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Lactuca indica extract as feed additive enhances immunological parameters and disease resistance in *Epinephelus bruneus* to *Streptococcus iniae*

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ABSTRACT

The present study evaluated the efficacy of dietary doses of *Lactuca indica* extract on immunological parameters and disease resistance against *Streptococcus iniae* infection in kelp grouper, *Epinephelus bruneus*. Fishes were fed with *L. indica* enriched diet at 1.0% and 2.0%; both the diets significantly enhanced NBT level and phagocytic activity on week 2 and 4 compared to control diet whereas the changes did not manifest on first week. The total immunoglobulin significantly enhanced in fish fed with 1.0% and 2.0% enriched diets whereas the lysozyme activity significantly enhanced all the diets from weeks 1 to 4. Further the enriched diets at 1% and 2% level resulted in lower mortality of 45% and 40% respectively indicating higher protection from *S. iniae* infection than 0.1% diet that resulted in 55% mortality. The results suggest that the dietary supplementation of *L. indica* extract stimulates the immunological parameters and increases disease resistance in *E. bruneus* against *S. iniae* infection.

1. Introduction

Groupers are found in the tropical and warm waters in the Eastern Atlantic Ocean and in the Mediterranean Sea; they are commonly known as groupers and belong to the subfamily *Epinephelinae* which include 159 species in 15 genera. The global production of groupers was 198,690 metric tonnes in 2007 (FAO Yearbooks of Fisheries Statistics, 2009). Large scale grouper production continues to encounter increasing difficulties due to different viral, bacterial, and parasitic pathogens such as *Vibrio carchariae* and *V. alginolyticus*, and *V. harveyi* (Lee, 1995; Saeed, 1995; Yii et al., 1997), *Pseudomonas* sp. (Nash et al., 1987), *Flexibacter* sp., (Danayadol et al., 1996), and *Streptococcus* sp. (Arthur and Ogawa, 1996). The streptococcal infection caused by *Streptococcus iniae* was first reported in rainbow trout in Japan during the year 1958 (Hoshina et al., 1958). Later the pathogen was reported in yellowtail, Ayu, and tilapia (Bowser et al., 1998; Kusuda et al., 1979; Ohnishi and Jo, 1981). In 1997 the estimated annual impact of this bacterium infection in USA aquaculture industry alone amounted to US$ 10 million and globally estimated as US$ 100 million (Shoemaker et al., 2001).

Antibiotics and chemotherapeutics used for prophylaxis and treatment in intensive aquaculture have been widely criticized for their negative impacts (FAO, 2003). Vaccines are the most promising method of preventing diseases. However, a single vaccine is effective against only one type of pathogen (Ardo et al., 2008). Recently, immunostimulants of herbal origin have been shown to possess the ability to increase disease resistance in fish against a number of diseases by enhancing non-specific and specific defence mechanisms (Harikrishnan et al., 2011).

Lactuca indica L. belonging to the family Compositae is an edible medicinal plant widely distributed in Asian countries including Korea. *L. indica* extract has been traditionally used as an anti-inflammatory, anti-bacterial, and anti-diabetic medicine (Hou et al., 2003; Kan, 1986); it is known to stimulate differentiation of the mouse melanoma cell line, B16 2F2 (Hata et al., 2003). In this backdrop, it was planned to systematically evaluate the dietary administration of *L. indica* extract on immunological parameters and disease resistance against *S. iniae* infection in *E. bruneus*.

2. Materials and methods

2.1. Fish and management

Kelp grouper, *Ephinephelus bruneus* (weight 27.7 ± 1.4 g) obtained from Dongbok fish farm located in Eastern Jeju Island, Republic of Korea and were transported to the Marine and Environmental Research Institute, Jeju National University. The fish were immediately examined to find out their health status upon arrival (Austin and Austin, 1989) and acclimatized in the indoor cement tanks (capacity:1000 L) with recirculating aerated seawater for 15 days. Continuous aeration was also provided to maintain dissolved oxygen levels at 8.5 ± 0.5 mg l^{-1} and 30% of the seawater was exchanged with sand-filtered water daily.

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During the experimental period water temperature, pH, and salinity were 26 ± 1 °C, at 7.7 ± 0.8, and at 31.2 ± 1.3‰. Fishes were provided with normal feed (without herbal extract) at the rate of 5% of their body weight twice a day at 09:00 and 15:00 h (Table 1).

2.2. Lactuca indica extract

L. indica was collected from locally in Jeju Island, South Korea and the identification was done by Department of Biotechnology, Jeju National University. The plants were washed thoroughly with sterile distilled water, shade dried, and grounded in a mechanical grinder and sieved. The powders were sieved through an 80 μ mesh. The collected powder was kept sealed in a plastic bag and stored at −20 °C until use. The herbal powder (100 g) was mixed with 1000 ml of 95% ethanol in a 2000 ml conical flask kept for 7 d at room temperature, and agitated daily to ensure complete digestion. The extracts were filtered through Whatman No. 2 filter paper and the filtrate was dried under reduced pressure. The residues obtained after evaporation of ethanol was kept in sterilized screw cap glass container and stored at −20 °C until use.

2.3. Preparation of herbal diets

The experimental diet was prepared with the locally available ingredients as shown in Table 1. All the ingredients were mixed thoroughly by adding water, and then pelletized by using a hand pelletizer (Xie et al., 2008) and then dried at 40 °C for 12 h. Four experimental pellet diets were prepared with 0.1%, 1.0%, and 2.0% of *L. indica* extract sprayed to the basal diet slowly and mixing evenly in a drum mixer; the feeds were then air dried under sterile conditions for 12 h. The control basal diet (0% extract) was added with the same volume of solvent without the extracts. The pellets were dried in an oven at 30 °C for 18 h, packed, and stored in a freezer at −20 °C until used. The proximate composition of the diets quantified following AOAC method comprised 53.5% crude protein, 8.2% crude lipid, 7.8% crude ash, and 14.7% crude carbohydrate.

2.4. Streptococcus iniae

S. iniae isolated from diseased olive flounder were kindly provided by Prof. Moon-Soo Heo, Jeju National University for the study. Stocks were grown in tryptic soy broth (TSB; Difco Laboratories, Sparks, MD) for 24 h at 27 °C and then kept frozen in 200 μl aliquots at −70 °C. The subculture was taken in TSB; after centrifugation the supernatant was discarded and the pellets were re-suspended in sterile phosphate buffer saline (PBS, pH 7.4). The cultures were adjusted to an optical density of 1.2 at 540 nm using a spectrophotometer to give an *S. iniae* suspension at 3.3×10^7 colony forming units (cfu) ml^{-1} after 24 h at 27 °C incubation. The bacterium was confirmed relevant biochemical and molecular studies.

Table 1
Basal diet composition for kelp grouper.

Ingredients	(%)
Fish meal	50
Krill meal	16
Fish oil[a]	10
Vitamin mixture[b]	5
Mineral mixture[c]	5
Guar gum	2
CMC Na[d]	4
Cellulose	8

[a] Riken feed oil Ω (Eiken Shoji Co. Ltd, Tokyo, Japan).
[b] Vitamins (mg/100 g dry diet): thiamine HCl, 1.6; pyridoxine HCl, 1.4; nicotinic acid, 4.7; inositol, 120; folic acid, 1.5; choline chloride, 725; calcium ascorbate, 124; menadione-$NaHSO_4$, 5.12; riboflavin, 2.85; calcium pantothenate, 9.20; biotin, 4.75; cyanocobalamin, 1.10; vitamin A palmitate, 2.75; α-tocopherol, 115; α-cellulose, 820.
[c] Minerals (mg/100 g dry diet): KH_2PO_4, 215; $Ca(H_2PO_4)_4 \cdot H_4O$, 255; calcium lactate, 110; iron citrate 67; $ZnSO_4 \cdot H_2O$, 7; $CuSO_4 \cdot 4H_2O$, 5.25; $CoCl_2 \cdot 6H_2O$, 0.04; KIO_3, 0.13; a-cellulose, 450.0; dextrin, 360.
[d] Carboxymethyl cellulose sodium salt.

2.5. Experimental design

The experiment was performed in 200 L plastic tanks in the department wet laboratory. The fishes were divided into four groups (0%, 0.1%, 1.0%, or 2.0%) of 25 fish each in triplicate. Fishes were provided with adequate aeration and fed at the rate of 5% of body weight twice a day with the respective diets till the end of the experiment. On 30th day of feeding, all groups were injected intraperitoneally (i.p.) with 100 μl PBS containing *S. iniae* at 3.3×10^7 cfu ml^{-1}. On weeks 1, 2, and 4 post-challenge, six fish randomly collected from each tank were anaesthetized with MS-222 ($NaHCO_3$ and tricaine methanesulphonate; Sigma Chemicals) 1:4000 in dechlorinated water for 2 min to collect blood samples for analyzing of immunological parameters. The cumulative mortality was calculated by following Amend (1981). Relative percentage survival (RPS) was calculated as: The Number of surviving fishes after challenge/Number of fishes injected with bacteria) x 100 (Misra et al., 2006a,b).

2.6. Bleeding and separation of serum

Blood from the fish were drawn directly from the heart with the help of a sterilized 1 ml hypodermal syringe containing EDTA as an anticoagulant using 24 gauge needles. For serum separation blood was collected without anticoagulant in serological tubes and stored in a refrigerator overnight. The clot was then spun down at 3000 g for 10 min. The collected serum 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.

2.7. Non-specific immune response assay

The respiratory burst activity was measured by NBT assay, the phagocytic activity, total immunoglobulin level, the plasma lysozyme activity in plasma were quantified following the modified method of Anderson and Siwicki (1995).

2.8. Statistical analysis

All the data are expressed as mean ± SE. Statistical analysis of data involved one way analysis of variance (ANOVA) followed by the comparison of means following Least Square Design (LSD) with SPSS windows 15.0 version. The level of significance was expressed as p-value at 0.05 levels.

3. Results

3.1. NBT assay

The NBT level did not significant change in all diets on first week against *S. iniae*. The NBT level significantly increased with 1.0% and 2.0% supplemented diets on weeks 2 and 4 compared to control. However, NBT level did not significantly change with 0.1% dose in comparison with control (Fig. 1).

3.2. Phagocytic activity

Phagocytic activity did not significantly enhance with 0.1%, 1.0%, and 2.0% enriched diet on first week against *S. iniae*. However with 1.0% and 2.0% doses the activity significantly increased on weeks 2 and

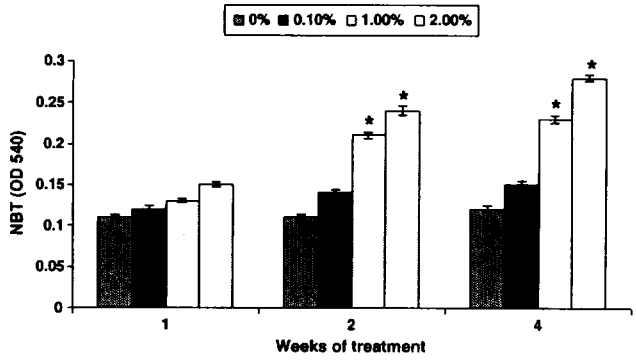

Fig. 1. NBT level of *E. bruneus* fed with different doses of *L. indica* extract against *S. iniae*. Data are mean ± S.E (n = 6) and the difference in values ($p < 0.05$) between groups is indicated by asterisks.

4, but not with 0.1% dose supplementation diet, as compared with the control (Fig. 2).

3.3. Total immunoglobulin in plasma

Total immunoglobulin (Ig) level did not significantly increase in fish fed with 0.1% dose supplementation diet from weeks 1 to 4 when compared to control against the pathogen. On the other hand, fish fed with 1.0% and 2.0% doses had significantly enhanced the phagocytic activity from weeks 1 to 4 when compared to control (Fig. 3).

3.4. Lysozyme activity

The lysozyme activity did not significantly enhanced fish fed with 0.1% supplementation diet whereas 1.0% and 2.0% enriched diets resulted in a significant increase on first week compared to control. The lysozyme activity significantly increased in all the doses on weeks 2 and 4, in comparison with the control against *S. iniae* (Fig. 4).

3.5. Disease resistance

The cumulative mortality was low (45% and 40%) when fed with 1.0% and 2.0% supplemented diets. Without the extract (0% dose) the mortality was 85%. However, the 0.1% dose yielded 55% mortality (Fig. 5).

4. Discussion

In aquaculture, the use of immunostimulants is of increasing interest for boosting the defence mechanisms and conferring

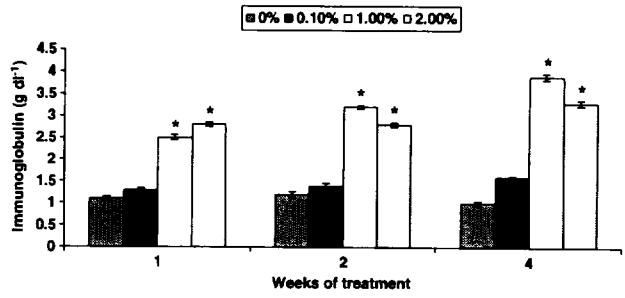

Fig. 3. Total immunoglobulin level of *E. bruneus* fed with different doses of *L. indica* extract against *S. iniae*. Data are mean ± S.E (n = 6) and the difference in values ($p < 0.05$) between groups is indicated by asterisks.

protection from infectious diseases. The effect of *L. indica* on the immune systems in aquatic animal has not been established though it has been used as an important herb for boosting the defence mechanisms. The present study revealed that NBT value did not significantly increase on first week at any doses against pathogen. However, it significantly increased with 1.0% and 2.0% doses on week 2 and 4. This result supports the findings in *C. carpio* fed with the diet containing oligodeoxynucleotides (Asmi et al., 2002) but do not fall in line with that of *L. rohita* fed with diet containing n-3 PUFA (Misra et al., 2006a,b) and in trout upon injection of yeast cell wall glucan (Jorgensen et al., 1993), which showed unchanged NBT level. NBT activity is a good indicator of oxygen dependent bactericidal activities (Weir and Stewart, 1993). In rainbow trout fed with *Allium sativum*, *Lupinus perennis*, *Mangifera indica*, and *Urtica dioica* against *Aeromonas hydrophila* infection the treatment also boosted the head kidney macrophage extracellular respiratory burst activity (Awad and Austin, 2010; Nya and Austin, 2009).

The phagocytic activity significantly increased when fed with 1.0% and 2.0% supplementation diets on weeks 2 and 4 but it did not any diet on first week against the pathogen. The present findings are in line with the report in *C. carpio* fed with the diet containing oligodeoxynucleotides (Asmi et al., 2002), greasy groupers *E. tauvina* fed with herbal diet containing purified active principle of *Ocimum sanctum*, *Withania somnifera*, and *Myristica fragrans* (Sivaram et al., 2004) and in Chinese sucker treated with TCM extracts (Zhang et al., 2009). An increase in phagocytic activity indicated in the present study indicates the significant role of *L. indica* in enhancing the non-specific immune response. Similar finding has been reported in rainbow trout fed with *A. sativum*, *L. perennis*, *M. indica*, and *U. dioica* against *A. hydrophila* infection (Awad and Austin, 2010; Nya and Austin, 2009).

The total Ig significantly increased with 1.0% and 2.0% dose supplementation diets but not with 0.1% diet against pathogen; the results are comparable with the findings in greasy groupers fed with

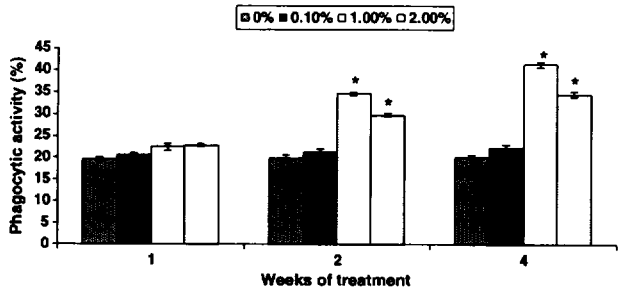

Fig. 2. Phagocytic activity (%) of *E. bruneus* fed with different doses of *L. indica* extract against *S. iniae*. Data are mean ± S.E (n = 6) and the difference in values ($p < 0.05$) between groups is indicated by asterisks.

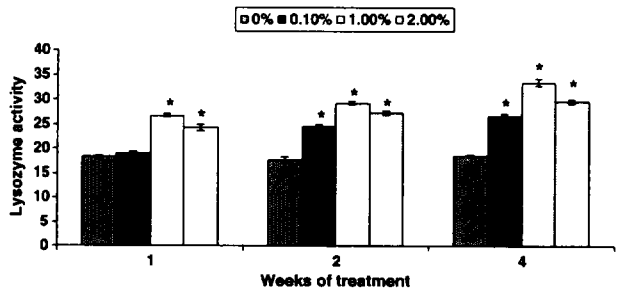

Fig. 4. Lysozyme activity of *E. bruneus* fed with different doses of *L. indica* extract against *S. iniae*. Data are mean ± S.E (n = 6) and the difference in values ($p < 0.05$) between groups is indicated by asterisks.

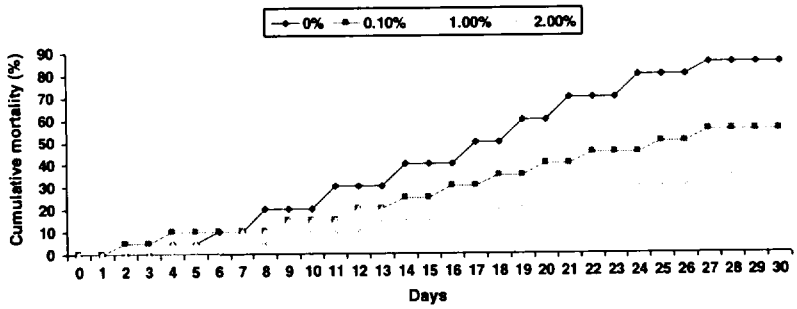

Fig. 5. The cumulative mortality of *E. bruneus* fed with different doses of *L. indica* extract against *S. iniae*.

herbal diet (Sivaram et al., 2004), in Siberian sturgeon, *Acipenser baeri* with glucan (Kolman et al., 1998) and epin (Kolman, 2001) respectively but differs with the finding in hybrid striped seabass *Morone chrysops* × *M. sexatilis* upon vitamin C and E treatment (Sealey and Gatlin, 2002) where unchanged level of total Ig were reported. In the present study the lysozyme activity significantly increased from weeks 1 to 4 with all *L. indica* supplemented diets but not with 0.1% dose diet on first week. Similar results have been reported in Chinese sucker treated with TCM extracts could enhance the lysozyme activity (Zhang et al., 2009), and in Jian carp (Jian and Wu, 2004). *Oreochromis niloticus* fed with *Astragalus radix* root also yielded the same effect (Yin et al., 2006). Administration of the Vitamin C also has increased the lysozyme activity in Atlantic salmon, *Salmo salar* (Lygren et al., 1999) and in rainbow trout, *Oncorhynchus mykiss* (Verlhac et al., 1996). Lysozyme activity functions as a primary defence factor of humoral immunity in preference to cellular defence mechanisms and its ability to disrupt the cell walls of certain pathogen makes lysozyme a natural antagonist to harmful invaders like parasite, bacteria, and virus. The lysozyme activity was found to increase in fish blood in response to infections (Maita, 2007). The increase of lysozyme activity in the present study revealed an increased response by the non-specific defence system of the when fish were fed with *L. indica* supplemented diet.

The challenge test with *S. iniae* decreased the cumulative mortality with 45% and 40% when fed with 1.0% and 2.0% supplementation diet; it was as high as 55% in 0.1% dose when compared to the control (85%). The decrease in mortality rate with dietary *L. indica* after injection of *S. iniae* is in agreement with previous study conducted in *O. mossambicus* fed with diet containing *O. sanctum* (Logambal et al., 2000), *L. rohita* fed with the diet containing *Achyranthes aspera* (Rao et al., 2006), *O. mossambicus* treated with *Eclipta alba* leaf extract (Christybapita et al., 2007) against *Aeromonas hydrophila* infection. In *A. hydrophila* infected rainbow trout a similar result was reported when fed with *A. sativum*, *L. perennis*, *M. indica*, and *U. dioica* (Awad and Austin, 2010; Nya and Austin, 2009). The increased level of total Ig as observed in the present study might be due to the secretion of humoral immune responsive proteins via cellular immune response as reported in BALB/c mice administered with standardized root extract of *W. somnifera* which elicited humoral and cell mediated immune responses by up regulation of Th1-dominant polarization (Malik et al., 2007). Similarly the decreased mortality rate on administration of *L. indica* is comparable to the immunomodulatory activity of *W. somnifera* (Davis and Kuttan, 2000; Ziauddin et al., 1996) as evident from increased NBT level, phagocytic activity, and lysozyme activity. Thus, from the present study it can be deduced that feed containing 1.0% and 2.0% of *L. indica* extract can influence the immunological parameters in kelp grouper. The plant's constituents may directly initiate activation of the innate defence mechanisms acting on receptors and triggering intracellular gene activation that may result in the production of antimicrobial molecules (Bricknell and Dalmo, 2005). The stimulation of specific and non-specific immune defence observed in the present study might be due to the presence of one or more than one components present in *L. indica*. The present results has confirmed the maximum response with 1.0% and 2.0% enriched diet against *S. iniae* which might suffice to activate the receptors and the corresponding genes responsible for the secretion of immune defence factors. The present study opens up new vistas of research to assess the most effective dose under field conditions, experimentation with purified extract of *L. indica*, degree, and duration of the resistance offered, administrative regime for different age group of fish and time of application to ensure improved harvest in culture ponds.

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References

Amend, D., 1981. Potency testing of fish vaccines. Dev. Biol. Stand. 49, 447–454.
Anderson, D.P., Siwicki, A.K., 1995. Basic hematology and serology for fish health programmes. In: Shariff, M., Arthur, J.R., Subasinghe, R.P. (Eds.), Disease in Asian aquaculture. Fish Health Section, Asian Fisheries Society, Manila, Philippines, pp. 185–202.
Ardo, L., Yin, G., Xu, P., Varadi, L., Szigeti, G., Jeney, Z., et al., 2008. Chinese herbs (Astragalus *membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. Aquaculture 275, 26–33.
Arthur, J.R., Ogawa, K., 1996. A brief overview of disease problems in the culture of marine finfishes in east and southeast Asia. In: Main, K.L., Rosenfeld, C. (Eds.), Aquaculture health management strategies for marine fishes. The Oceanic Institute, Makapu'u Point, HI, USA, pp. 9–31.
Asmi, C.M., Tassakka, A.R., Sakai, M., 2002. CpG oligonucleotides enhance the non-specific immune responses in carp, *Cyprinus carpio*. Aquaculture 209, 1–10.
Austin, B., Austin, D.A., 1989. Microbiological Examination of Fish and Shellfish. Ellis Horwood, Chichester.
Awad, E., Austin, B., 2010. Use of lupin, *Lupinus perennis*, mango, *Mangifera indica*, and stinging nettle, *Urtica dioica*, as feed additives to prevent *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis. 33, 413–420.
Bowser, P.R., Wooster, G.A., Getchell, R.G., Timmons, M.B., 1998. *Streptococcus iniae* infection of tilapia *Oreochromis niloticus* in a recirculation production facility. J. World Aquacult. Soc. 29, 335–339.
Bricknell, I., Dalmo, R.A., 2005. The use of immunostimulants in fish larval aquaculture. Fish Shellfish Immunol. 19, 457–472.
Christybapita, D., Divyagnaneswari, M., Michael, R.D., 2007. Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus*. Fish Shellfish Immunol. 23, 840–852.
Danayadol, Y., Krachaiwong, V., Ruangpan, L., Direkbusarakom, S., 1996. Causative agents and control measures of red boil disease in cultured grouper (*Epinephelus malabaricus*). Thai Fish Gazette 49, 29–35.

Davis, L., Kuttan, G., 2000. Immunomodulatory activity of withania somnifera. J. Ethnopharmacol. 71, 193–200.
FAO, 2003. Yearbook on fishery statistics. Rome, Italy.
Food Agriculture Organization (FAO), 2009. Yearbooks of Fisheries Statistics. Food Agriculture Organization of the United Nations, Rome.
Harikrishnan, R., Balasundaram, C., Heo, M.S., 2011. Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. Aquaculture 317, 1–15.
Hata, K., Hori, K., Mukaiyama, T., Sakamoto, K., Takahashi, S., 2003. Activators of melanin biosynthesis from *Lactuca indica*. Nat. Med. 57, 238–241.
Hoshina, T., Sano, T., Morimoto, Y., 1958. A streptococcus pathogenic to fish. J. Tokyo Univ. Fish. 44, 57–68.
Hou, C.-C., Lin, S.-J., Cheng, J.-T., Hsu, F.-L., 2003. Antidiabetic dimeric guianolides and a lignan glycoside from *Lactuca indica*. J. Natu. Products. 66, 625–629.
Jian, J., Wu, Z., 2004. Influences of Traditional Chinese medicine (TCM) on non specific immunity of Jian carp (*Cyprinus carpio* var. Jian). Fish Shellfish Immunol. 16, 185–191.
Jorgensen, J.B., Sharp, G.J.E., Secombes, C.J., Robertsen, B., 1993. Effect of yeast cell wall glucan on the bactericidal activity of rainbow trout macrophages. Fish Shellfish Immunol. 3, 51–58.
Kan, W.S., 1986. Pharmaceutical Botany, 7th ed. National Research Institute of Chinese Medicine, Taipei, Taiwan, ROC.
Kolman, H., 2001. The humoral effects of epin in Siberian sturgeon (*Acipenser baeri* Brandt). Arch Polish Fish. 9, 61–69.
Kolman, R., Kolman, H., Siwicki, R., 1998. The effect of some immunomodulators on the growth rate of sturgeon fry (Acipenseridae). Arch Polish Fish. 6, 383–390.
Kusuda, R., Kawai, K., Toyoshima, T., Komatsu, I., 1979. A new pathogenic bacterium belonging to the genus *Streptococcus*, isolated from a epizootic of cultured yellowtail. Nihon Suisan Gakkai Shi 42, 1345–1352.
Lee, K.K., 1995. Pathogenesis studies on *Vibrio alginolyticus* in the grouper, *Epinephelus malabaricus* Bloch et Schneider. Microb. Pathog. 19, 39–48.
Logambal, S.M., Venkatalakshmi, S., Michael, R.D., 2000. Immunostimulatory effect of leaf extract of *Ocimum sanctum* Linn. in *Oreochromis mossambicus* (Peters). Hydrobiologia 430, 113–120.
Lygren, B., Sveier, H., Hjeltnes, B., Waagbo, R., 1999. Examination of the immunomodulatory properties and the effect on disease resistance of dietary bovine lactoferrin and vitamin C fed to Atlantic salmon (*Salmo salar*) for a short-term period. Fish Shellfish Immunol. 9, 95–107.
Maita, M., 2007. Fish health assessment. In: Nakagava, H., Sato, M., Gatlin III, D.M. (Eds.), Dietary supplements for the health and quality of cultured fish. CABI International, Japan, pp. 11–35.
Malik, F., Singh, J., Khajuria, A., Suri, K.A., Satti, N.K., Singh, S., et al., 2007. A standardized root extract of *Withania somnifera* and its major constituent withanolide-A elicit humoral and cell mediated immune responses by up regulation of Th1- dominant polarization in BALB/c mice. Life Sci. 80, 1525–1538.
Misra, C.K., Das, B.K., Mukherjee, S.C., Pattnaik, P., 2006a. Effect of multiple injections of β-glucan on non-specific immunoresponse and disease resistance in *Labeo rohita* fingerlings. Fish Shellfish Immunol. 20, 305–331.
Misra, S., Sahu, N.P., Pal, A.K., Xavier, B., Kumar, S., Mukherjee, S.C., 2006b. Pre and Postchallenge immune-haematological changes in *Labeo rohita* juveniles fed gelatinized or non-gelatinised carbohydrate with n-3 PUFA. Fish Shellfish Immunol. 21, 346–356.
Nash, G., Anderson, I.G., Shariff, M., Shamsudin, M.N., 1987. Bacteriosis associated with epizootic in the giant sea perch, *Lates calcarifer*, and the estuarine grouper, *Epinephelus tauvina*, cage cultured in Malaysia. Aquaculture 67, 105–111.
Nya, E.J., Austin, B., 2009. Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis. 32, 963–970.
Ohnishi, K., Jo, Y., 1981. Studies on streptococcal infection in pondcultured fishes-I Characteristics of a beta-hemolytic *Streptococcus* isolated from cultured Ayu and Amago in 1977–1978. Fish Pathol. 16, 63–67.
Rao, Y.V., Das, B.K., Jyotyrmayee, P., Chakrabarti, R., 2006. Effect of Achyranthes aspera on the immunity and survival of Labeo rohita infected with Aeromonas hydrophila. Fish Shellfish Immunol. 20, 263–273.
Saeed, M.O., 1995. Association of *Vibrio harveyi* with mortalities in cultured marine fish in Kuwait. Aquaculture 136, 21–29.
Sealey, W.M., Gatlin III, D.M., 2002. Dietary vitamin C and vitamin E interact to influence growth and tissue composition of juvenile hybrid striped bass (*Morone chrysops* (female)*M. sexatilis* (male)) but have limited effects on immune responces. J. Nutr. 132, 748–755.
Shoemaker, C.A., Klesius, P.H., Evans, J.J., 2001. Prevalence of *Streptococcus iniae* in tilapia, hybrid striped bass, and channel catfish on commercial fish farms in the United States. Am. J. Vet. Res. 62, 174–177.
Sivaram, V., Babu, M.M., Immanul, G., Murugadass, S., Citarasu, T., Petermarian, M., 2004. Growth and immunoresponse of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemented diets against *Vibrio harveyi* infections. Aquaculture 237, 9–20.
Verlhac, V., Gabaudan, J., Obach, A., Schup, W., Hole, R., 1996. Influence of dietary glucan and vitamin C on non-specific responses of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 143, 123–133.
Weir, D.M., Stewart, J., 1993. Immunology, 2nd ed. Longman Group, UK Ltd, p. 372.
Xie, J., Liu, B., Zhou, Q., Su, Y., He, Y., Pan, L., et al., 2008. Effects of anthraquinone extract from rhubarb *Rheum officinale* Bail on the crowding stress response and growth of common carp *Cyprinus carpio* var. Jian. Aquaculture 281, 5–11.
Yii, K.C., Yang, T.I., Lee, K.K., 1997. Isolation and characterization of *Vibrio carchariae*, a causative agent of gastroenteritis in the groupers, *Epinephelus coioides*. Curr. Microbiol. 35, 109–115.
Yin, G., Jeney, G., Racz, T., Xu, P., Jun, X., Jeney, Z., 2006. Effect of two Chinese herbs (*Astragalus radix* and *Scutellaria radix*) on nonespecific immune response of tilapia, *Oreochromis niloticus*. Aquaculture 253, 39–47.
Zhang, G., Gong, S., Yu, D., Yuan, H., 2009. Propolis and *Herba epimedii* extracts enhance the non-specific immune response and disease resistance of Chinese sucker, *Myxocyprinus asiaticus*. Fish Shellfish Immunol. 26, 467–472.
Ziauddin, M., Phansalkar, N., Patki, P., Diwanay, S., Patwardhan, B., 1996. Studies on the immunomodulatory effects of Ashwagandha. J. Ethnopharmacol. 50, 69–76.