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Jerusalem artichoke enriched diet on growth performance, immuno-hematological changes and disease resistance against *Aeromonas hydrophila* in Asian seabass (*Lates calcarifer*)

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ABSTRACT

A 45 days feeding trial was conducted to study the effect of Jerusalem artichoke (JA) on growth performance, body composition, biochemical, immuno-hematological parameters and disease resistance in Asian seabass (*Lates calcarifer*) fingerlings against *Aeromonas hydrophila*. JA was supplemented at three different levels viz., control 0, 5, 10, and 20 g kg^{-1} in the commercial diet (403 g kg^{-1}protein and 89 g kg^{-1}lipid) in *L. calcarifer*. The results showed that there were no significant ($P > 0.05$) differences in various growth parameters, while the whole body composition showed significant differences ($P < 0.05$) between control and treatment groups. Hematological parameters showed that red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), pack cell volume (PCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were not significantly ($P > 0.05$) affected by dietary supplementation of JA at different concentration. However, the mean corpuscular volume (MCV) was significantly ($P < 0.05$) higher in the fish fed with 20 g kg^{-1} JA supplemented diet. Biochemical parameters revealed that glucose, urea, cholesterol, and triglyceride showed significant ($P < 0.05$) differences between control and treatments groups. Interestingly, 20 g kg^{-1} JA supplemented diet significantly modulates the innate immune response and disease resistance against *Aeromonas hydrophila* compared with control and other treatment groups. The results of the study revealed that 20 g kg^{-1} JA supplementation has a beneficial effect in the biochemical, immunological and disease resistance in *L. calcarifer* juveniles.

1. Introduction

Asian seabass (*Lates calcarifer*) an economically important candidate species is commonly known as bhetki or barramundi widely cultured in Southeast Asia and Australia region under extensive or intensive system in brackish-marine water resource [1]. This species is being considered in India as a potential alternate candidate species for coastal aquaculture [2] while large scale production of this species under crowded condition are exposed to stress often leading to disease susceptibility accompanied with mass mortalities resulting in serious economic losses [3]. Aquaculture activities are suffering mortalities particularly in hatcheries due to several disease outbreaks by bacterial pathogens. Among, *Aeromonas hydrophila* is a Gram-negative bacterium considered as a major threat and easily spread through accidental abrasions, known as many symptoms namely, haemorrhagic septicaemia, ulcers, exophthalmia, abdominal distension, etc. [4–6]. Traditionally use of antibiotics and chemicals to prevent and control of many fish diseases [7,8]. The abuses of antibiotics or chemicals are led to the development of antibiotic-resistant bacterial strains and environmental hazards [8,9]. Prebiotics have recently attracted extensive attention in aquaculture because of its natural origin and less influence on natural environment [3]. However, prebiotics are often used as a prophylactic strategy rather than curative, thereby reducing the need for antibiotics [10].

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Prebiotics are commonly fermented ingredients that lead to specific changes in the gastrointestinal microbiota resulting benefits for the host's comfort and health [11]. Inulin is considered as an important prebiotic substrate contains reserve carbohydrate in the roots and tubers of plants such as Jerusalem artichoke (JA), chicory, dahlia and yacon [12] well-studied due to its effects on intestinal bifidobacteria [13]. It has been reported that stimulate good gut microbiota, suppress pathogens, and enhance immune response [14]. However, information concerning the influence of inulin in fish is relatively scanty [15]. Our earlier studies using prebiotics, such as mannanoligosaccharide (MOS) and fructooligosaccharide (FOS) showed the beneficial effect in improving the growth and health performance in seabass [2,3]. The efficacy of JA in diet of Asian seabass has not been reported so far. Therefore the present study was carried out to assess the effect of JA supplementation as a dietary feed additive on growth, body composition, immuno-hematological and disease resistance in *L. calcarifer* fingerlings against *A. hydrophila*.

2. Materials and methods

2.1. Preparation of experimental diets

Jerusalem artichoke (JA) was supplemented in a standard commercial diet for seabass at four different concentrations viz., control (0), 5, 10, and 20 g kg^{-1} (403 g kg^{-1} protein; 89 g kg^{-1} lipid). The ingredients and proximate composition of the experimental diets and JA are shown in Table 1 and Table 2. All ingredients and supplement were ground in an electrical grinder and passed through a 0.5 mm sieve. They were mixed along with additives and homogenized thoroughly in an electrical blender. The diet mix was made into soft dough by adding 400 ml water. It was steam cooked (at atmospheric pressure) for 5 min, cooled, and pelletized in a hand pelletizer using a 2.0-mm in our feed mill located at the Muttukadu experimental station, Central Institute of Brackishwater Aquaculture (CIBA), Chennai. The pellets were air dried in a hot air oven at 50 °C till the moisture content was well below 10% than packed in air tight containers and stored in refrigerator for future use.

Table 1
Formulation and proximate composition of experimental diets (% dry matter).

Diets	Control	5 g	10 g	20 g
Ingredients (g kg^{-1})				
Fish meal[a]	400	400	400	400
Squid	50	50	50	50
Soya	200	200	200	200
Wheat	130	130	130	130
Rice	80	75	70	60
Maize	50	50	50	50
Fish oil[a]	40	40	40	40
Lecithin	20	20	20	20
Vitamins & minerals mix[b]	20	20	20	20
Binder[c]	10	10	10	10
Jerusalem artichoke[d]	0	5	10	20
Proximate composition (g kg^{-1})				
Moisture	75.2	72.0	73.0	72.9
Crude protein	401.6	403.0	404.3	408.5
Crude lipid	89.1	89.2	89.7	89.8
Crude fiber	22.0	24.2	26.5	33.6
Total ash	114.9	115.2	118.3	121.9
NFE	297.2	296.4	288.2	273.3

[a] Sardine fishmeal and fish oil (Bismifisheries, Mayiladuthurai, Tamil Nadu, India).
[b] Commercially vitamins and minerals premix (Sarabhai Zydus Animal Health Ltd, Vadodara, Gujarat, India) each kg contains: Vitamin A, 2000000 IU; Vitamin D, 400000 IU; Vitamin E, 300 U; Vitamin K, 450 mg; Riboflavin, 800 mg; Panthothenic acid, 1 g; Nicotinamide, 4 g; Vitamin B12, 2.4 mg; Choline chloride, 60 g; Ca, 300 g; Mg, 11 g; I, 400 mg; Fe, 3 g; Zn, 6 g; Cu, 800 mg; Co, 180 mg.
[c] Pegabind, Bentoli Agri nutrition Asia Pvt. Ltd, Singapore.
[d] Jerusalem Artichoke-Adept impex Pvt Ltd, UP.

Table 2
Proximate composition (g kg^{-1}) of JA.

Parameters	Values
Moisture	108
Crude protein	58.4
Crude lipid	4.8
Crude fiber	31.7
Total ash	38.9
NFE	758.2

2.2. Pathogen

A. hydrophila (MTCC 646) was obtained from Institute of Microbial Technology, Chandigarh, India which is isolated from diseased fish. The pathogenic of *A. hydrophila* was confirmed to inoculate into Seabass and reisolation [16,17]. *A. hydrophila* was grown with agitation at 37 °C in a 250 ml conical flask containing with tryptic soy broth (TSB; Merck) to log phase. The culture was harvested by centrifugation at 3500 × g for 20 min at 4 °C and the bacterial pellets washed twice with sterile 0.15 M phosphate buffered saline (PBS) at pH 7.2. The bacterial pellets were resuspended, divided into aliquots, and stored in TSB supplemented with 15% (v/v) glycerol at −70 °C for further experiment. The identity of the bacterium was confirmed by morphological, pictorial, and biochemical characteristics including the following reactions: motile, Gram-negative, cytochrome oxidase positive, glucose positive, arginine dihydrolase positive, ornithine decarboxylase negative, ONPG positive, esculin positive, sucrose positive, L-arabinose utilization and fermentation of salicin [18]. It was further confirmed by PCR in the genus and species level [19].

2.3. Fish rearing and experimental design

Hatchery bred and farm-reared Asian seabass juveniles were procured from a farm at Pulicat, 60 KM North of Chennai, India and transported to our nutrition wet-laboratory at Muttukadu Experimental Station, CIBA. They were acclimatized for a fortnight and fed with the control diet. The juveniles (average body weight: 7.84 ± 0.02 g) were randomly distributed into twelve 1000L oval Fiber Reinforced Plastic (FRP) tanks. The tanks were supplied with sand-filtered seawater with provisions for continuous aeration through air diffuser stones and the water exchanged daily once in the morning and evening. Fishes were hand-fed in excess twice a day at 10.00 and 16.00 h and unconsumed feed was siphoned out and dried to determine the actual feed consumption after 30 min. After two week acclimatization, fish were divided into five groups namely, (i) non-infected fed with control diet, (ii) infected fed with control diet, infected fed with (iii) 5, (iv) 10 and (v) 20 g kg^{-1} JA supplementation diets. A completely randomized design was used with three replicates in each experiment (5 × 20 x 3 = 300 fish). After 45 days of JA supplementation feeding, all groups except the non-infected control were challenged with 50 µl PBS containing *A. hydrophila* at 1.7 × 10^7 cfu ml^{-1}. Fishes were maintained under a natural photoperiodicity (12 h L: 12h D) and the water quality parameters measured once a week by standard methods APHA [20] viz. temperature, 26–29 °C; salinity, 28–31‰, pH, 7.4–8.2; dissolved oxygen, 6.0–7.3 mg l^{-1} and total ammonia nitrogen 0.08–0.11 mg l^{-1}.

2.4. Chemical analysis

The proximate composition of the ingredients, experimental diets, and the whole body composition of the experimental fishes were analyzed by standard procedures as per AOAC [21].

At the termination of the experiment, 6 fish from each tank were collected and killed by over dose of anesthesia for determination of whole body composition. The fish samples were homogenized and dried at 105 °C for 24 h. The dried samples within a tank were pooled and analyzed. All analyses were carried out in triplicate and the results were expressed in wet weight basis. Moisture content (MC) was estimated by gravimetric analysis after oven drying at 105 °C for 12 h and the crude protein (CP) was determined by Kjeldahl method (Kjeltec 2100, FOSS, Tecator, Sweden) after acid hydrolysis [22]. The crude lipid (CL) was calculated gravimetrically after extraction with petroleum ether in a Soxhlet system (SOCS, Pelican, India). Total ash (TA) was determined gravimetrically by ignition at 600 °C for 6 h in muffle furnace and the Crude fiber (CF) was estimated gravimetrically after acid and alkali digestion and loss in mass by combustion at 600 °C for 3 h. The nitrogen free extract (NFE) was calculated from 1000 − (CP + CL + CF + TA).

2.5. Measurement of hepatosomatic index (HSI), viscerosomatic index (VSI) and growth performance

On termination of the experiment, fish were anaesthetized using 2-phenoxyethanol at a dose of 0.3 mL L^{-1} and the total length and weight of each fish recorded. Three fish from each tank were randomly selected to measure the biometric indices. Liver and viscera of fish were dissected out and weighed for computation of hepatosomatic index (HSI) and viscerosomatic index (VSI) [2]. Growth parameters were calculated as detailed below.

Hepatosomatic index (HSI, %) = (liver weight, g / body weight, g) x 100

Viscerosomatic index (VSI, %) = (visceral weight, g / body weight, g) x 100

The growth parameters were calculated as below:

IBW (g) = Initial body weight

FBW (g) = Final body weight

Survival (%) = (final count of fish / initial count of fish) x 100

Feed conversion ratio (FCR) = feed consumed (g, dry weight) / weight gain (g)

Condition factor (CF, g $(cm^3)^{-1}$) = [(live weight, g) / (length, cm) ^{3}] x 100

2.6. Blood sample collection

About 1 ml of blood was withdrawn from the caudal vein puncture using a 2 ml syringe with 26-G needle. One half of blood sample was transferred to heparinized tubes while the half was transferred to non-heparinized tubes to analyze biochemical and immuno-hematological parameters. The blood samples were allow to clot for 2 h at 4 °C than the blood serum was separation by centrifugation at 1000 rpm for 5 min and the serum stored at -20 °C until used for further study [23].

2.7. Hematology

The heparinized blood samples were used for analysis of hematological parameters. Red blood cell count, (RBC) and white blood cell count (WBC) were determined using a Neubaeur hemocytometer [24]. Hemoglobin (Hb) levels were estimated by cyanomethemoglobin method [24]. The packed cell volume (PCV) was measured using standard micro hematocrit method and reported as percentages [25]. The erythrocytes indices like, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated by Blaxhall and Daisley [24].

2.8. Immunological assay

The head kidney macrophages (HKM) were isolated and prepared for the evaluation of immunological parameters according to Secombes [26]. The phagocytic activity of macrophages was determined by the following Sakai et al. [27] and Houwen [28]. Reactive oxygen species (ROS) production of the intracellular respiratory burst activity was measured by NBT method [26] and the lysozyme activity determined by turbidimetric assay [29].

2.9. Biochemical parameters

Serum glucose level was estimated using a commercial kit (Sigma Diagnostics Pvt. Ltd., Baroda, India) according to Trinder [30]. Urea in the serum was estimated by using a commercial diagnostic kit based on the method of Fawcett and Scott [31]. Serum cholesterol and triglyceride levels were estimated according to Parekh and Jung [32] and Rice [33].

2.10. Challenge study (disease resistance) with A. hydrophila

For challenge study or disease resistance study, after 45 days of JA supplementation feeding, 20 fish were collected from each experimental group and challenged with virulent *A. hydrophila* intraperitoneally (i.p.) with 100 µl PBS containing *A. hydrophila* at 3.1×10^7 cfu ml^{-1} as determined using a Neubauer haemocytometer except non-infected control. The bacterial culture, challenge study, and the concentration of bacterial suspension as mentioned previously. The mortality was observed after 30 days of challenge. The tissues were collected from the dead fish for bacteriological examination to confirm of *A. hydrophila* as the cause of death. The cumulative mortality (CM) and relative percent survival (RPS) in different treatment groups were calculated as follows [34].

$$\text{Cumulative mortality (CM; \%)} = \frac{\text{Total mortality in each treatment after challenge}}{\text{Total number of fish challenged for same treatment}} \times 100$$

$$\text{Relative percent survival (RPS; \%)} = \frac{\text{\% of Mortality in treated group}}{\text{\% of Mortality in control group}} \times 100$$

2.11. Statistical analysis

Data were analyzed using ANOVA to compare significant differences between treatments. Significance of treatments was tested

Table 3
Biometric indices of Asian seabass fed experiment diets containing JA in different levels for 45 days.

Parameters	Control (0 g)	5 g	10 g	20 g
CF (k)	1.21 ± 0.02	1.24 ± 0.01	1.22 ± 0.01	1.26 ± 0.05
HSI (%)	1.34 ± 0.02	1.36 ± 0.01	1.32 ± 0.06	1.39 ± 0.07
VSI (%)	6.14 ± 1.23	6.29 ± 0.77	6.27 ± 1.14	6.41 ± 0.99

CF: condition factor, Hepatosomatic index (HIS), Viscerosomatic index (VSI). All values are mean ± SE of three observation.

Table 4
Growth performance and survival rate for 45 days of Asian seabass fed experiment diets containing JA in different levels.

Parameters	Control (0 g)	5 g	10 g	20 g
IBW (g)	7.84 ± 0.02	7.87 ± 0.02	7.85 ± 0.27	7.89 ± 0.07
FBW (g)	20.05 ± 1.01	20.85 ± 1.32	20.79 ± 1.45	19.95 ± 0.72
WG (g)	12.51 ± 1.02	12.89 ± 1.14	12.75 ± 1.20	13.95 ± 1.52
Survival rate (%)	83.33 ± 7.21	79.16 ± 12.50*	83.33 ± 7.22	87.50 ± 12.50*
FCR	1.63 ± 0.14	1.56 ± 0.08	1.44 ± 0.15	1.36 ± 0.25

IBW: initial body weight; FBW: final body weight; WG: weight gain; FCR: feed conversion ratio. All values are mean ± SE of six observations.

Table 5
Whole body composition (% dry matter basis) of Asian seabass fed experiment diets containing JA in different levels for 45 days.

Parameters	Control (0 g)	5 g	10 g	20 g
Moisture (M)	72.89^{a} ± 0.58	71.74^{b} ± 0.29	69.63^{c} ± 0.27	69.71^{c} ± 0.44
Crude protein (CP)	57.36 ± 0.14	57.39 ± 0.07	57.33 ± 0.13	57.38 ± 0.03
Crude lipid (CL)	10.42^{a} ± 0.08	10.29^{a} ± 0.21	11.21^{b} ± 0.11	11.55^{b} ± 0.14
Total ash (TA)	24.93^{a} ± 0.04	24.19^{b} ± 0.07	23.99^{c} ± 0.20	22.15^{d} ± 0.06

All values are mean ± SE of six observation and the means with different superscript in a row differ significantly ($P < 0.05$).

by Duncan's multiple range tests. All data were analyzed using SPSS version 16.0 software (SPSS, Chicago, IL, USA).

3. Results

3.1. Biometric indices

The biometric indices of seabass fed diet with JA supplemented diets are presented in Table 3. The condition factor (CF), hepatosomatic index (HSI), and viscerosomatic index (VSI) of the fish fed with JA supplemented diets showed non-significant ($P > 0.05$) difference among the experimental groups.

3.2. Growth performance, survival rate and whole body composition

The growth performance and survival of seabass fed with JA supplemented diets are shown in Table 4. The results showed that there was no significant ($P > 0.05$) difference in final body weight (FBW), weight gain (WG) and feed conversion ratio (FCR) among the various treatment groups. The survival rate significantly varies in the seabass fed with 5 g and 20 g kg^{-1} diet groups as compared to control while it did not in 10 g diet group. The whole body composition of seabass fed with JA supplemented diets is presented in Table 5. Analysis of moisture (M), crude lipid (CL), and total ash (TA) content of seabass fed with different experimental diets showed significant differences ($P < 0.05$). However, the crude protein showed non-significant ($P > 0.05$) differences among various diet groups.

Table 6
Hematological parameters of Asian seabass fed experiment diets containing JA in different levels for 45 days.

Parameters	Control (0 g)	5 g	10 g	20 g
RBC (10^6 mm^{-3})	3.30 ± 0.29	3.57 ± 0.51	3.54 ± 0.51	3.17 ± 0.39
WBC ($10^3 mm^{-3}$)	7.26 ± 0.09	7.56 ± 0.09	7.16 ± 0.06	7.44 ± 0.42
Hb (g dL^{-1})	8.20 ± 0.30	7.80 ± 0.53	7.59 ± 0.70	7.56 ± 0.81
PCV (%)	34.3 ± 1.70	36.0 ± 1.85	35.3 ± 0.54	34.6 ± 0.75
MCV (fl)	92.0^{a} ± 0.95	92.9^{b} ± 0.59	92.4^{a} ± 1.05	93.56^{c} ± 0.72
MCV (pg)	20.9 ± 0.70	22.3 ± 0.59	21.36 ± 0.06	20.9 ± 0.62
MCHC (g dL^{-1})	21.5 ± 1.04	22.2 ± 1.07	21.6 ± 0.55	21.6 ± 0.68

RBC: red blood cell count, WBC: white blood cell count, Hb: hemoglobin, PCV: packed cell volume, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, and MCHC: mean corpuscular hemoglobin concentration. All values are mean ± SE of six observation and the means with different superscript in a row differ significantly ($P < 0.05$).

3.3. Hematological parameters

Hematological parameters of seabass fed with JA supplemented diets are presented in Table 6. Results of the hematological parameters revealed that RBC, WBC, Hb, PCV, MCH and MCHC values were not significantly ($P > 0.05$) affected by dietary supplementation of JA. However, significantly higher MCV was observed in the fish fed with 20 g kg^{-1} JA supplemented diet compared to the rest.

3.4. Biochemical parameters

The blood biochemical parameters (glucose, urea, cholesterol, and triglyceride) results showed in the present study showed significant ($P < 0.05$) difference among the experimental group when compared with control group (Table 7).

3.5. Immune response

The phagocytic activity in head kidney leucocytes of seabass fed with any supplementation diets significantly enhanced as compared with control as shown in Fig. 1. The respiratory burst

Table 7
Biochemical parameters of Asian seabass fed experiment diets containing JA in different levels for 45 days.

Parameters	Control (0 g)	5 g	10 g	20 g
Glucose (mg dL^{-1})	$49.10^{a} \pm 4.43$	$42.90^{a} \pm 3.82$	$66.80^{b} \pm 6.73$	$64.86^{b} \pm 7.07$
Urea (mg dL^{-1})	$17.73^{d} \pm 4.90$	$12.53^{a} \pm 3.11$	$19.83^{b} \pm 0.36$	$18.46^{c} \pm 2.85$
Cholesterol (mg dL^{-1})	$72.20^{b} \pm 3.76$	$64.43^{a} \pm 5.16$	$71.66^{ab} \pm 7.03$	$91.86^{c} \pm 4.96$
Triglyceride (mg dL^{-1})	$150.4^{a} \pm 3.04$	$168.73^{b} \pm 9.70$	$175.53^{c} \pm 7.65$	$209.4^{d} \pm 9.32$

All values are mean ± SE of six observation and the means with different superscript in a row differ significantly ($P < 0.05$).

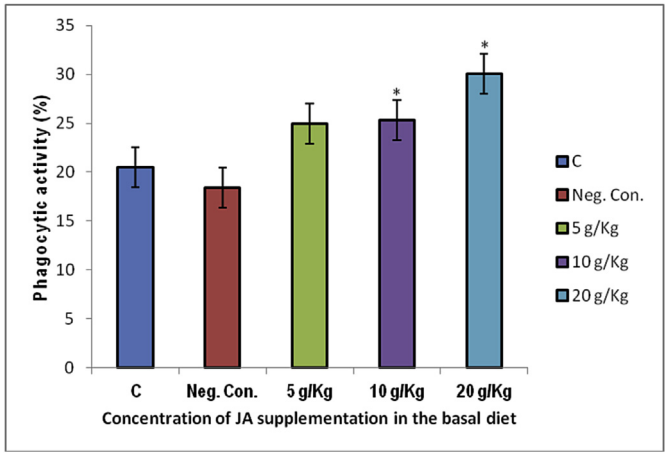

Fig. 1. Phagocytic activity (%) of seabass (mean ± SEM, n = 6) fed experiment diets containing JA in different levels against *A. hydrophila* for 45 days. Significant different ($p < 0.05$) from the control are indicated by asterisks.

activity isolated from phagocytic cells indicate that there was no significant ($P > 0.05$) difference in seabass fed with 5 and 10 g kg^{-1} JA supplemented diet groups as compared with control. However, the respiratory burst activity significantly enhanced in seabass fed with 20 g kg^{-1} JA supplemented diet compared with control (Fig. 2). The lysozyme activity in serum samples of seabass fed with 5 g kg^{-1} JA supplemented diet was not statistically significant ($P > 0.05$) as compared to control. On the other hand, the lysozyme activity was observed in fish fed with 10 g and 20 g kg^{-1} JA supplemented diets significantly increase over control (Fig. 3).

3.6. Disease resistance

The cumulative mortality was 35% and 30% in infected seabass fed with 10 and 20 g kg^{-1} JA supplementation diets while it was high (50%) with 5 g kg^{-1} JA supplementation diet. The mortality was observed 80% in the infected seabass fed without supplementation of JA diet for 30 days as shown in Fig. 4.

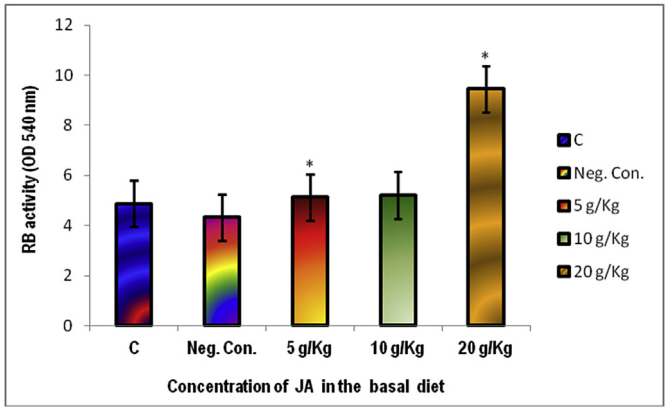

Fig. 2. Respiratory burst activity (%)of seabass (mean ± SEM, n = 6) fed experiment diets containing JA in different levels against *A. hydrophila* for 45 days. Significant different ($p < 0.05$) from the control are indicated by asterisks.

4. Discussion

This is the first study to investigate the efficacy of JA on growth performance, body composition, biochemical, immuno-hematological parameters and disease resistance in Asian seabass (*Lates calcarifer*) fingerlings against *Aeromonas hydrophila*. The FBW, WG, survival, and FCR results in the present study showed that there was no significant effect of different levels of JA supplementation diets. On the contrary, Van Doan et al. [35] have reported that JA (the source of inulin) significantly improved specific growth rate (SGR), feed conversion ratio (FCR) in catfish. Studies on the effect of JA in Nile tilapia, *Oreochromis niloticus* [36]; turbot larvae, *Psetta maxima* [37]; rainbow trout, *Oncorhynchus mykiss* [38]; sea cucumber, *Apostichopus japonicus* [39] juvenile ovate pompano, *Trachinotus ovatus* [40]; and juvenile white shrimp, *Litopenaeus vannamei* [41] have been reported in beneficial growth performance. JA has an abundant source of fructose and inulin; fresh tuber contains 50–70 g kg^{-1} of inulin-type fructan [3]. Inulin is a fructooligosaccharide commonly used as a prebiotic in human and animal feedstuffs [42,43]. JA have been shown antimicrobial and antifungal activities [44,45]; it also reported as anti-cancer [45–47], anti-tumour, anti-inflammatory, cytotoxic, and anti-microbial properties [48]. Carbohydrates of inulin or FOS can modulate the immune system through the interaction with immune related cells that exhibiting the complement carbohydrates receptor [49,50]. Recently its applications helpful in aquaculture to stimulate beneficial gut microbiota, suppress pathogens, and enhance the immune response in fish [51]. However in the present investigation, supplementation of JA supplementation at 5, 10 and 20 g kg^{-1} no beneficial effect on various growth parameters in seabass. The reason may be the main source of inulin in addition to certain other components which might have played an antagonistic role against inulin, so this hypothesis needs further investigations. Hence attempts to extract inulin from JA is one of the adopt strategy to explore beneficial effect of inulin. The effects of inulin on growth performance have been evaluated in different aquaculture species obtained various results [52].

The whole body composition of seabass fed with different level of JA supplementation diets showed significant difference in moisture, crude lipid, and total ash content between control and treatment groups. On the contrary, Akrami et al. [53] have been reported that there was no significant difference in the proximate composition in the carcass of beluga fed with the test feeds supplemented with inulin a 10–30 g kg^{-1} for eight week feeding trial. However, fish fed with the basal diet had higher protein content than those fed with prebiotic inulin 10–30 g kg^{-1} supplemented diet. Similarly, Ortiz et al. [38] had reported that in *Oncorhynchus mykiss*, the addition of inulin showed no significant difference in protein. The whole body composition of fish is often used as an indicator of fish quality. The value of any food product, including

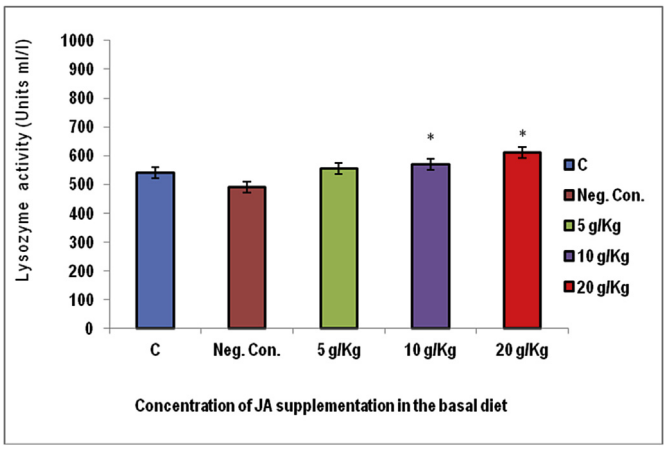

Fig. 3. Lysozyme activity (%) of seabass (mean ± SEM, n = 6) fed experiment diets containing JA in different levels against *A. hydrophila* for 45 days. Significant different ($p < 0.05$) from the control are indicated by asterisks.

fish is a function of nutritional properties. Both fresh-water and marine-water exhibit variations in the biochemical composition of whole body as well as individual organs. These variations are attributed to many factors including season, feeding, growth, maturation, and spawning etc. [54]. The condition factor, HSI and VSI showed slightly differences between control and experimental groups. Condition factor is used to compare the condition, fatness or well being of fish and are based on the hypothesis that heavier fish of a given length are in better condition [55].

The hematological parameters did not significantly affected by the dietary JA supplementation in the present study. Ahmadifar et al. [56] had reported that the supplementation of diets with 1% inulin significantly increased WBC and lymphocyte values in beluga juvenile. With increasing levels of supplementation of inulin, the mean values of MCH and MCHC increased but the mean value of WBC, RBC, Hb, PCV, and MCV slightly decreased from control value. In fish, RBC, WBC, and Hg are frequently used as indicators of health status and it was also involved in regulation of immunological function in the organism. Blaxhall and Daisley [24] have reported the possibility of using PCV as a tool in aquaculture for checking anemic condition. The fish PCV are normally between 20 and 35% and scarcely attain values greater than 50%. The MCV, MCH, and MCHC have a particular importance in the diagnosis of anemia in most animals. Although, the hematological parameters of fish are reported to be affected by many factors, including species, size, age, physiological status, environmental conditions, dietary regime, quality and quantity of food, dietary ingredients, protein sources, vitamins, and probiotics [57].

Improvement of the immune system through dietary enrichment is the imperative strategy in preventing fish diseases. The innate immune system of fish is considered to be the first line of defense against invading pathogens, and is more important for fish rather than mammals [58]. The innate immune response depends on the function of macrophage activity such as phagocytosis and chemotaxis. Phagocytic cells are the most important cellular components of the innate immune system of fish [59]. In the present study, infected *L. calcarifer* fed with 10 and 20 g kg^{-1} JA supplementation diets were able to enhance the phagocytic activity and respiratory burst activity of leukocytes. The present results are in agreement in catfish fed with JA supplementation diet significantly enhanced the phagocytic activity after 9 and 12 weeks [35]. However, no significant effect was found with on 5 g kg^{-1} JA supplementation diet group in this study.

Lysozyme is an imperative defense molecule of the innate immune system, that playing an important role in mediating protection against microbial intrusion [60]. Lysozyme is known as muramidase or N-acetylmuramide glycanhydrolase enzyme that attack peptidoglycans in the cell walls of Gram-positive bacteria and hydrolyzing the glycosidic link with N-acetylmuramic acid with 4th carbon atom of N-acetylglucosamine. It does this by binding to the peptidoglycan molecule in their binding site, causing distortion to the 4th sugar molecules in the hexasaccharide (the D ring). In this stressed condition, the glycosidic bond is easily broken leading to damage to the bacterial cell walls by hydrolyzing the 1, 4-beta-linkages between N-acetylmuramic acid and N-acetyl-D glucosamine residues in the peptidoglycan [60]. Fish lysozyme possesses lytic activity against pathogens and it can activate the complement and phagocytes. Since O^{-2} is the first product released during the respiratory burst, it has been accepted as an accurate parameter to quantify the intensity of respiratory burst [34,61]. In the present study, the lysozyme activity was observed significantly increased with 20 g kg^{-1} JA supplementation diet group whereas, there was no significant effect in other experimental diet groups. It was suggested that a recent study indicate that cat fish fed with JA supplementation diet significantly increased the lysozyme activity after 12 weeks [35]. Diet with inulin had stimulated the immune system in gilthead *seabream, Sparus aurata* that the serum complement, leucocyte phagocytic, leucocyte respiratory burst activities and IgM level [62]. Similarly, Nile tilapia fed 5 g kg^{-1} inulin significantly improved respiratory burst and serum lysozyme activities [63]. However, it has been reported in gilthead seabream [64] and hybrid surubim, *Pseudoplatystoma* sp [65]. in different

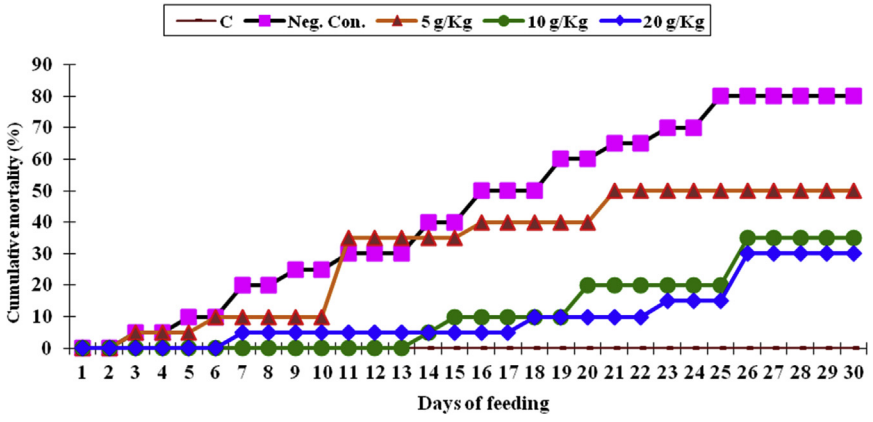

Fig. 4. Cumulative mortality (%) of seabass (n = 20) fed experiment diets containing JA in different levels against *A. hydrophila* for 30 days.

results. The cumulative mortality was 30% and 35% in the infected fish fed with 10 and 20 g kg^{-1} JA supplementation diets indicating a more direct effect than the serum indicators increasing the immune function. A similar result was reported recently in cat fish fed with JA supplementation diet [35].

The results of the biochemical parameters showed that there was a significant difference in the glucose, urea, cholesterol, and triglyceride between control and treatments groups. Akrami et al. [53] have reported that cholesterol, glucose, triglycerides, and uric acid were not significantly affected by inulin supplemented diets. Glucose in serum is a major metabolite of carbohydrate metabolism. The amount of glucose in fish blood depends on fish species/type in the range 25–350 mg dl^{-1}. In the present study, the amount of glucose was 49–66 mg dl^{-1} and the highest value was observed in fish fed with 10 g kg^{-1} JA supplemented diet. The fish fed with 20 g kg^{-1} JA supplemented diet showed highest cholesterol level compared to the rest of diet group.

The results in the present study revealed that JA supplementation in the diet of Asian seabass had slightly improving the growth and survivals, while significantly modulate the immuno-hematological parameters against pathogens. However further detailed studies are necessary to explore inulin from JA is one of the adopt strategy and explore beneficial effect of inulin in practical diets as a prebiotic feed additive.

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