh, C. Ghosh, Recent developments on polyphenol–protein interactions: effects on tea and coffee taste, antioxidant properties and the digestive system, Food & function 3 (6) (2012) 592–605.
[111] L. Zhang, H. Zeng, J. Zhang, Q. Ai, K. Mai, A study on feeding attraction activity of Chinese herbal medicine for Japanese sea bass *Lateolabrax japonieus*, Mar. Fish. Res. 29 (4) (2008) 101–104.
[112] H. Pu, X. Li, Q. Du, H. Cui, Y. Xu, Research progress in the application of Chinese herbal medicines in aquaculture: a review, Engineering 3 (5) (2017) 731–737.