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Asparagus racemosus aqueous root extract induced effects on cellular immune reaction of *Labeo rohita* (Hamilton)

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ABSTRACT

The present study was undertaken to evaluate the effect of *Asparagus racemosus* aqueous root extract on cellular immune reaction of *Labeo rohita* (Hamilton) fingerlings with response to bacterial infection caused by *Aeromonas hydrophila* at cool hilly mid altitude region of Meghalaya. Four concentrates mixtures of pelleted diet were formulated viz. without *A. racemosus* aqueous root extract (control); with 50 mg *A. racemosus* aqueous root extract/kg of diet (AR_1); with 100 mg *A. racemosus* aqueous root extract/kg of diet (AR_2); and with 150 mg *A. racemosus* aqueous root extract/kg of diet (AR_3). Feeding trial was conducted for 60 days. Immune reactions, viz. NBT level, phagocytic activity, total immunoglobulin level, lysozyme activity, antiprotease activity and myeloperoxidase activity of fish were determined at 0, 15, 30, 45 and 60 days of feeding. Fish were infected with *A. hydrophila* 60 days post feeding, mortalities (%) and agglutination antibody titre were recorded over 14 days post infection. The results showed that in the treatment group AR_2, AR_3, there was significantly enhanced NBT level, phagocytic activity, lysozyme activity, immunoglobulin level, antiprotease activity and myeloperoxidase activity compared to control. The treatment group AR_1 NBT level, phagocytic activity, lysozyme activity, myeloperoxidase activity were significantly enhanced whereas immunoglobulin level and antiprotease activity were nonsignificant compared to control. The highest survival was recorded in the AR_2 (43.36±0.65) group, followed by AR_3 (24.32±0.14), AR_1 (17.26±0.45) and lowest were recorded in the control (3.42±0.02). The highest agglutination antibody titre was recorded in the AR_2 (87.36±0.65) group followed by AR_3 (49.32±0.14) and AR_1 (38.26±0.45) group and lowest in control (17.42±0.02) against *A. hydrophila* infection. Thus, from the present study it can be deduced that feed containing *A. racemosus* aqueous root extracts/kg diet can influence immune reaction in *L. rohita*; however, AR_2 group showed better result in terms of immune reaction and protection against pathogenic *A. hydrophila* at cool hilly mid altitude region of Meghalaya.

Key words: *Asparagus racemosus*, *Aeromonas hydrophila*, Immune reaction, *Labeo rohita*, Root extract

The fish cultured in ponds in the cool hilly mid altitude region of India frequently suffer from different diseases viz. bacterial (http://www.dcfr.res.in res_achievements. php), fungal (Das *et al*. 2012) resulting in mass mortality strongly associated with increased susceptibility to infections due to lower immunity (Janeway 2001) particularly during the winter months from November to February. As a result of it, various chemotherapeutants have been used for treatment and the irrelevant antibiotics usage may lead to genes selection encoding the resistance (Orozova *et al*. 2010).

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Herbs are staging a comeback and 'herbal renaissance' is happening all over the globe (Rajashree *et al*. 2012). *Asparagus racemosus* wild commonly known as 'Shatavari' is one such important medicinal plant which is regarded as a 'rasayana' (plant drugs promoting general well-being by increasing cellular vitality and resistance) in the Ayurvedic system of medicine (Goyal *et al*. 2003) and is abundantly found in the forests in India (Hayes *et al*. 2008). Different parts of the plants were used root powder effect on broilers chicks growth performance and general health (Rekhate *et al*. 2010), production of monosexnile tilapia, *Oreochromis niloticus* (Mukherjee *et al*. 2015), anti-cancerous effect of *A. racemosus* leaf extract on UOK 146, a renal cell carcinoma cell line (Verma *et al*. 2014). Reports indicate that the pharmacological activities of *A. racemosus* root extract include antiulcer (Sairam *et al*. 2003), digestive activity (Dange *et al*. 1969), diuretic activity (Gaitonde and Jetmalani 1969), antihepatotoxic activity (Muruganadan *et al*. 2001), immunomodulatory activities (Dahanukar *et al*. 1986), antitussive activity (Mandal *et al*. 2000). The

bioactive phytoconstituents of *A. racemosus* roots includes five steroidal saponins, shatavarins VI-X, together with five known saponins (known as shatvarins), shatavarin I, shatavarin IV, shatavarin V, immunoside and schidigerasaponin D5 (Hayes *et al*. 2008); a new isoflavone, 8-methoxy-5,6,4'-trihydroxyisoflavone-7-O-ß-D-glucopyranoside (Saxena and Chaurasia 2001) and sitosterol, 4, 6-dihydryxy-2-O (-2-hydroxy isobutyl) benzaldehyde and undecanylcetanoate (Singh and Tiwari 1991). Borkar *et al*. (2014) found that experimental diet formulated with different levels of Shatavari powder (20, 30, 40 g/kg diet) increased body weight of the freshwater fish *Channa punctatus*.

In the present study, it was planned to systematically evaluate the immunomodulatory effect of dietary administration of the root of *A. racemosus* on *Labeo rohita* (Hamilton) infected by bacterial pathogen *Aeromonas hydrophila* at cool hilly mid altitude region of Meghalaya, India.

MATERIALS AND METHODS

Experimental animal and feeding management: Clinically healthy fish (*Labeo rohita;* 600) with average weight 16.51±0.18 g and average length 8.22±0.72 cm, were collected from the private fish farm of Asom and brought to institute. Fishes were acclimatized in the laboratory condition in 500 l cemented tanks for 15 days at 26–28°C before the commencement of the experiment. Fishes were provided with adequate aeration and fed with normal feed (rice bran and oil cake, 1: 1) at the rate 3% of the body weight twice a day. *Asparagus racemosus* roots were obtained from reliable sources and were correctly identified and authenticated as *A. racemosus* Willd. (Asparagaceae) by Department of Botany, St. Xavier's College, Mumbai, India (vide accession no. 75583). A voucher sample was retained and deposited at Department of Botany, St. Xavier's College, Mumbai, India. Fresh root of *A. racemosus* was clean and shade dried. The dried plants were pulverized by an electrical blender and passed through 20 µm mesh sieve. Powdered roots were extracted as aqueous decoction and water used was distilled and deionized (Milipore, USA). About 100 g of *A. racemosus* root powder were immersed in aqueous solution in a 500 ml flat bottom flask and was cold extracted for 7 days with occasional shaking and warming. At the end of the seventh day, the clear filtrate was obtained by filtering through a Buchner funnel. The filtrate was further concentrated by vacuum distillation, cooled, transferred into a petri dish and dried in an oven at 60°C for a period of five minutes. Finally, the aqueous extract was kept in a desiccator for 15 days to remove the excessive moisture. The procedure resulted in 30 g of extract obtained from 100 g of powdered roots (Wani *et al*. 2011). The experimental diet was prepared with the locally available ingredients containing 0.005%, 01%, 0.015% of *A. racemosus* aqueous root extract (Table 1). Initially all ingredients except vitamin mineral mixture and extracts were weighed up to desired quantity, blended properly with water to make dough. The dough was steam cooked for 20 min in a pressure cooker at 15 psi. The different herbal extracts prepared were added at different doses along with Vitamin-mineral pre-mix to the steam ingredients mixture so as to prepare experimental feed and then made into pellets by using a hand pelletizer (Xie *et al*. 2008) and then dried at 40°C for 12 h. The dried pellets were stored in an air sealed container and stored in cool dry place for further use. The experiment was performed in 500 litres cemented tanks in the Fishery Division wet laboratory. The fishes were divided into four groups (Control, AR_1, AR_2 and AR_3) and each group was maintained in triplicate set containing 50 nos. of fishes. The control group diet was devoid of aqueous root extract. The remaining groups AR_1, AR_2 and AR_3 were fed with feed containing 0.005, 0.01, 0.015% of *A. racemosus* aqueous root extract. Fishes were provided with adequate aeration and fed at the rate of 3% of body weight twice a day in the 7: 00 AM and 7: 00 PM The experiment was conducted for 60 days and the sampling for various immunological parameters was carried out on 0 day, 15th day, 30th day, 45th day and 60th day of feeding trial. For each sampling, 8 fishes were selected randomly from each tank and analysed for various parameters.

Table 1. Composition of control and experimental diets (in 100g of feed)

Ingredient	Control	AR_1	AR_2	AR_3
Ground nut oil cake (g)	40	40	40	40
Fish meal (g)	25	25	25	25
Rice bran (g)	20	19.995	19.990	19.985
Soyabean meal (g)	12	12	12	12
Vitamin-mineral mix[a] (g)	2	2	2	2
Starch (g)	1	1	1	1
Herbal extract (g)	0	0.005	0.01	0.015

[a]Vitamin–mineral mix (EmixTM plus) (quantity/2.5 kg): vitamin A, 55,00,000 IU; vitamin D_3, 11,00,000 IU; vitamin B_2, 2,000 mg; vitamin E, 750 mg; vitamin K, 1,000 mg; vitamin B_6, 1,000 mg; vitamin B_{12}, 6 mg; calcium panthothenate, 2,500 mg; niacinamide, 10 g; choline chloride, 150 g; Mn, 27,000 mg; iodine, 1,000 mg; Fe, 7,500 mg; Cu, 2,000; Zn, 5,000 mg; Co, 450 mg; Ca, 500 g; P, 300 g; Se, 50 ppm; L, lysine-10 g; DL-methionine, 10 g.

Collection of blood from the fish and separation of serum: Blood from the 8 fishes from each tank were drawn with the help of a sterilized 2 ml hypodermal syringe and 24 gauge needles directly from the caudal vein containing EDTA as an anticoagulant. Before drawing blood, fishes were anaesthetized with CIFECALM (50 µl/l) (Verma *et al*. 2007). For serum separation, the blood was collected without anticoagulant in serological tubes and stored in a refrigerator overnight. The clot was then spun down at 3000g for 10 min. The serum collected was stored in sterile serum tubes at –20°C until used for assays. All the procedures were carried out in the sterilized condition. After drawing blood fishes were given 1% $KMnO_4$ dip treatment and released in to the tank.

Culture of pathogens: Pathogenic strain of *Aeromonas*

hydrophila (ATCC 35654) was procured from Himedia, India. *A. hydrophila* was grown on nutrient broth (HiMedia Ltd., India) for 24 h at 37°C.The culture broth was centrifuged at 3000g for 10 min. The supernatant was discarded and the pellet was re-suspended in sterile phosphate buffer saline (PBS, pH 7.2) and the OD of the solution was adjusted to 0.5 at 456 nm, which corresponded to 1×10^7 cells/ml.

Determination of immune reaction: Nitro Blue tetrazolium (NBT) assay, phagocytic activity assay, total immunoglobulin assay and lysozyme activity assay were performed as per Sharma *et al*. (2010). Serum antiprotease activity by modification of the method described by Ellis (1990). Briefly, 10 µl of serum were incubated with the same volume of standard trypsin solution for 10 min at 22°C. To this, 100 µl of 0.1 M phosphate buffer, pH 7.0 and 125 µl 2% azocasein were added and incubated for 1 h at 22°C. Then 250 µl of 10% trichloro acetic acid (TCA) was added and incubated for 30 min at 22°C. The mixture was centrifuged at 6000g for 5 min. 100 µl of the supernatant was transferred to a 96 well non-absorbent microtray (Nunc) containing 100 µl/well of 1 N NaOH. The O.D. was read at 430 nm. The blank was phosphate buffer in place of serum and trypsin and the reference sample was phosphate buffer in place of serum. The percentage inhibition of trypsin activity compared to the reference sample was expressed for each serum sample as described by Zuo and Woo (1997). Trypsin inhibition (%) = (Trypsin blank OD- sample OD/ Trypsin blank OD) ×100. Myeloperoxidase content was measured according to Quade and Roth (1997). Agglutination antibody titre assay were determined according to Plumb and Areechon (1990).

Challenge study and relative percentage survivals (RPS): After feeding trial of different herbal extracts through feed over 60 days to the fish in various experimental groups, 10 fish from each experimental tank were injected intraperitoneally with 100 µl of bacterial suspension maintained in separate tanks and also the mortality was observed for 14 days. Sampling of the survivors was carried out on the 14th day of *A. hydrophila* infection. The confirmation of the infection was accomplished after re-isolating the bacteria from kidney, liver and muscle of the dead fish. The reconfirmation was done after performing all the biochemical and the confirmatory tests. Relative percentage survivals (RPS) = (Number of surviving fishes after challenge/Number of fishes injected with bacteria) ×100 (Misra *et al*. 2006).

Statistical analysis: All the data were expressed as arithmetic mean±SE. Statistical analysis of data involved one way analysis of variance (ANOVA) followed by the comparison of means following Least Square Design (LSD) available with SPSS windows 16.0 software. The level of significance were expressed as P-value less or greater than 0.05.

RESULTS AND DISCUSSION

Determination of immune reaction: In the present study the NBT level in all the groups of fishes fed with diet containing *Asperagus racemosus* aqueous root extract was insignificant on 15th day of sampling. The groups AR_1, AR_2 and AR_3 showed increasing trend from 30th to 60th days of sampling then decreased. AR_3 group was significant ($P< 0.05$) on 30th, 45th and 60th days of sampling and AR_2 group was significant ($P <0.05$) on 45th and 60th days of sampling compared to control. Whereas, AR_1 group were insignificant on all the days of sampling except on 60th day in comparison with control. The maximum level was observed in AR_2 group recorded on day 60th (Fig. 1). The enhancement of NBT level in the present study supports the findings of Laitha *et al*. (2017) in tilapia, *Oreochromis niloticus* administered with *Excoecaria agallocha* leaf extract. Similar observations were reported by Nan *et al*. (2015) in grouper (*Epinephelus coioides*) administered with *Curcuma zedoaria* and *Zingiber zerumbet* extract; Uthayakumar *et al*. (2014) in striped murrels (*Channas triatus*) administered with *Lawsonia inermis* against *Aphanomyces invadans* infection; Anusha *et al*. (2014) in ornamentel gold fish *Carrasius auratus* by treating *Ixora coccinea* active principles against *Aeromonas hydrophila*. Present study suggests the production of super oxide anion and stimulation of phagocytes as NBT reduced form formazan can precipitates only on the cytoplasm of phagocytes (neutrophils, eosinophils, basophils, monocytes and macrophages) upon stimulation (Abreu *et al*. 2009) which could be beneficial for the fish in protecting them from invading pathogen.

In present study, phagocytic activity in AR_1, AR_2 and AR_3 groups of fishes fed with diet containing *A. racemosus* root extract showed increasing trend from 30th to 60th days of sampling then decreased noticeably. The group AR_2 was significantly ($P <0.05$) higher from 30th days onwards with maximum value observed on 60th day. The group AR_1 was observed to be significant ($P<0.05$) on 60th day of sampling, whereas, AR_3 was significant on 45th and 60th day of sampling as compared to control (Fig. 2). The enhancements of phagocytic activity in the present study signifies the role of *A. racemosus* in enhancing the nonspecific immune response and was in line with the report in Laitha *et al*.

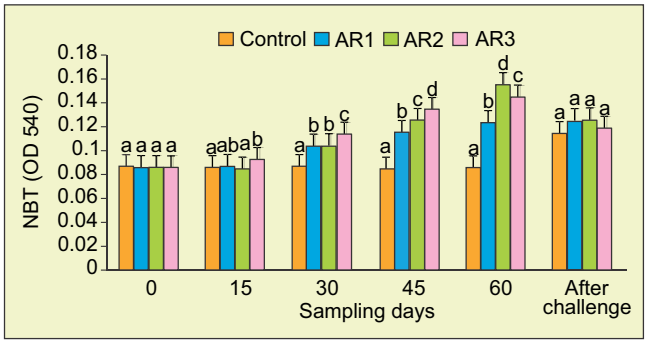

Fig. 1. NBT level on various sampling days of different experimental groups fed with *A. racemosus* roots aqueous extract (values are mean±SE). Mean values with different superscript within a column for a parameter are significantly different ($P<0.05$).

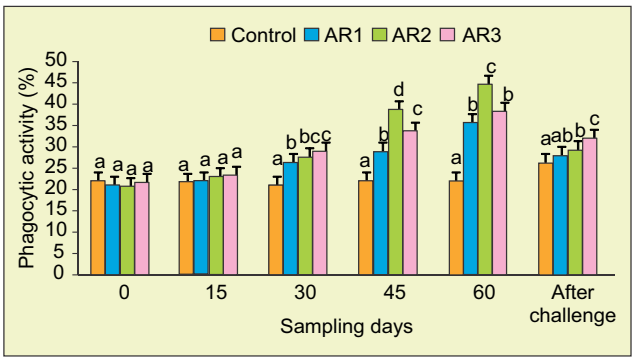

Fig. 2. Phagocytic activity (%) on various sampling days of different experimental groups fed with *A. racemosus* roots aqueous extract (values are mean±SE). Mean values with different superscript within a column for a parameter are significantly different (P<0.05).

(2017) in tilapia, *Oreochromis niloticus* administered diet with *Excoecaria agallocha* leaf extract. This observation corroborates with the findings of Subeenabegum and Navaraj (2016) who concluded that phagocytic activity increased significantly (P<0.05) in fish administered with different concentrations of methanolic extract of plants *Solanum trilobatum* and *Ocimum sanctum*. Similar results were also observed by Yeasmin *et al*. (2015) who reported that phagocytic activity significantly increased in Nile tilapia, *Oreochromis niloticus* at 2.0% enriched diet of *Lactuca indica* extract; in gold fish (*Carassius auratus*) fed extract of *Asparagus racemosus* was an adjuvant (Thangaviji *et al*. 2012). The highest phagocyte activity in this study was recorded in group AR_2 fed with 100 mg *A. racemosus* root extract, suggesting that this level was adequate in fighting off the protruding bacteria by enhancing total phagocytosis activity.

The level of serum lysozyme activity in all the groups AR_1, AR_2 and AR_3 of fishes fed with diet containing *A. racemosus* root extract showed increasing trend from 15th to 60th days of sampling then decreased noticeably. The groups AR_2 and AR_3 was significantly (P<0.05) higher on 30th, 45th, and 60th days of sampling in comparison to control; whereas, AR_1 was significant on 60th day of sampling as compared to control. The highest value was observed in AR_2 on 60th day of sampling (Fig. 3). Our results revealed an increase in lysozyme activity in groups fed with diets containing *A. racemosus* root extract were in line with Laitha *et al*. (2017) in tilapia, *Oreochromis niloticus* administered with *Excoecaria agallocha* leaf extract. Similar observations were documented by Soltanian and Fereidouni (2016) in common carp, *Cyprinus carpio* administered with Henna (*Lawsoni ainermis*) extract infected with *Aeromonas hydrophila*; Alishahi *et al*. (2014) in *Heros severus* after administration of 100 and 200 mg/kg *Dunaliel lasalina* extract. Increase of lysozyme activity in the present study revealed its strong lytic activity against bacterial pathogens supplemented with *A. racemosus* aqueous root extract diet.

Total immunoglobulin level in all the groups AR_1, AR_2 and AR_3 of fishes fed with diet containing *A. racemosus* root extract showed increasing trend from 30th to 60th days of sampling then decreased noticeably. The groups AR_2 was significantly (P<0.05) higher on 30th, 45th, and 60th days of sampling in comparison to control. The group AR_3 was observed to be significant (P<0.05) on 45th and 60th days of sampling whereas, AR_1 was insignificant on all the days of sampling as compared to control. The highest total immunoglobulin was observed in AR_2 and lowest in AR_1 on 60th day of sampling (Fig. 4).The total immunoglobulin enhanced value in the present study were in line with Soltanian and Fereidouni (2016) who revealed that administration of both medium and high dose of Henna (*Lawsoni ainermis*) extract could significantly increase serum immunoglobulin level in common carp infected with *Aeromonas hydrophila*. Simillarly, Pratheepa and Sukumaran (2014) reported enhanced immunoglobulin response in *Cyprinus carpio* administered with *Euphorbia hirta* plant leaf extract against *A. hydrophila* infection. Likewise, Alishahi and Abdy (2013) also reported an elevated level of immunoglobulin in *Cyprinus carpio* administered with different level of *Aloe vera* challenged against *A. hydrophila*. Enhanced immunoglobulin level indicates the ability of *A. racemosus* aqueous root extract in stimulation of specific immune system (Wijendra and

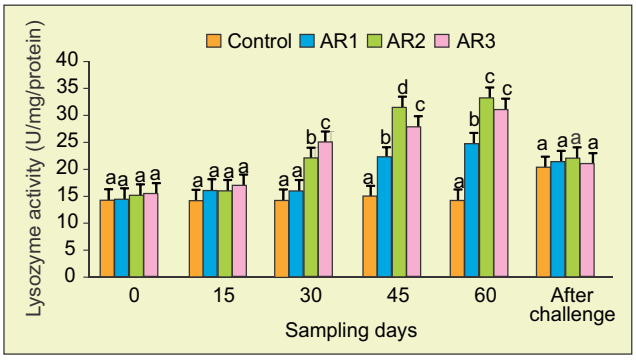

Fig. 3. Lysozyme activity (U/mg/protein) on various sampling days of different experimental groups fed with *A. racemosus* roots aqueous extract (values are mean±SE). Mean values with different superscript within a column for a parameter are significantly different (P<0.05).

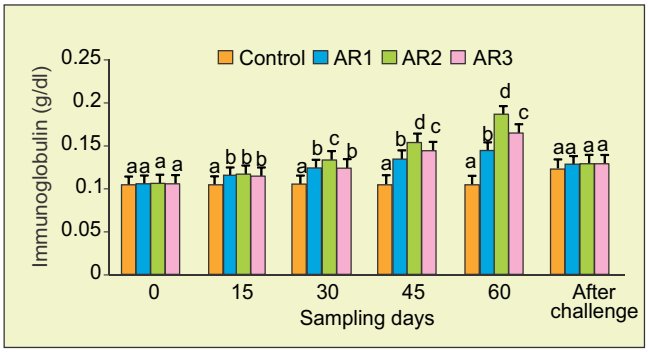

Fig. 4. Total immunoglobulin level (g/dl) on various sampling days of different experimental groups fed with *A. racemosus* roots aqueous extract (values are mean±SE). Mean values with different superscript within a column for a parameter are significantly different (P<0.05).

Pathiratne 2007) and killing of micro-organisms (Gerwich *et al*. 2002).

Serum antiprotease activity in the groups AR_2 and AR_3 of fishes fed with diet containing *A. racemosus* root extract showed increasing trend from 15th to 60th days of sampling then decreased noticeably. The group AR_3 was significantly ($P<0.05$) higher on 30th, 45th, and 60th days of sampling in comparison to control. The group AR_2 was observed to be significant ($P<0.05$) on 45th and 60th days of sampling whereas, AR_1 was insignificant on all the days of sampling as compared to control. The highest serum antiprotease activity was observed in AR_3 on 60th day of sampling (Fig. 5). The present study showed a significant elevation of the serum antiprotease activity in *L. rohita*. This finding gets the support from the works in *Mystus keletius* administered with *Solanum trilobatum* and *Ocimum sanctum* extract (Subeenabegum and Navaraj 2016). Similarly, in rainbow trout (*Oncorhynchus mykiss*) administered with 1.0% of lupin (*Lupinus perennis*), mango (*Mangifera indica*) and stinging nettle (*Urtica dioica*) (Elham 2010), in *O. mossambicus* administered with 20mg/kg of *Nyctanthus arbortristis* seed extracts (Kirubakaran 2009). The enhancement of the serum antiprotease activity observed in this study indicates the contribution of

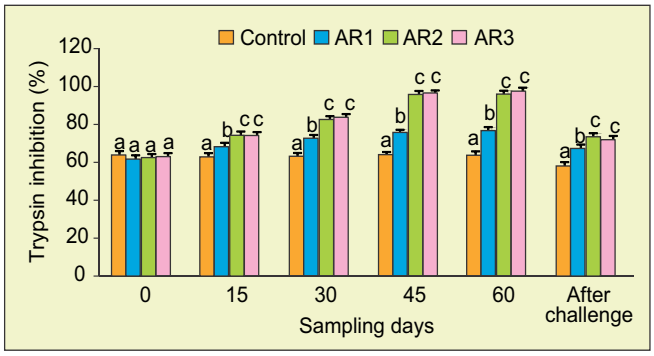

Fig. 5. Antiprotease activity (%) on various sampling days of different experimental groups fed with *A. racemosus* roots aqueous extract (values are mean±SE). Mean values with different superscript within a column for a parameter are significantly different ($P<0.05$).

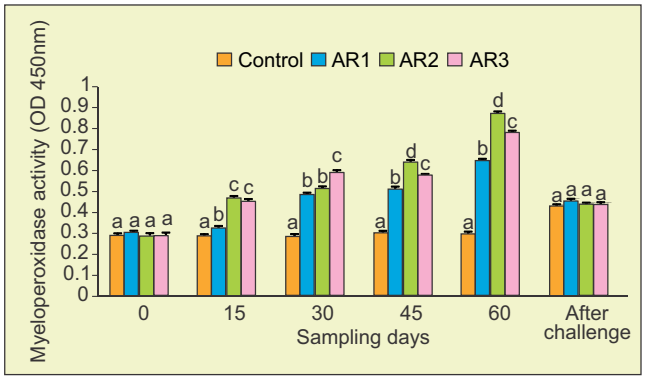

Fig. 6. Myeloperoxidase activity on various sampling days of different experimental groups fed with *A. racemosus* roots aqueous extract (values are mean±SE). Mean values with different superscript within a column for a parameter are significantly different ($P<0.05$).

A. racemosus roots extracts potent bioactive substances role in up-regulation of the mechanism of immune defence against pathogens by influencing the enzyme activity and hydrolysis of pathogens protein *in vivo* (Tremacoldi and Pascholati 2002) and restricting the replication of microbial pathogens without adverse toxicity to the fish (James *et al*. 1999).

Myeloperoxidase, a member of the heme peroxidase superfamily, is contained in azurophilic granules in neutrophils and monocytes. It is released upon leukocyte activation, contributing to innate immunity (Nicholls and Hazen 2005). Myeloperoxidase activity in the groups AR_2 and AR_3 was significantly ($P<0.05$) higher on 15th, 30th, 45th, and 60th days of sampling in comparison to control. The group AR_1 was observed to be significant ($P<0.05$) on 30th, 45th and 60th day of sampling as compared to control. The highest myeloperoxidase activity was observed in AR_2 group on 60th day of sampling (Fig. 6). From the results obtained in the present study, it is clear that the root extract at different concentrations was able to stimulate the serum peroxidase activity which was in line with Subeenabegum and Navaraj (2016), who had shown a substantial increase in myeloperoxidase activity in *Mystus keletius* administered with *Solanum trilobatum* and *Ocimum sanctum* extract. Present results corroborates with the finding of Pratheepa and Sukumaran (2014), who reported enhanced myeloperoxidase activity in *Cyprinus carpio* administered with *Euphorbia hirta* plant leaf extract against *A. hydrophila* infection. Similarly, it was supported by the findings in *Catla catla* fed with *Cynodon dactylon* (Kaleeswaran *et al*. 2010), in *Oreochromis mossambicus* administered with *Eclipta alba* leaf extract (Christybapita *et al*. 2007). In the present, study enhanced serum myeloperoxidase activity indicates the synergism of phyto components (Subeenabegum and Navaraj 2016) of the plant extracts in release of myeloperoxide enzyme from the azurophilic granules of neutrophils through the activation of leukocytes catalyses the formation of several reactive species (Pratheepa and Sukumaran 2014), as indicated by increase respiratory burst activity (NBT level), thus plays a role in host defence against microorganisms (Klebanoff 2005).

Challenge study and agglutination antibody titre: After injection with *Aeromonas hydrophila*, the first mortality was recorded after 12 h. The highest survival was recorded in the AR_2 (43.36±0.65) group, followed by AR_3 (24.32±0.14), AR_1 (17.26±0.45) and lowest percentage survival was recorded in the control (3.42±0.02) group (Table 2). Interestingly, after challenge with *A. hydrophila*, all the experimental groups showed higher survival rate compared to the control. This might be due to the enhancement of the non-specific immune system of fish by plant extracts. Similar results were reported in *Cyprinus carpio* administered with Henna (*Lawsoni ainermis*) extract challenged with *A. hydrophila* (Soltanian and Fereidouni 2016). Similarly, in *Cyprinus carpio* administered with *Euphorbia hirta* plant leaf challenged with *A. hydrophila*

Table 2. Relative percentage survival (RPS) and agglutination antibody titre of *Labeo rohita* after challenge with *A. hydrophila*

Group	RPS (%)	Agglutination antibody titre
Control	3.42±0.02[a]	17.42±0.02[a]
AR_1	17.26±0.45[b]	38.26±0.45[b]
AR_2	43.36±0.65[c]	87.36±0.65[c]
AR_3	24.32±0.14[d]	49.32±0.14[d]

All the data are represented as mean±SE. Mean values with different superscript within a column for a parameter are significantly different (P<0.05).

(Pratheepa and Sukumaran 2014), in gold fish (*Carassius auratus*) *A. racemosus* extract used as an adjuvant (Thangaviji *et al*. 2012).

The highest agglutination antibody titre was recorded in the AR_2 (87.36±0.65) group followed by AR_3 (49.32±0.14) and AR_1 (38.26±0.45) and lowest in control (17.42±0.02) group (Table 2); supported by the findings in pacu, *Piaractus mesopotamicus* fed with levamisole immunized with inactive *A. hydrophila* (Biller-Takahashi *et al*. 2014). Similarly, in *Catla catla* feeding with *Cynodon dactylon* ethanol extract (Kaleeswaran *et al*. 2010), in *Oreochromis niloticus* challenged with *A. hydrophila* (Bailone *et al*. 2010).

Decreased mortality rate and better protection level against infections in present study may be considered mainly due to combination of non-specific immune reaction such as production of super oxide anion, strong lytic activity of lysozyme, production of hypochlorous acid, hydrolysis of pathogens protein *in vivo*, stimulation of phagocytes and specific immune reaction such as increase in serum immunoglobulin and agglutination antibody titre provided protection from the invading pathogen and facilitated the antigen neutralization and the quick removal from the host (Kaleeswaran *et al*. 2010). The activation of immune response in the present study might be due to bioactive phaytoconstituents of *A. racemosus* roots which includes five saponins: shatavarin I, shatavarin IV, shatavarin V, immunoside and schidigerasaponin D5 (Hayes *et al*. 2008), steroidal saponins, shatavarins VI-X, isoflavone (Saxena and Chaurasia 2001), sitosterol, benzaldehyde and undecanylcetanoate (Singh and Tiwari 1991) may directly initiate activation of the defence mechanisms by acting and up-regulating on different immune targets (Eui-joon *et al*. 2006) which results triggering proliferative effects on immune cells and lymphocytes (Shive *et al*. 2000) and provided protection.

Thus, from the present study, it can be concluded that feed containing 100 mg *A. racemosus* aqueous root extracts/ kg diet (AR_2) might be the most appropriate dose in *L. rohita* which activated the receptors and the corresponding genes responsible for the secretion of immune defence factors and protection against pathogenic *A. hydrophila* bacterial infection at cool hilly mid altitude region. However, present study opens up new avenues for future study on most effective dose under pond conditions, as well as the effect of *A. racemosus* through different modes of administration should be further investigated in order to ascertain its molecular mechanism, administrative regime for different age group of fish and the time of application to ensure enhanced growth and production in culture system.

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NUTRIENT REQUIREMENTS OF ANIMALS

A nutritionally balanced 'livestock feed basket' improves the productivity of animals and simultaneously the economic condition of animal keepers. Feed requirement varies from species to species and from one geographic zone to another depending upon the animal potential and plant-soil-animal relationship. Several institutes of the Indian Council of Agricultural Research, have been working on these crucial aspects of animal nutrition since their

Poultry in 1985 and 1998. Changing
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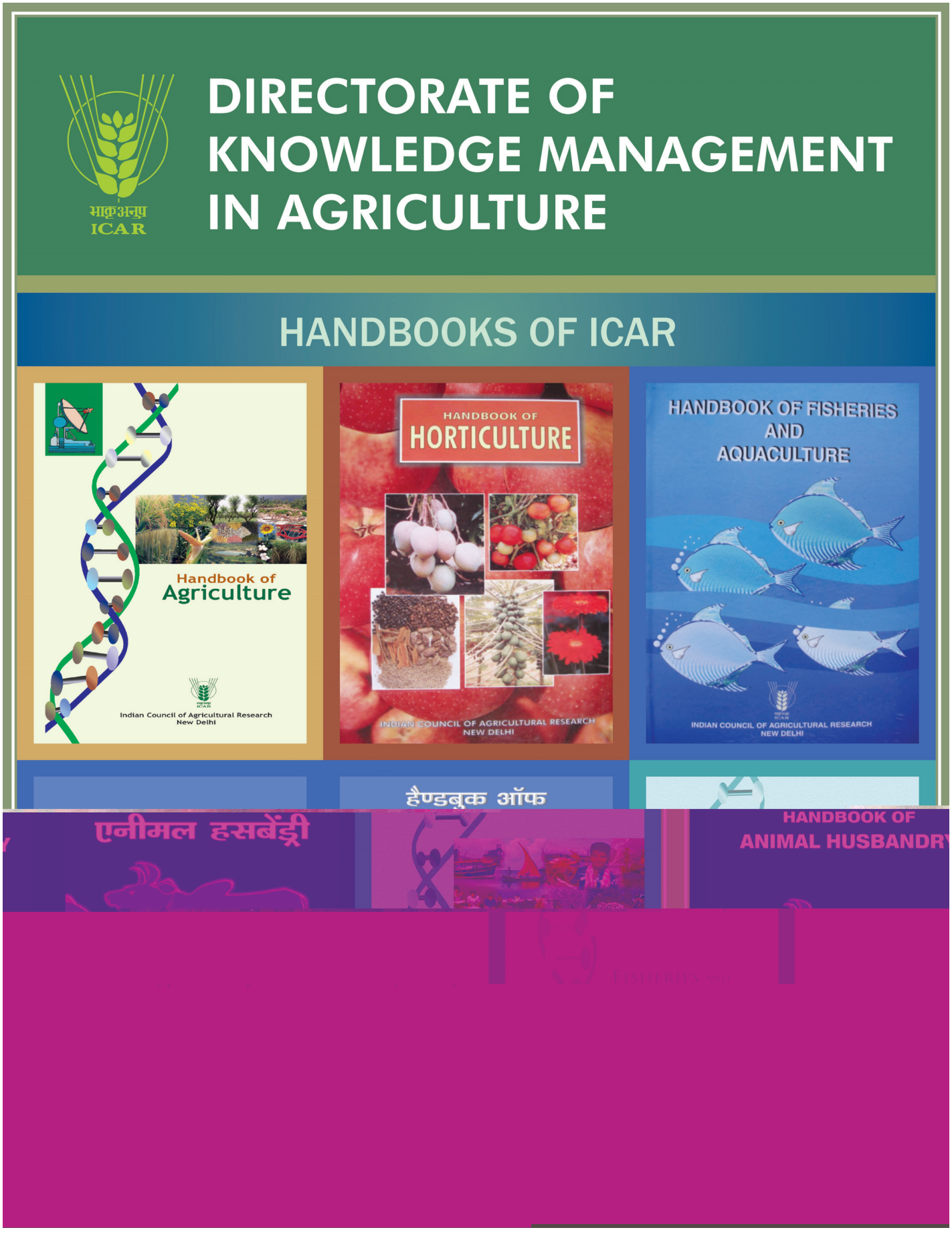
inception. Earlier, ICAR published Nutrient Requirement of Livestock and
climate, vegetation cover and expectations of human population from anim
animal sector scenario. Realizing the fact that detailed information is require
feeds and fodders, the Council constituted a National Committee on Nu
compilation of information generated by these institutes.

In this present attempt the Committee has brought out 'Nutrient Requi
publications. For the first time nutrient requirements of Camel, Yak and
captive wild animals besides Finfish and shellfish have been compiled. This se
for livestock policy-framers, researchers, academicians, extension officials an
changes in the societies' nutritional security and social integration.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