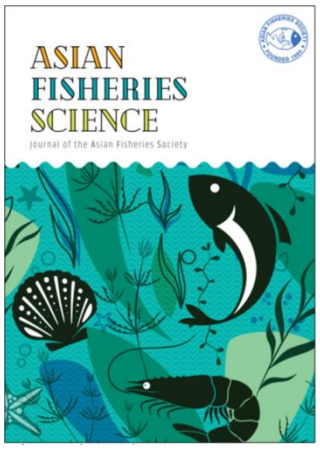

Enhanced Growth Performance, Haemato-biochemical and Immune Parameters of Asian Seabass, *Lates calcarifer* (Bloch, 1790) Fed Dietary Supplementation with *Polygonum chinense*

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Abstract

The present study evaluated the effects of *Polygonum chinense* extract (PCE) on the growth performance, haemato-biochemical and innate immunity parameters of Asian seabass, *Lates calcarifer* (Bloch, 1790). The fish with an average weight of 9.5 ± 0.2 g were fed for 60 days using three supplementation diets of PCE at 0.2 %, 0.5 % and 1.0 %, and a non-supplemented diet as a control. Fish fed on 0.5 % PCE diet revealed significant higher body weight gain, specific growth rate, protein efficiency ratio, condition factor, and significantly lower feed conversion ratio. Dietary supplementation with 0.5 and 1.0 % showed a significant increase in red blood cell count and haemoglobin level, but the white blood cell count significantly increased only in 1.0 % diet. The alkaline phosphatase level significantly increased in fish group fed 0.2 and 0.5 % diets, but the aspartate transaminase level significantly decreases in 0.2 and 1.0 % groups. Meanwhile, all PCE supplemented diets showed significant increase in total protein, albumin, globulin, albumin: globulin ratio, total immunoglobulin, lysozyme, respiratory burst and phagocytic activities, and significantly reduced the alkaline transaminase level. These results suggest that dietary *P. chinense* supplementation at 0.5 % was found to be suitable to enhance the growth performance and immune status of Asian seabass.

Keywords: fish; plant extract; growth trial; feed evaluation; haematology; innate immune response

Introduction

Asian seabass or commonly known as barramundi is an economically important species in Malaysia and other countries in the Indo-Pacific region (Mathew 2009). This euryhaline species is being extensively or intensively cultured in earthen ponds and sea cages using fresh, brackish or marine water (Glencross 2006; Hutson 2014). Intensification of fish production together with poor environmental conditions and management increases the susceptibility of the fish to pathogens, which lead to disease outbreak (Chen 1991). Numerous diseases have been reported in Asian seabass, and the causative agents varied from parasites, bacteria, fungi, viruses, malnutrition or environmental stresses (Talpur and Ikhwanuddin 2012). These aquatic diseases are being controlled with the use of antibiotics, chemicals, disinfectants and chemotherapeutics (Austin and Austin 2007; Rico et al. 2012, 2013; Reverter et al. 2014). However, the indiscriminate use of antibiotics have resulted in the development of resistant fish bacterial pathogens (Cabello 2006). With the increasing awareness on the negative impact of antibiotics usage, researchers have been inspired to find natural alternatives to overcome the disease problems in aquaculture. One of the natural solutions for controlling and preventing fish diseases is the development of immunostimulant diets (Mastan 2015).

Immunostimulant diets using natural products and plant extracts have shown promising results in improving the growth and immune response of fish. A large numbers of plant products have been investigated for growth performance and immune response in many fish species: *Aloe barbadensis* (Adegbesan et al. 2018) and *Vernonia amygdalina* and *Carica papaya* (Olusola and Nwokike 2018) in *Clarias gariepinus* (Burchell, 1822) (African catfish); *Cissus quadrangularis* (Devakumar and Chinnasamy 2017) and *Camellia sinensis* (Al Ngada et al. 2017) in *Lates calcarifer* (Bloch, 1790) (Asian seabass); *Nigella sativa* (Altunoglu et al. 2017), *Capparis spinosa*

(Bilen et al. 2016) and *Stachys lavandulifolia* (Moghanlou et al. 2018) in *Oncorhynchus mykiss* (Walbaum, 1792) (rainbow trout).

One of the local herbal plants *Polygonum chinense* used for the treatment of enteritis, diarrhoea, hepatitis and dysentery (Li 1994; Ren and He 2001; Tang 2003) was selected for the present study. *Polygonum chinense* known as Chinese knotweed belongs to the family Polygonaceae (Ezhilan and Neelamegam 2012) can be found in the sub-tropical and warm temperate regions of Asia such as China, Japan, India, Indonesia, Malaysia and Vietnam (Maharajan et al. 2012). More recently, Xiao et al. (2013) have proven that *P. chinense* contained two components, ellagic acid and corilagin which contributes to its anti-diarrheal effect. Moreover, studies on rats that received a diet containing *P. chinense* extract could significantly prevent gastric ulcer (Ismail et al. 2012) and protect against induced liver injury (Das 2015). Furthermore, Maharajan et al. (2012) found that ethanolic extract of *P. chinense* contained antibacterial and antifungal properties. Despite the excellent potential of medicinal properties of this plant, as yet no study has evaluated its potential use as a growth enhancer and immunostimulant in aquaculture. Therefore, the present study was aimed to investigate the effects of different doses of *P. chinense* supplementary diet on the growth performance, haemato-biochemical parameters and immune status of *L. calcarifer*.

Materials and Methods

Plant material and preparation of the plant extract

Ten kilograms of fresh leaves of *P. chinense* were purchased from a local plant nursery in Johor, Malaysia. Identification of the plant was carried out by the staff of University Agriculture Park, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. The samples were washed with distilled water, dried under shade and coarsely powdered using an electric grinder. The samples were homogenised with 80 % methanol at a ratio of 1:10 ($w.v^{-1}$) and shaken for 48 h at room temperature (Eloff 1998). The crude extracts were centrifuged at 3000 g for 20 min and filtered through a Whatman filter paper (Whatman, Maidstone, England). These procedures were repeated thrice to obtain the maximum extract. The solvent was evaporated using a rotary vacuum evaporator (Rotavapor R-215, Buchi, Switzerland) at 30 - 35 °C and the residues obtained were freeze-dried (Labconco, USA) to powder form. The powdered extracts were stored at -20 °C until use.

Diet preparation

A commercial basal diet (Star Feed, Star Feedmills (M) Sdn Bhd, Malaysia) was purchased from a local fish pellet supplier in Rawang, Selangor, Malaysia. The composition of this commercial diet was 42 % crude protein, 6 % crude fat, 12 % moisture, 5 % crude fibre and 10 % crude ash. The feed pellet was coarsely powdered using an electric household blender, and PCE was added at 0.2 %, 0.5 % and 1.0 % as a supplement. The non-supplemented basal diet was treated as a control. The diets were manually pelleted using a mould for pressing cakes with the inclusion of 40 % distilled water to achieve proper pelleting consistency. The pellets were dried in an oven overnight at 35 °C and stored at 4 °C until use.

Experimental system and fish husbandry

Four hundred and eighty Asian seabass juveniles with an average weight of 9.5 ± 0.2 g were purchased from a local fish farm (Banting, Selangor, Malaysia) and transferred to Aquatic Animal Health Unit, UPM, Selangor, Malaysia. On arrival, the fish were kept for 2 weeks in two 1000 L fibre glass tanks for acclimatisation and fed with commercial diet, twice daily. After acclimatisation, the fish were randomly distributed into 200 L aquarium tanks allotted for the three treatments and the control in triplicates (40 fish per tank). The fish were hand fed for 60 days with their respective diet at 4 % of body weight, twice daily (9.00 am and 4.00 pm). The fish were maintained at water temperature of 29.1 ± 0.3 ºC, dissolved oxygen 6.14 ± 0.13 $mg.L^{-1}$, pH 7.2 ± 0.1, salinity 21.4 ± 0.5 $g.L^{-1}$ (YSI multi-probe system, YSI Inc., USA) and total ammonia 0.68 ± 0.09 $mg.L^{-1}$ (API® Ammonia Test Kit, USA). Each tank was supplied with filtered seawater, continuous aeration through air diffuser stones and a filter and protein skimmer were fixed to maintain good water quality. The water in the tank was partially (40-50 %) replaced daily in the evening throughout the experiment. The Institutional Animal Care and Use Committee of Universiti Putra Malaysia approved all procedures involving Asian seabass treatments (UPM/IACUC/AUP-R077/2017).

Growth performance and survival

At days 30 and 60, the body weight gain (BWG), specific growth rate (SGR), feed efficiency (FE), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF), thermal growth condition (TGC) and survival of the different groups were calculated according to the following equations (Bullerwell et al. 2016; El Basuini et al. 2016):

BWG = Final body weight (ωf) – Initial body weight (ωi);

SGR = ln ωf – ln ωi / Number of days (t) × 100;

FE = Final weight of feed (ff) – Initial weight of feed (fi) / number of fish;

FCR = ff - fi / ωf - ωi;

PER = ωf – ωi / Feed consumed × percentage of protein in feed;

CF = Body weight (g) / [Standard length] 3 (cm) × 100;

TGC = ($\sqrt[3]{\omega f}$ - $\sqrt[3]{\omega i}$ / Temperature × t) × 1000;

Survival (%) = Final number of fish – Initial number of fish × 100.

Blood sampling

At day 30 and 60, five fish caught randomly from each treatment group and anesthetised with MS-222 (0.1 $mg.L^{-1}$) for the collection of blood samples. One millilitre of blood was drawn from the caudal vein with a 25G needle (Terumo, Australia) and 1 ml syringe (Terumo, Australia). For each treatment, blood samples were pooled from the five fish and were divided into two halves. One half of the blood sample was treated with heparin (BD Vacutainer® Lithium Heparin^{N} (LH), Becton Dickinson, USA) for immediate analysis of haematological parameters, and respiratory and phagocytic activities. The other half was transferred to a plain tube for biochemical and immunological studies. The blood specimens were left to coagulate at room temperature for 1 h, followed by overnight at 4 °C, centrifuged at 3000 g for 15 min, and the serum obtained was kept at -80 °C until assayed (Adel et al. 2015).

Determination of haematological and biochemical parameters

The collected blood samples were immediately subjected to haematological analysis. The red blood cells (RBC), white blood cells (WBC) and haemoglobin (Hb) were analysed using Cell-Dyn® 3700 Haematology Analyser (Abbott Diagnostics, USA). Haematocrit (Ht) or packed cell volume (PCV) was analysed using a micro-haematocrit reader (Hawksley Micro Haematocrit Reader, Hawksley and Sons Ltd, UK). The mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) were calculated according to the following formulae:

1) $\mathrm{MCV\ (fL)} = (\mathrm{PCV} \times 1000)/\mathrm{RBC}$

2) $\mathrm{MCHC\ (g/L)} = \mathrm{Hb/PCV}$

The blood serum was used to determine the total glucose (GLU), total protein (TP), albumin (ALB), alkaline transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) using automatic chemistry analyser (Biolis 24i Premium, Tokyo Boeki Machinery Ltd, Japan) and using optimised tests of Boehringer Mannheim GmBH by means of spectrophotometers. The globulin (GLO) was calculated by subtracting ALB value from the TP value, and albumin: globulin ratio (A: G) was calculated by dividing ALB value with GLO value.

Determination of immune parameters

Respiratory burst activity

The respiratory burst activity of the phagocytes was done by nitroblue tetrazolium (NBT) assay following the method of Anderson and Siwicki (1995). Blood (100 µL) was placed into a microtiter plate well, and 100 µl of 0.2 % NBT solution was added. The plate was incubated at room temperature for 30 min. After incubation, 50 µL of NBT-blood cell suspension was taken and added into a glass tube containing 1.0 ml N, N-dimethyl formamide (DMF). The suspension was centrifuged at 3000 g for 5 min. The optical density of the supernatant was recorded in a spectrophotometer at 540 nm using a glass cuvette.

Serum lysozyme activity assay

An assay based on the lysis of *Micrococcus lysodeikticus* was used for determining serum lysozyme activity. The serum lysozyme activity was measured according to the method of Ellis (1990) with some modifications. Briefly, 0.015% ($w.v^{-1}$) *M. lysodeikticus* (ATCC No. 4698, Merck, Germany) in 50 mM potassium phosphate buffer (pH 6.2) was used as a substrate. Fifty microlitres of fish serum was added to 950 µL of bacterial suspension, and the reduction of absorbance at 450 nm was determined after 1 min and 5 min of incubation using a spectrophotometer (Shimadzu, Japan). A unit of lysozyme activity was defined as the amount of sample causing a decrease in absorbance of 0.001 per min.

Phagocytosis assay

The phagocytosis activity of fish blood was determined according to Anderson and Siwicki (1995) with some modifications. One hundred microlitres of blood was placed into a microtiter plate well, followed by 100 µL of *Vibrio harveyi* (1×10^{7} $CFU.mL^{-1}$) suspended in phosphate buffered saline (PBS). The blood-bacteria suspension was mixed with a pipette, then the plate was incubated at room temperature for 20 min. After incubation, 5 µL of the suspension was removed and place on a glass slide to make a blood smear, and air dried at room temperature. The slide was fixed with ethyl alcohol (95 %) for 5 mins and air dried. Then, the slide was stained with 7 % Giemsa's stain for 10 min. Three smears were made from each group of fish. A total of 100 cells from each smear were observed under a microscope (Nikon Eclipse 80i, Japan) and the percentage of phagocytic activity was derived by dividing the number of phagocytising cells by 100.

Serum total immunoglobulin assay

The total immunoglobulin was quantified based on the biuret method by Anderson and Siwicki (1995). One hundred microliters of serum and 100 µL of 12 % polyethylene glycol (PEG) were added into a centrifuge tube. After 2 h of incubation at room temperature with continuous shaking, the tube was centrifuged at 5000 g for 10 min. The supernatant was removed, and the protein concentration was determined. The value obtained from the analysis was subtracted from the total protein level, to give the total immunoglobulin concentration of the serum.

Statistical analysis

All data obtained were checked for homogeneity of variances (Levene) and normality (Kolmogorov–Smirnov) prior to statistical analysis. The statistical analysis between dietary groups was then analysed using one-way variance (ANOVA) followed by Duncan's multiple range comparison test using IBM SPSS Statistics 21.0 (IBM Corporation, 2012) computer software.

Results

Growth performance, feed utilisation and survival

The effects of experimental diets containing PCE on growth performance of Asian seabass are summarised in Table 1. Fish fed on 0.5 % PCE had significantly higher BWG, SGR, FE and PER compared to other experimental diets on day 30 and 60. Similarly, the TGF was significantly higher, and the FCR was significantly lower in all treatment diets on day 30 and 60. However, the CF was not significantly different ($P > 0.05$) in all experimental diets on day 30, but significantly higher in fish fed on 0.5 % PCE on day 60. After 60 days of feeding, the survival rate was significantly higher in 0.2 % and 0.5 % compared with the control.

Haematological and biochemical parameters

On days 30 and 60, the RBC content, Hb and PCV levels were significantly higher in fish fed with 0.2 %, 0.5 % and 1.0 % PCE supplementation diets. The WBC content was significantly higher in all treatment diets on day 30, but only in 1.0 % PCE supplementation diet on day 60. However, the MCV and MCHC levels not significantly affected by dietary inclusion of PCE on days 30 and 60 (Table 2). Feeding with 1.0 % PCE significantly reduced the GLU level on days 30 and 60. Moreover, the TP, ALB, GLO, A:G ratio and ALP levels significantly increased, but the AST and ALT levels were significantly reduced in fish fed with all doses of PCE diets on day 60 (Table 3).

Immune status

The serum lysozyme activity significantly increased in fish fed with 1.0 % PCE on day 30 (Fig. 1a). Likewise, on day 60, significantly higher serum lysozyme activity and total immunoglobulin were noticed in all treatment groups compared to the control group. However, there were no significant variations in the respiratory burst activity and total immunoglobulin of all experimental diets on day 30 (Figs. 1b and 1d). The phagocytic activity increased in 0.2, 0.5 and 1.0 % PCE diet supplementations as compared to the control group on days 30 and 60 (Fig. 1c).

Table 1. Effects of feeding *Polygonum chinense* extract on the growth performance and feed utilisation of Asian seabass, *Lates calcarifer* over 60 days period.

	Experimental diet (%)			
	0	0.2	0.5	1.0
Body weight gain (g.fish^{-1})				
Day 30	6.10 ± 0.67^{a}	7.97 ± 0.54^{a}	14.89 ± 1.61^{b}	8.93 ± 1.04^{a}
Day 60	28.82 ± 1.88^{a}	33.59 ± 2.18^{ab}	47.24 ± 1.59^{c}	38.81 ± 2.36^{b}
Specific growth rate				
Day 30	0.57 ± 0.05^{a}	0.74 ± 0.04^{ab}	1.03 ± 0.08^{c}	0.77 ± 0.07^{b}
Day 60	0.86 ± 0.04^{a}	0.96 ± 0.03^{b}	1.14 ± 0.02^{c}	1.03 ± 0.03^{b}
Feed efficiency				
Day 30	8.14 ± 0.89^{a}	11.02 ± 0.72^{ab}	18.91 ± 2.15^{c}	11.91 ± 1.39^{ab}
Day 60	22.17 ± 1.44^{a}	26.06 ± 1.68^{ab}	35.80 ± 1.22^{c}	29.86 ± 1.81^{bc}
Feed conversion ratio				
Day 30	2.96 ± 0.17^{c}	2.47 ± 0.17^{abc}	1.81 ± 0.32^{a}	2.80 ± 0.39^{bc}
Day 60	2.07 ± 0.20^{c}	1.7 ± 0.12^{b}	1.17 ± 0.04^{a}	1.47 ± 0.09^{ab}
Protein efficiency ratio				
Day 30	0.15 ± 0.02^{a}	0.20± 0.01^{a}	0.34 ± 0.04^{b}	0.21 ± 0.02^{a}
Day 60	0.69 ± 0.04^{a}	0.81 ± 0.05^{ab}	1.11 ± 0.04^{c}	0.92 ± 0.06^{b}
Condition factor				
Day 30	1.36 ± 0.04^{a}	1.36 ± 0.04^{a}	1.38 ± 0.02^{a}	1.40 ± 0.04^{a}
Day 60	1.24 ± 0.02^{a}	1.26 ± 0.03^{a}	1.39 ± 0.02^{b}	1.31 ± 0.03^{a}
Thermal growth coefficient				
Day 30	0.360 ± 0.040^{a}	0.638 ± 0.140^{b}	0.732 ± 0.066^{b}	0.518 ± 0.005^{b}
Day 60	0.650 ± 0.031^{a}	0.676 ± 0.076^{ab}	0.892 ± 0.019^{c}	0.801 ± 0.003^{bc}
Survival (%)				
Day 60	91.25 ± 0.40^{a}	97.50 ± 0.00^{b}	98.75 ± 0.40^{b}	93.75 ± 0.40^{a}

Data are mean ± SE (n = 15) and different superscripts within rows indicate significant differences ($P < 0.05$) between means among dietary treatments but not between parameters.

Discussion

Dietary application of medicinal plants have shown positive effects on the fish growth (Abdel-Tawwab et al. 2010; Yilmaz and Ergün, 2012; Samad et al. 2014; Giri et al. 2015; Ngugi et al. 2017). In the present study, the growth of Asian seabass was enhanced by the administration of the methanolic extract of *P. chinense* into the diet. The significant increase of BWG, SGR, FE, PER, CF, TGC and survival indicated that diet supplementation with 0.5 % PCE for 60 days promoted the growth and nutrient utilisation in the fish. A similar result was reported by Giri et al. (2015) that the BWG and SGR of rohu, *Labeo rohita* (Hamilton, 1822) significantly increased after dietary supplemented with 0.5 % *Psidium guajava* extract for 60 days. Abdel-Tawwab et al. (2010) also demonstrated that Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fed with 0.5 % green tea, *Camellia sinensis* extract had significant higher BWG, SGR, FCR, PER, feed intake and energy utilisation. The high

Table 2. Effects of feeding *Polygonum chinense* extract on the haematological parameters of Asian seabass, *Lates calcarifer* over 60 days period.

	Experimental diets (%)			
	0	0.2	0.5	1.0
Red blood cell ($\times 10^{12}$ L^{-1})				
Day 30	3.3 ± 0.1^{a}	3.7 ± 0.1^{b}	3.9 ± 0.1^{c}	3.8 ± 0.1^{bc}
Day 60	3.6 ± 0.1^{a}	4.1 ± 0.1^{b}	4.4 ± 0.1^{c}	4.3 ± 0.1^{bc}
Haemoglobin (g.L^{-1})				
Day 30	77.5 ± 1.5^{a}	89.5 ± 0.3^{b}	92.5 ± 3.5^{b}	89.0 ± 2.0^{b}
Day 60	93.0 ± 1.0^{a}	110.5 ± 5.5^{b}	120.0 ± 4.0^{b}	115.0 ± 4.0^{b}
PCV (L.L^{-1})				
Day 30	0.27 ± 0.01^{a}	0.30 ± 0.01^{b}	0.32 ± 0.01^{c}	0.30 ± 0.01^{b}
Day 60	0.30 ± 0.01^{a}	0.34 ± 0.04^{b}	0.37 ± 0.01^{c}	0.36 ± 0.01^{bc}
MCV (fL)				
Day 30	77.5 ± 1.5^{a}	79.8 ± 2.4^{a}	81.1 ± 0.7^{a}	77.5 ± 0.1^{a}
Day 60	83.1 ± 0.9^{a}	81.6 ± 0.7^{a}	82.8 ± 0.1^{a}	82.9 ± 0.1^{a}
MCHC (g.L^{-1})				
Day 30	287.0 ± 2.3^{a}	303.4 ± 2.0^{a}	293.6 ± 0.1^{a}	301.7 ± 1.7^{a}
Day 60	310.0 ± 3.3^{a}	330.2 ± 8.7^{a}	328.7 ± 6.5^{a}	324.0 ± 2.4^{a}
White blood cells ($\times 10^{7}$ L^{-1})				
Day 30	2.8 ± 0.1^{a}	3.2 ± 0.1^{b}	3.2 ± 0.1^{b}	3.6 ± 0.1^{c}
Day 60	2.9 ± 0.3^{a}	3.3 ± 0.5^{a}	3.1 ± 0.3^{a}	5.1 ± 0.7^{b}

Data are mean ± SE (n = 3) and different superscripts within rows indicate significant differences (P < 0.05) between means among dietary treatments but not between parameters.

amount of bioactive compounds in *P. chinense* that possessed antimicrobial properties (Ezhilan and Neelamegam 2012) may account for the improved fish growth performance.

The evaluation of haematological and biochemical parameters in fish blood has been used to monitor fish health (De Pedro et al. 2005) as the changes in these parameters can be an indicator for stress (Kumar and Banerjee 2016; Odedeyi and Odo 2017) or diseases infection (Aydin et al. 2005; Sebastião et al. 2011). In the present study, feed supplementation with PCE did not affect the haematological parameters except for RBC and WBC counts, and Hb level. According to Moghanlou et al. (2018), the increase of these haematological parameters indicates the immunostimulant effects and anti-infection properties of the plant. Similar findings of immunostimulant effects and anti-infection

Table 3. Effects of feeding *Polygonum chinense* extract on the biochemical parameters of Asian seabass, *Lates calcarifer* over 60 days period.

	Experimental diets (%)			
	0	0.2	0.5	1.0
Glucose (mmol.L^{-1})				
Day 30	3.7 ± 0.3^{b}	3.1 ± 0.2^{ab}	3.6 ± 0.5^{ab}	2.5 ± 0.3^{a}
Day 60	1.6 ± 0.1^{b}	1.2 ± 0.1^{ab}	1.2 ± 0.1^{ab}	1.0 ± 0.1^{a}
Total protein (g.L^{-1})				
Day 30	45.0 ± 0.6^{a}	50.6 ± 1.9^{b}	53.6 ± 3.1^{b}	44.5 ± 1.3^{a}
Day 60	48.5 ± 1.8^{a}	58.1 ± 0.1^{b}	58.5 ± 0.5^{b}	58.3 ± 0.4^{b}
Albumin (g.L^{-1})				
Day 30	10.4 ± 0.1^{a}	11.6 ± 0.9^{a}	13.0 ± 0.7^{a}	11.2 ± 0.4^{a}
Day 60	13.6 ± 0.4^{a}	18.4 ± 0.3^{b}	19.9 ± 0.4^{c}	18.9 ± 0.2^{b}
Globulin (g.L^{-1})				
Day 30	34.5 ± 0.8^{b}	39.0 ± 1.1^{b}	40.6 ± 1.9^{b}	33.3 ± 0.9^{a}
Day 60	35.0 ± 1.4^{a}	39.7 ± 0.2^{b}	38.6 ± 0.3^{b}	39.4 ± 0.2^{b}
Albumin: globulin ratio (g.L^{-1})				
Day 30	0.29 ± 0.0^{a}	0.30 ± 0.02^{a}	0.31 ± 0.01^{a}	0.34 ± 0.04^{b}
Day 60	0.39 ± 0.01^{a}	0.46 ± 0.01^{b}	0.51 ± 0.01^{c}	0.48 ± 0.01^{b}
Alkaline phosphate (U.L^{-1})				
Day 30	57.8 ± 4.6^{a}	68.5 ± 1.2^{b}	62.3 ± 2.6^{a}	61.8 ± 1.3^{a}
Day 60	46.3 ± 2.3^{a}	67.8 ± 2.3^{c}	57.0 ± 2.0^{b}	61.3 ± 4.1^{c}
Aspartate aminotransferase (U.L^{-1})				
Day30	120.0 ± 1.6^{b}	77.8 ± 3.3^{a}	73.8 ± 3.4^{a}	71.5 ± 5.7^{a}
Day 60	111.8 ± 4.5^{b}	72.8 ± 3.9^{a}	77.8 ± 2.4^{a}	74.0 ± 0.4^{a}
Alanine aminotransferase (U.L^{-1})				
Day 30	46.3 ± 2.5^{b}	40.8 ± 1.8^{ab}	42.5 ± 1.8^{ab}	37.0 ± 0.4^{a}
Day 60	50.8 ± 2.3^{b}	41.5 ± 0.6^{a}	34.3 ± 1.7^{a}	35.0 ± 2.5^{a}

Data are mean ± SE (n = 3) and different superscripts within rows indicate significant differences (P < 0.05) between means among dietary treatments but not between parameters.

properties were reported in catfish, *Clarias gariepinus* fed on the extract of *Aloe barbadensis* (Adegbesan et al. 2018), beluga, *Huso huso* (Linnaeus, 1758) fed on *Urtica dioica* extract (Binaii et al. 2014), *C. gariepinus* fed on *Vemonia amydalina* and *Carica papaya* extracts (Olusola and Nwokike 2018), and rainbow trout, *O. mykiss* fed on *Echinacea purpurea* extract (Oskoii et al. 2012).

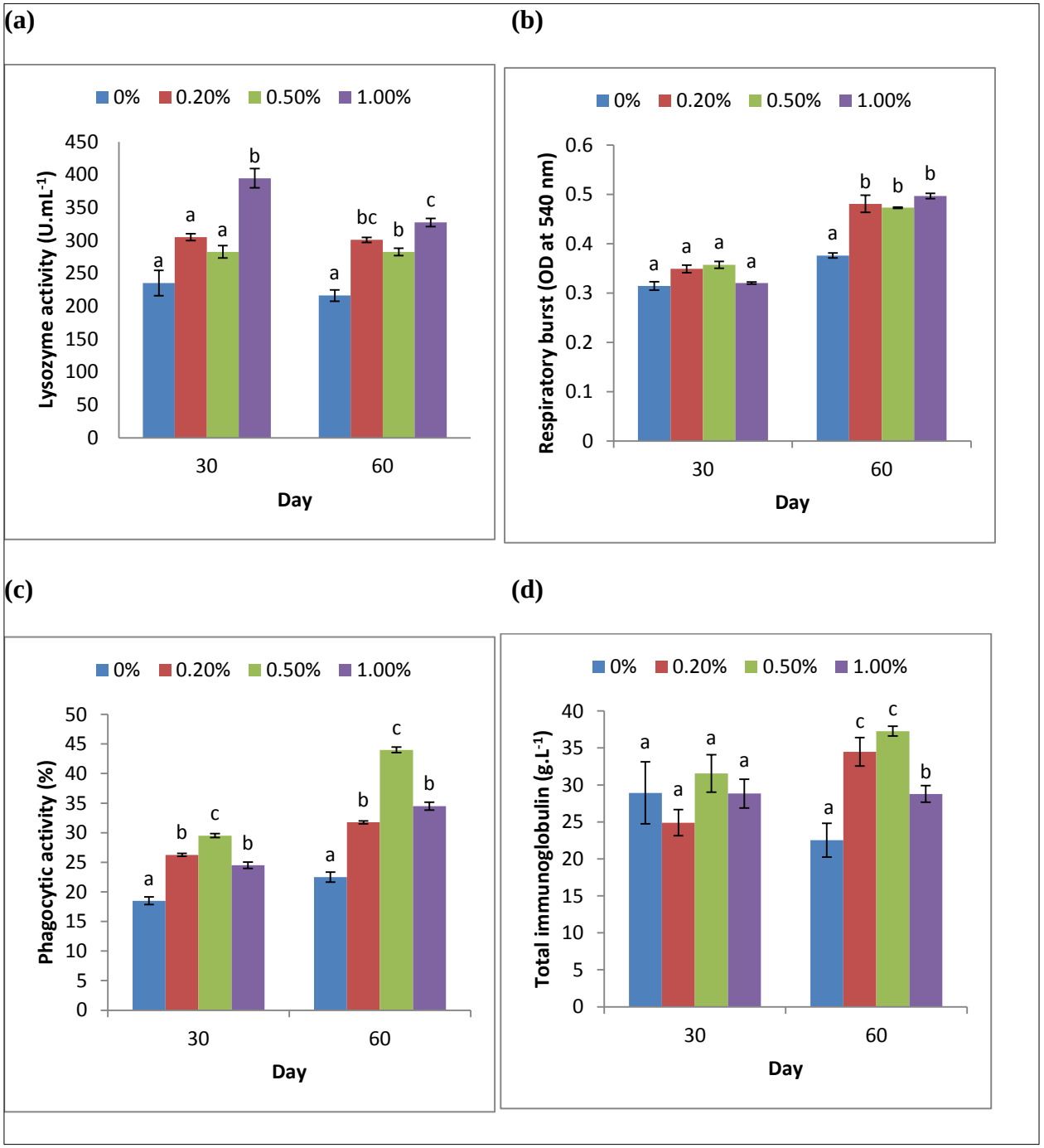

Fig. 1. The immunological status of Asian seabass, *Lates calcarifer* fed with different doses of *Polygonum chinense* extract. (a) Lysozyme activity; (b) respiratory burst activity; (c) phagocytic activity; (d) total immunoglobulin. Data are mean ± SE (n = 3) and the difference in values between groups is indicated by different superscripts (ANOVA, Duncan, $P < 0.05$).

In the present study, feeding fish with 1.0 % PCE significantly decreased the serum glucose level on days 30 and 60, but no significant variation was noticed in the fish group supplemented with other experimental diets. The reduction of glucose level could have been due to the capability of the plant extract to reduce the effect of stressors (Baba et al. 2016). Changes in the total protein, albumin and globulin levels also can be an indicator in determining the fish health and nutritional status (Svetina et al. 2002; John, 2007). In the present study, the total protein, albumin and globulin levels significantly increase in Asian seabass fed at all levels of PCE. The significant increase in total protein albumin and globulin are in agreement with other researchers who observed an increase of similar parameters when supplementation with *Ocimum sanctum* extract into *L. rohita* diet (Das et al. 2015), *Zingiber officinale* extract into *L. calcarifer* diet (Talpur et al. 2013) and *Allium sativum* and *Z. officinale* oil extracts into *Dicentrarchus labrax* (Linnaeus, 1758) diet (Yilmaz and Ergün 2012). These findings might be associated with a stronger innate immune response by the fish fed with plant extracts (Wiegertjes et al. 1996), as the albumin and globulin are the important components to maintain the healthy immune system (Jha et al. 2007).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are the most important liver enzymes that can be used as biomarkers for liver damage (Bhardwaj et al. 2010). These blood serum enzymes are released into the blood plasma if their cells are injured or infected by disease (Banaee et al. 2011). The increase of AST and ALT levels indicated abnormal liver function and stress response in fish (Tan et al. 2017), and the decreased in ALP activity can be a sign of disturbance of membrane transport system (Oner et al. 2008). However, the dietary *P. chinense* supplementation increases the ALP activity as shown in the present study. The increase of ALP indicates that fish fed with PCE enhanced the enzyme activity. The increase of ALP activity may be due to increasing of the macrophage cells which consecutively produced a higher amount of phosphate enzyme (Pratheepa et al. 2010). Moreover, dietary *P. chinense* inclusion also resulted in a reduction of AST and ALT activities in the present study indicating that *P. chinense* supplementation may have hepatoprotective effects that prevent liver damage (Deng et al. 2011). Previous studies have also reported similar observations as in the present study for ALP, AST and ALT activities in *O. mykiss*, rainbow trout fed with extract of propolis (Deng et al. 2011), rohu, *L. rohita* fed with *Curcuma longa* extract (Sahu et al. 2008), and golden pompano, *Trachinotus ovatus* (Linnaeus, 1758) fed with *Taraxacum officinale* extract (Tan et al. 2017) respectively.

Serum immunoglobulin is part of the immune response of fish that functions to identify and neutralise the invading microbial pathogens (Ngugi et al. 2017). In the present study, feeding fish with the diet supplemented with PCE resulted in statistically increase of total immunoglobulin, which could have been due to the extract of *P. chinense* that can actively stimulate the production of immunoglobulin. Lysozyme is an important defence molecule and is an indicator of the fish innate immune system. It can act as a defence barrier against foreign bacterial pathogens (Misra et al. 2006), promoting phagocytosis by activating the leucocytes and macrophages (Saurabh and Sahoo 2008), and play a role in leucocyte respiratory burst activity (Biller-Takahashi et al. 2013). In the present study, the 60 days of PCE diet supplementation significantly increased the lysozyme, phagocytic and respiratory burst activities in Asian seabass. This result suggests that dietary supplementation with PCE enhanced the non-specific cellular immune responses, and such phenomena as reported by Pourmoghim et al. (2015) promote the phagocytic activity of neutrophils with dietary supplementation of *Origanum vulgare* extract. Phagocytosis is a process where microorganisms and other cellular particles are engulfed and destroyed (Lau et al. 1991). These neutrophils can also consecutively increase the respiratory burst activity in fish (Talpur and Ikhwanuddin 2012) by producing the reactive oxygen species (ROS) rapidly, which is toxic and capable of destroying the phagocytised bacterial pathogens (Musthafa et al. 2017). This result is in agreement with studies by Ardó et al. (2008) and Harikrishnan et al. (2010) which showed similar trends when Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) were fed to Nile tilapia, *O. niloticus* and mixed herbs (*Azadirachta indica*, *Oscimum sanctum*, and *C. longa*) fed to goldfish, *Carassius auratus* (Linnaeus, 1758) respectively. Therefore, the mode of action of *P. chinense* extract on the Asian seabass immunity was monitored with the activation of the cellular and humoral immune response, i.e. phagocytosis, respiratory burst, immunoglobulin and lysozyme with the effective dose of 0.5 % PCE. Moreover, in the present study, there was a significant correlation between phagocytic activity, and weight gain and specific growth rate implying fish immunity plays a significant role in the growth of Asian seabass.

Conclusion

In summary, the dietary supplementation of 0.5 and 1.0 % PCE significantly improved the growth performance and feed efficiency in Asian seabass. In addition, supplementation with 1.0 % significantly increased the RBC and WBC counts, and Hb level, but all levels of PCE supplementation significantly increased the total protein, albumin, globulin, albumin:globulin ratio, lysozyme, respiratory burst and phagocytic activities, and total immunoglobulin. These results indicate the potential use of *P. chinense* as a growth promoter and immunostimulant for Asian seabass.

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