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Protective efficacy of Azomite enriched diet in *Oreochromis mossambicus* against *Aeromonas hydrophila*

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ABSTRACT

The effect of dietary supplementation with Azomite, a natural mineral of volcanic ash, on growth performance, innate immune response, and disease resistance in *Oreochromis mossambicus* against *Aeromonas hydrophila* is reported. On being fed with 4 g kg^{-1} and 6 g kg^{-1} enriched diets in infected fish the survival rate, weight gain (WG), protein efficiency ratio (PER),specific growth rate (SGR), feed conversion rate (FCR), and feed efficiency (FE) increased significantly from weeks 1 to 4; the complement activity registered a significant increase from weeks 1 to 4; however the phagocytic activity increased significantly between weeks 2 and 4. With any supplementation diet the respiratory burst activity significantly increased between weeks 2 and 4. The lysozyme activity significantly increased the infected fish fed with 4 g kg^{-1} and 6 g kg^{-1} Azomite diets from weeks 1 to 4. The cumulative mortality was lower in fish fed with 4 g kg^{-1} and 6 g kg^{-1}diets (10% and 15%) than with2 g kg^{-1}diet (20%). The present results suggest that Azomite enriched diet at 4 g kg^{-1} and 6 g kg^{-1}positively enhance the innate immunity and disease resistance in *O. mossambicus* against *A. hydrophila*.

Statement of relevance: Aeromonas hydrophila is one among the most common bacterial pathogens in freshwater habitats throughout the world (Larsen and Jensen, 1977) affecting fish species including carps both in farms and field. The traditional use of chemotherapeutics and antibiotics to combat fish disease has the risk of generating resistant pathogens, alter gut microbiota, bioaccumulation, and environmental pollution. Commercial vaccines are expensive for fish farming practices and are specific against particular pathogens. Azomite is a mineral ore that occurs in natural state prior to being ground is the registered trademark for a complex silica ore (hydrated sodium calcium aluminosilicate, HSCAS) and contains over 70 minerals and trace rare elements, including calcium, phosphorus, magnesium, iron, selenium, copper, zinc, and manganese. Undersea volcanic sediment of Azomite was mixed with a large amount of plant and animal residues and minerals. It was especially rich in rare earth elements. The growth performance was enhanced with dietary supplementation of rare earth elements at low concentrations in various farming animals, including beef cattle, sheep, pigs, rabbits, ducks, chickens, shrimps, and fish (Shen et al., 1991; Rosewell, 1995; Duan et al., 1998; Tang et al., 1998; Liu, 2005; Yang and Chen, 2000; Yang et al., 2005). Dietary supplementation of rare earth elements in aquaculture increases in output and survival rate of several fish species including grass carp, blunt-snoup carp, black carp, common carp, silver carp, and prawn (Tang et al., 1998; Tang et al., 1997). Liu et al. (2009) have been reported that 2.5 and 5.0 g kg^{-1} Azomite supplementation in the diets improve the growth, pepsin activity, and nutrient digestibility in tilapia (*Oreochromis niloticus* × *O. aureus*). Dietary supplementation with 2.0 g kg^{-1} Azomite in grass carp (*Ctenopharyngodon idellus*) improved the efficiency of feed utilization, activities of intestinal digestive enzymes, and serum non-specific immune function (Liu et al., 2011). Based on the above information, Azomite was anticipated to have the ability to promote growth, innate immune function, and disease resistance in fish culture. So far, there was no study reported on the impact of

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Azomite on innate immune response and diseases resistance in fish against bacterial diseases. Therefore, the present study investigated dietary supplementation with Azomite on growth performance, innate immune response, and disease resistance in *Oreochromis mossambicus* against *A. hydrophila*.

1. Introduction

Aeromonas hydrophila is one of the most common opportunistic bacterial pathogens throughout the world (Larsen and Jensen, 1977) affecting fish species both in farm and field. The infection is frequently associated with hemorrhagic septicemia (Kuge et al., 1992; Thune et al., 1993; Angka et al., 1995; Roberts et al., 1992) and a variety of symptoms including septicemia, tail rot, fin rot, ulcer, dropsy, abnormal distension, etc. (Sarkar and Rashid, 2012; Ye et al., 2013).

The traditional chemotherapy generates resistant pathogens, alter gut microbiota, bio-accumulates, and causes environmental pollution. The commercial vaccines are often specific against particular pathogens (Verschuere et al., 2000; Kaskhedikar and Chhabra, 2010).In this regard, phytotherapy is one of the most promising methods; many herbs have been shown to act as immunostimulants and confer disease resistance (Raa et al., 1992; Harikrishnan et al., 2009, 2011; Harikrishnan and Balasundaram, 2008, Kumar et al., 2005).

Azomite a mineral ore that occurs in natural state prior to being ground is the registered trademark for a complex silica ore (hydrated sodium calcium aluminosilicate, HSCAS); it contains over 70 minerals and trace rare elements (http://rcpm-hosting.com/client_sites/dairydoo/wp-content/uploads/2014/04/Azomite-Info.pdf). It is recognized by Organic Materials Review Institute (OMRI, Eugene, OR, USA) as a natural mineral for use in organic agriculture.

The incorporation of rare earth elements with animal feeds significantly enhances the growth performance, innate immune response, and disease resistance in several animal species (Wan et al., 1998). Poultry, shrimp, and tilapia farmers have been using Azomite in their feed in combination with their regular trace mineral mix since last two decades to improve weight gain, feed conversion, survival rate, and livability (http://www.azomiteinternational.com/resources/Azomite_Tilapia_Fodge.pdf, Liu et al., 2009, Fodge et al., 2011) and also in several fish species including grass carp, blunt-snout carp, black carp, common carp, silver carp, and prawn (Tang et al., 1997; Tang et al., 1998; Liu et al., 2009). In tilapia (*Oreochromis niloticus* X *Oreochromis aureus*) dietary supplementation of Azomite at 2.5 and 5 g kg^{-1} improves the growth, pepsin activity, and nutrient digestibility. In grass carp (*Ctenopharyngodon idellus*) the Azomite enriched diet at 2 g kg^{-1} improved the feed utilization efficiency, activity of intestinal digestive enzymes, and serum non-specific immune function (Liu et al., 2011). Based on the above information, Azomite was anticipated to have the ability to promote growth performance, innate immune function, and disease resistance in fish culture. So far, there is no study reported on the impact of Azomite on innate immune response and diseases resistance in fish against bacterial diseases. Therefore in the present study the protective effect of dietary supplementation with Azomite on growth performance, innate immune function, and disease resistance in *Oreochromis mossambicus* against *A. hydrophila* was investigated.

2. Materials and methods

2.1. Diet

The basal diet (control) comprised mackerel meal, dehulled soybean meal, and corn gluten meal as the protein sources; wheat flour, α-potato starch, and wheat gluten as carbohydrate and fish oil as lipid source in addition with vitamin and mineral premix (Table 1). The dietary Azomite (V5 Organic Biotech Private Limited, Chennai, India) was incorporated with the basal diet at doses of 2, 4, and 6 g kg^{-1} by evenly mixing with the basal diet thoroughly. The enriched feeds were dried in a vacuum freeze drier for 15 h, ground, and extruded by passing through 5 mm mesh sieve. The prepared diets were stored at −20 °C until used for the experiment. The proximate composition of the experimental diets quantified following AOAC method comprised the percentage of crude protein, crude lipid, crude ash, and crude carbohydrate.

2.2. Pathogen

A. hydrophila (MTCC 646) was obtained from Institute of Microbial Technology in Chandigarh, India which is isolated from infected fish. The pathogenic of *A. hydrophila* was confirmed to inoculate into *O. mossambicus* and re isolation according to Krieg and Hold (1984); Yogananth et al. (2009).*A. hydrophila* was grown with agitation at 37 °C in a 250 ml conical flask containing tryptic soy broth (TSB; Merck) to log phase. The culture was harvested by centrifugation at 3500 × *g* for 20 min at 4 °C. Bacterial pellets were washed twice with sterile 0.15 M phosphate buffered saline (PBS) at pH 7.2. The bacterial pellets were resuspended and divided into aliquots and stored in (TSB) supplemented with 15% (*v*/*v*) glycerol at −70 °C until used. 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 (Deng et al., 2009) and the followed by PCR for confirmation of genus and species, using the methods described by Ghatak et al. (2007).

2.3. Fish and experimental design

The freshwater fish, *O. mosambicus* (30.5 ± 1.7 g) was obtained from a commercial fish farm (Ram Raghu Seed Fish Walajapet, Chennai, India); the fish were maintained in 60 L aerated fiber tanks. They were examined for their health status immediately upon arrival. After two week's acclimation, the fish were divided into five groups of 25 each

Table 1
Ingredients and proximate composition (g) of experimental diets.

Ingredients (g)	Diets groups (%)				
	C	I	2 g kg^{-1}	4 g kg^{-1}	6 g kg^{-1}
Mackerel meal	55	55	55	55	55
Dehulled soybean meal	12	12	12	12	12
Corn gluten meal	5	5	5	5	5
Wheat flour	12	12	12	12	12
α-potato starch	2	2	2	2	2
Wheat gluten	6	6	4	2	0
Fish oil	5	5	5	5	5
Vitamin premix[a]	2	2	2	2	2
Mineral premix[b]	1	1	1	1	1
Azomite	0	0	2	4	6
Proximate composition in dry matter (%)					
Moisture	7.12	7.16	7.11	7.34	7.02
Crude protein	54.3	53.4	50.7	50.2	51.8
Crude lipid	8.6	8.7	8.7	8.9	9.2
Crude carbohydrate	14.2	13.8	13.5	13.2	13.0
Crude ash	7.6	7.4	7.1	6.8	6.4

[a] Vitamin premix per kg: Vitamin A = 700,000 IU; Vitamin D = 140,000 IU; Vitamin E = 500 mg; Vitamin B_{12} = 1000 mcg; Folic Acid = 100 mg; Nicotinamide = 1000 mg.
[b] Mineral premix per kg: Copper = 1200 mg; Cobalt = 150 mg; Iron = 1500 mg; Zinc = 3000 mg; Iodine = 325 mg; Selenium = 10 mg; Magnesium = 6000 mg; Manganese = 1500 mg; Potassium = 100 mg; Calcium = 270 g; Phosphorus = 1 30 g; Sulfur = 7.2 g; Fluorine = 300 mg.

in triplicate (5 × 25 × 3 = 375 fish) and the fish were fed with (i) control group, without Azomite diet (C), (ii) infected group, fed without Azomite diet (I), (iii) infected, fed with 2 g kg^{-1} Azomite supplementation diet, (iv) infected, fed with 4 g kg^{-1} Azomite supplementation diet, and (v) infected, fed with 6 g kg^{-1} Azomite supplementation diet at the rate of 5% of their body weight twice a day. Feeding with the respective diets continued till the end of experiment. On 30thday of feeding, all fish were challenged intraperitoneally (i.p.) with 100 μl PBS containing *A. hydrophila* at 3.1 × 10^7 cfu ml^{-1} as determined using a Neubauer hemocytometer. On weeks 1, 2, and 4 post-infection, six fish were randomly collected from each experimental tank to collect blood samples for immunological assays after anesthetizing with MS-222 ($NaHCO_3$ and tricaine methanesulphonate; Sigma Chemicals) 1:4000 in dechlorinated water for 2 min.

2.4. Growth performance

The growth performance, including percentage weight gain (RGR), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) for each group was determined by Olmedo Sanchez et al. (2009).

$$\text{Specific growth rate (SGR)} = (\text{Ln Final weight} - \text{Ln Initial weight}) \div \text{No of days in trial} \times 100.$$

$$\text{Feed conversion ratio (FCR)} = \text{Feed given (dry wt)} \div \text{Weight gain (wet weight)}.$$

$$\text{Protein efficiency ratio (PER)} = \text{wet weight gain by fish (g)} \div \text{Protein intake(g)}.$$

2.5. Preparation of serum and head kidney macrophages

O.mosambicus were sacrificed with an overdose of anesthetic and exsanguinated by caudal venipuncture using 1 ml capacity Vacuettes containing a Z Serum Sep. Clot Activator (Greiner Bio-one). The collected blood samples were kept separately and were maintained at −70 °C until used for the experiment. The blood was allowed to clot for 2 h at 4 °C and the serum was separated by centrifugation at 3500 g for 25 min at 4 °C. The samples were maintained at −70 °C for subsequent analysis. The head kidney macrophages were isolated and prepared for the evaluation of immunological parameters according to Secombes (1990).

2.6. Immunological assays

The phagocytic activity of macrophages was determined following Sakai et al. (1995), Houwen (2002). Reactive oxygen species (ROS) production of the intracellular respiratory burst activity was measured by NBT method according to Secombes (1990). The alternative complement activity was examined by the following method of Yano (1992) using rabbit red blood cells (RBC; Oxoid); the lysozyme activity was determined by turbidimetric assay as described by Parry et al. (1965).

2.7. Challenge study with A. hydrophila

After 30 days of feeding trial, 20 fish in each group were challenged with virulent *A. hydrophila*. The bacterial culture, challenge study, and the concentration of bacterial suspension as mentioned previously. Mortality was observed for 30 days. The tissues were collected from the dead fish for bacteriological study to confirm *A. hydrophila* as the cause of death. The cumulative and relative percent survival (RPS) in different treatment groups were calculated as follows (Amend, 1981).

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

$$\text{Relative percent survival (RPS)} = 1 - \frac{(\%\ \text{of Mortality in treated group}}{(\%\ \text{of Mortality in control group}} \times 100.$$

2.8. Statistical analysis

The data of each parameter were expressed as the mean ± standard error of mean (SEM) and the effects of Azomite were tested using one-way analysis of variance (ANOVA) followed by Tukey's pair wise comparison test using SPSS (version 16 for windows). Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Growth performance and feed utilization

Growth performance of *O. mossambicus* fed with Azomite enriched diets was measured at the end of fourth week (Table 2). All the enriched diet fed groups were found active and healthy. The survival rate of the fish during the experimental period ranged from 91.3% to 95.3% of Azomite enriched diets. When infected fish were fed with 4 g or 6 g kg^{-1} Azomite enriched diets better growth rate; a moderate growth observed with control diet and 2 g kg^{-1} Azomite supplementation diet. A significant ($p < 0.05$) difference was found in the growth performance across the different Azomite concentrations. A significant ($p < 0.05$) increase in the feed conversion ratio (FCR), specific growth rate (SGR), feed efficiency (FE), and protein efficiency ratio (PER) was noted in fish fed with 4 g or 6 g kg^{-1} Azomite diets over the control group (Table 2). The overall differences in growth performance between doses of Azomite significantly varied between the treated groups and the control.

3.2. Phagocytic activity

The phagocytic activity in head kidney leucocytes did not significantly vary in any Azomite supplementation diet fed group on first week. However, in infected fish fed with 4 and 6 g kg^{-1}diets, the phagocytic activity was significantly ($p < 0.05$) elevated on weeks 2 and 4 when compared to the control but not with 2 g kg^{-1} diet (Fig. 1).

Table 2
Mean growth performance and feed utilization after fourth week of *O. mossambicus* fed Azomite supplementation diets against *A. hydrophila*.

Parameter	Control	I	2 g kg^{-1}	4 g kg^{-1}	6 g kg^{-1}
IBW(g)	31.8 ± 1.8	29.1 ± 1.2	33.8 ± 1.1*	35.4 ± 2.4*	33.0 ± 1.4
FBW(g)	47.9 ± 1.8	33.4 ± 1.8	50.3 ± 2.0*	53.1 ± 2.4*	51.9 ± 2.8*
LWG(g)	16.1 ± 0.8	4.3 ± 0.7	16.5 ± 1.1	17.7 ± 1.4	18.9 ± 1.1
SGR(% d^{-1})	2.55 ± 0.02	2.48 ± 0.01	3.18 ± 0.02	4.26 ± 0.03*	3.64 ± 0.02*
FCR	0.80 ± 0.02	0.71 ± 0.02	0.70 ± 0.02	0.62 ± 0.03*	0.63 ± 0.03*
FE	1.59 ± 0.02	2.06 ± 0.03	2.84 ± 0.02*	2.91 ± 0.03*	2.82 ± 0.02*
PER	4.41 ± 0.03	3.94 ± 0.04	4.78 ± 0.02	4.83 ± 0.02	4.76 ± 0.02
Survival rate (%)	100 ± 0.0	93.3 ± 0.12	95.3 ± 0.14	91.3 ± 0.12	91.3 ± 0.14

The values are expressed as mean ± standard errors of mean. Significant different ($p < 0.05$) from the control are indicated by asterisks.IBW = Initial body weight, FBW = Final body weight, SGR = Specific growth rate, FCR = Feed conversion ratio, FE = Feed efficiency ratio, PER = Protein efficiency ratio.

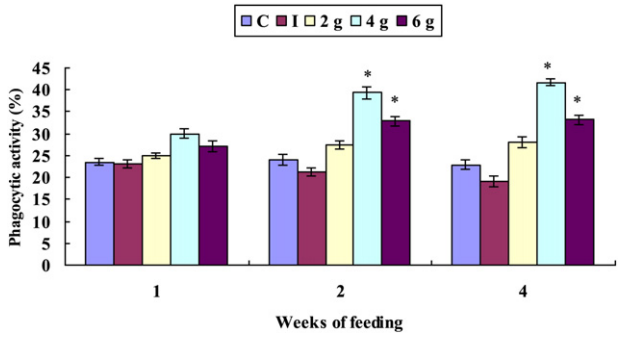

Fig. 1. Phagocytic activity (%) of *O. mossambicus* (mean ± SEM, n = 6) fed dietary supplementation diets with different concentration (2, 4, and 6 g kg^{-1}) of Azomite against *A. hydrophila*. Significant different ($p < 0.05$) from the control are indicated by asterisks.

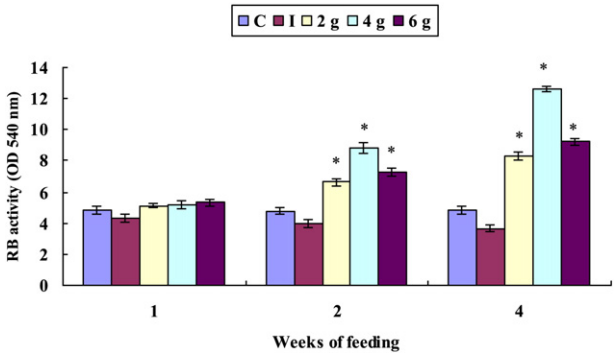

Fig. 3. Respiratory burst (RB) activity of *O. mossambicus* (mean ± SEM, n = 6) fed dietary supplementation diets with different concentration (2, 4, and 6 g kg^{-1}) of Azomite against *A. hydrophila*. Significant different ($p < 0.05$) from the control are indicated by asterisks.

3.3. Complement activity

The complement activity was significantly enhanced ($p < 0.05$) with 4 and 6 g kg^{-1} diets from week 1 to 4 when compared to control. However, it did not significantly increase ($p > 0.05$) with 2 g kg^{-1} diet at any time (Fig. 2).

3.4. Respiratory burst activity

The impact of different doses of Azomite supplementation diets on respiratory burst activity isolated from phagocytic cells as shown in Fig. 3 indicate that the activity significantly increased ($p < 0.05$) with all enriched diets on weeks 2 and 4 but did not on first week.

3.5. Lysozyme activity

The lysozyme activity was significantly enhanced ($p < 0.05$) in 4 and 6 g kg^{-1} Azomite supplementation diet groups from weeks 1 to 4 as compared with control but did not 2 g kg^{-1} Azomite supplementation diet group (Fig. 4).

3.6. Disease resistance

The cumulative mortality was 10% and 15% in infected fish fed with 4 and 6 g kg^{-1} Azomite supplementation diets. The mortality was high (20%) when fed with 2 g kg^{-1} diet. However, the maximum mortality was observed (90%) when infected fish were fed with nonAzomite diet for 30 days (Fig. 5).

4. Discussion

In aquaculture, application of traditional medicine is more advantageous to overcome the drawbacks in traditional chemotherapy; hence application of natural mineral supplements or herbals with multifunctional active principles can be ideal alternatives. A number of studies demonstrated that rare earth minerals possess certain antibacterial properties (Burkes and McCloskey, 1947; Wurm, 1951; Evans, 1990);the effects are dose-dependent.For example, Ceriumions inhibit the growth of several bacteria including *Escherichia coli*, *Bacillus pyocyaneus*, *Staphyloccous aureus*, *Leuconostoc*, and *Streptococcus faecalis* at concentrations ranging from 10^{-3} mol/l to 10^{-2} mol/l (Zhang et al., 2000; Ruming et al., 2002). Dietary supplementations of rare earth elements cause bacterial flocculation by changing the structure and altering the surface charge of bacterial membranes (Sobek and Talbut, 1968. Peng et al. (2004) has reported that La^{3+} may possibly change the structure of outer cell membrane of Gram-negative bacteria, *E. coli*.

Fish fed with enriched diets showed weight gain by 29.6% in trout and by 16% in carps (Tang et al., 1997).A number of studies demonstrated that rare earth elements enriched diets enhance the growth performance in pigs. However, some experiments observed little or no effect in pigs (Kraatz et al., 2004; Gebert et al., 2005).In China and other fish farming countries it has been recorded that fish fed with rare earth elements supplemented diets enhance growth and increase resistance to pathogens (Flachowski, 2003; Tautenhahn, 2004; Renard, 2005; Yeng, 1990).

Azomite® is a natural mined, hydrated sodium calcium aluminosilicate (HSCAS) product from an ancient mineral deposit in Utah (USA) that comprises a broad spectrum of over 70 minerals and trace elements, including calcium, phosphorus, copper, iodine, iron, magnesium, manganese, selenium, zinc, etc. It plays a pivotal role in the physiology

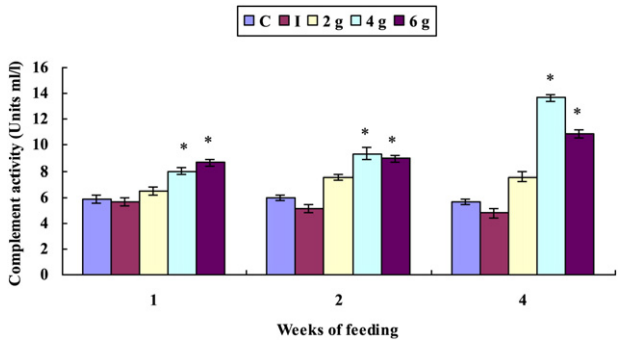

Fig. 2. Complement activity of *O. mossambicus* (mean ± SEM, n = 6) fed dietary supplementation diets with different concentration (2, 4, and 6 g kg^{-1}) of Azomite against *A. hydrophila*. Significant different ($p < 0.05$) from the control are indicated by asterisks.

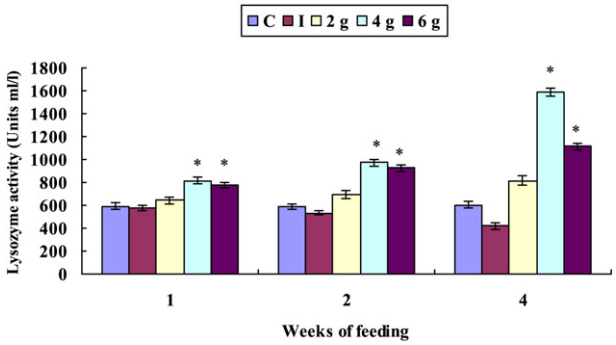

Fig. 4. Lysozyme activity of *O. mossambicus* (mean ± SEM, n = 6) fed dietary supplementation diets with different concentration (2, 4, and 6 g kg^{-1}) of Azomite against *A. hydrophila*. Significant different ($p < 0.05$) from the control are indicated by asterisks.

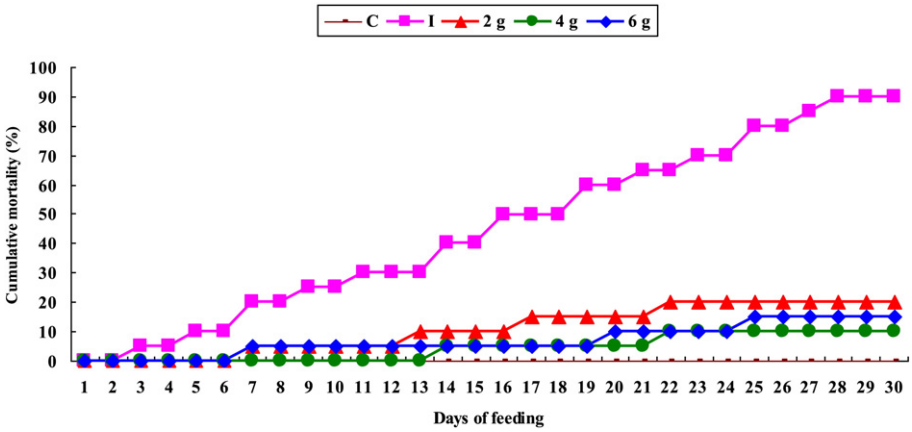

Fig. 5. Cumulative mortality(%) of *O. mossambicus* (n = 20) fed dietary supplementation diets for 30 days with different concentration (2, 4, and 6 g kg^{-1}) of Azomite against *A. hydrophila*.

of organisms. For example zinc directly increases the production and function of leucocytes and indirectly affects the immune system by acting as a cofactor of enzymes and improving the organ functions in man (Rink, 2000). In fish, the role of dietary zinc, immune response and disease resistance has been reviewed by Lim et al. (2001). Selenium is another important trace element for fish because it is a constituent of selenoproteins and has structural and enzymatic roles similar to glutathione peroxidase which is an antioxidant enzyme. Selenium modulates the immune functions such as inflammation and virulence development (Rayman, 2000). In channel catfish seleno yeast and selenomethionine are better selenium sources compared to sodium selenite, in increasing the antibody titer (Wang et al., 1997). Administration of 0.4 mg kg^{-1} from the organic sources improved the macrophage chemotactic response offering protection against *E. ictaluri* in channel catfish. Poultry, shrimp, and tilapia farmer use in the feed in conjunction with their regular trace mineral mix add Azomite for many years for improvements to improve the weight gain, feed conversion, and survival (Fodge and Fodge, 2014).

In the present study the growth performance parameters such as FCR, SGR, FE, and PER increased significantly when infected fish were fed with 4 and 6 g kg^{-1} Azomite over the control. These results are consistent with the above-mentioned reports.

Enhancement of the immune system is the most promising strategy in preventing fish diseases. The nonspecific 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 (Narnaware et al., 1994). The nonspecific 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 (Zhang et al., 2009); their phagocytic activity constitutes a primitive defense mechanism (MacArthur and Fletcher, 1985) which is an important characteristic of the nonspecific immune system (Seeley et al., 1990). In the present study, infected *O.mosambicus* fed with 4 and 6 g kg^{-1} Azomite supplementation diets were able to significantly enhance the phagocytic activity of leukocytes on weeks 2 and 4. However, no significant effect was found with any diet on first week.

Complement is among the main mechanism involved in the initiation of the innate immune response mounting an adaptive response further. The complement cascade is part of the phylogenetically ancient innate immune response and is crucial to the natural ability to ward off infection (Gasque, 2004). In the present study the complement activity; this was significantly enhanced on being fed with 4 and 6 g kg^{-1} Azomite supplementation diets from weeks 1 to 4. On the other hand, the respiratory burst activity significantly increased with any enriched diet on weeks 2 and 4.

Lysozyme is an important defense molecule of the innate immune system, playing a role in mediating protection against microbial invasion. It is a mucolytic enzyme produced by leucocytes, especially monocytes, and neutrophils. Fish lysozyme possesses lytic activity against bacteria and can activate 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 (Harikrishnan et al., 2011). In the present study, the lysozyme activity increased significantly with 4 and 6 g kg^{-1} Azomite enriched diets from week 1 to 4. A recent study in *Litopenaeus vannamei*has reported that dietary administration with 4 g kg^{-1} Azomite significantly increased the lysozyme activity (Tan et al., 2014).The cumulative mortality was 10% and 15% in the infected fish fed with 4 and 6 g kg^{-1} Azomite supplementation diets indicating a more direct effect than the serum indicators increasing the immune function.

To our knowledge, there were no detailed immunological studies in aquatic species using supplementation diet with Azomite. Hence, the present study is a more detailed immunological study reporting a positively enhanced nonspecific immune function in *O.mosambicus* fed with 4 and 6 g kg^{-1} Azomite supplementation diets against *A. hydrophila*. Further detailed immunological and molecular studies are needed to strengthen the role of this mineral in aquaculture before recommending the Azomite supplemented diet as a potential immunostimulant in other cultured fish species.

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