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Immunomodulatory effects of Curcumin (C*urcuma longa*) on fresh water edible fish Catla catla (H*amilton-buchanan*)

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Abstract

Natural agents such as curcumin may have the potential to enhance disease resistance in aquaculture. The incidence of infectious diseases combined with intensive commercial aquaculture practices are calling for additional and new strategies for disease management. The administration of antibiotics and other chemical anti-microbial agents is the most commonly used approach to control bacterial infections in fish. Among the renowned category of such immunostimulants, medicinal herbs, rank first due to their broad-spectrum activity, cost effectiveness and environmental-friendly disease preventative measure. Natural plant extracts could be one such alternative to enhance non-specific innate immunity and resistance to diseases caused by fish pathogens such as *Aeromonas hydrophila*. Curcumin is an orange-yellow, hydrophobic and polyphenolic compound of turmeric (*Curcuma longa*) that has been shown to be an anti-inflammatory and immunomodulatory agent in fish species (Kang et al., 1999).

Key Words: *Disease resistance Fish culture, Catla catla, clove oil, anti-inflammatory and immunomodulatory agent*

1. Introduction

The global trend of commercial aquaculture is towards intensification of culture practice in the aim to increase productivity per unit area (Elgendy et al., 2015a). Consequently, fish vulnerability to infectious agents is boosted with major stock mortalities and substantial economic losses (Elgendy et al., 2015b, c; Moustafa et al., 2015). Natural agents such as curcumin may have the potential to enhance disease resistance in aquaculture. The incidence of infectious diseases combined with intensive commercial aquaculture practices are calling for additional and new strategies for disease management. The administration of antibiotics and other chemical anti-microbial agents is the most commonly used approach to control bacterial infections in fish.

Recently, researchers have detected the serious threats associated with antibiotics in global public health. This effect is due to poor efficacy, potential toxicity and increased bacterial resistance from long-term usage of antimicrobial agents (Sasidharan et al., 2014). Strengthening fish defense mechanisms through prophylactic administration of immunostimulants has long been considered a stronghold in the control and management of diseases in aquaculture (Dugenci et al., 2003; Galina et al., 2009). Consequently, fish vulnerability to infectious agents is boosted with major stock mortalities and substantial economic losses (Elgendy et al., 2015b, c; Moustafa et al., 2015). Among the renowned category of such immunostimulants, medicinal herbs, rank first due to their broad-spectrum activity, cost effectiveness and environmental-friendly disease preventative measures (Alishahi and Nejad, 2012).

Alternative environment friendly, non-toxic and natural strategies that are both preventative and curative are being investigated. Natural plant extracts could be one such alternative to enhance non-specific innate immunity and resistance to diseases caused by fish pathogens such as *Aeromonas hydrophila*. Curcumin is an orange-yellow, hydrophobic and polyphenolic compound of turmeric (*Curcuma longa*) that has been shown to be an anti-inflammatory and immunomodulatory agent in fish species (Kang et al., 1999).

2. Materials and Methods

Diet preparation: Curcumin powder was purchased from (SIGMA-ALDRICH). A commercial pellet diet was crushed, mixed with tap water containing 2% of curcumin powder (2/100 g of powder) and made again into pellets. Remade pellets were dried and stored at 4°C.

2.1 Fish and experimental design:

Family :Cyprinidae
Sub-family :Cyprininae
Species :Catla catla (Hamilton-Buchanan)

Catla catla (Hamilton-Buchanan) fingerlings with average weight of 103±5 g were obtained from

Tamilnadu Fisheries Development Corporation Limited (TNFDC) Aliyar, Coimbatore district, Tamilnadu, India. Fish were transported alive to laboratory, before acclimatized for 15 days in glass aquaria (90×45×45 cm) supplied with chlorine free tap water under continuous aeration. The water was maintained at 25±2°C, pH (7.6), dissolved oxygen (6.6 mg L–1) and ammonia (0.01-0.005 mg L–1). The photoperiod was adjusted to 12 h light:12 h dark. Fish were fed on commercial diet 40% protein at the rate of 2% of body weight. Uneaten feed and faecal matters were siphoned out daily with about 75% water exchange. The physio-chemical water parameters were maintained within the optimum range as required for the *Catla catla* juveniles.

Fish were divided randomly into 2 groups each with 30 fish (10 per replicate) and fed for 30 days. First group fed with diet supplemented with 2% of curcumin while, other group were fed with commercial diets as control.

2.2 Determination of immune parameters in vivo

2.2.1Collection of blood and serum:

By the end of feeding experiment, after 30 days, blood samples were obtained from the caudal vein of experimental fish within the two groups for serum analysis and leukocytes isolation. Firstly, fish were anesthetized with clove oil (*Syzygium aromaticum*) (Merck, Germany) at 50 µL L–1 of water. Blood was drawn via syringe without anticoagulant for serum collection, tubes were kept in slanting position for about 2 h and thereafter centrifuged at 1600xg for 25 min at 4°C, followed by collection of dark coloured serum with micropipette and stored at -20°C for further analysis.

2.2.2 Serum proteins:

Different sera collected from fish groups were analysed for total protein and albumin content following the methods adopted from Doumas et al. (1971) and Lowry et al. (1951) respectively. Furthermore, globulin content was calculated by subtracting albumin from the total protein then albumin: globulin ratio was determined.

2.2.3 Challenge Study

After 10 days of initial sampling, all the fish in the experimental groups were injected intraperitoneally with the bacterial suspension of 0.2 ml (1.8 x 10^8 CFU ml^1). Mortality was observed for all the groups for 10 days. Sampling of the surviving fish was carried out on the tenth day. *A. hydrophila* was confirmed after reisolating it from the dead fish. Survival was calculated by using the following formula:

$$\% \text{ of survival} = \frac{\text{Number of surviving fish after challenge}}{\text{Number of fish injected with bacteria}} \times 100$$

2.2.4 Bacteria

Aeromonas hydrophila 018 was received from the, Central Institute of Fisheries Education (CIFE), Mumbai. *A. hydrophila* was grown on nutrient broth (HiMedia Ltd, India) for 24 h at 30 C. The culture broth was centrifuged at 3000g for 10 min. The supernatant was discarded, and the pellet was resuspended in sterile phosphate buffer saline (PBS, pH 7.4). The final bacterial concentration was adjusted to 1.8 x 108 CFU ml^1 by serial dilution.

3. Results and Discussion

Serum proteins: The total protein, albumen and globulin increased significantly with the administration of curcumin in comparison with the control group including the post challenge period.

3.1 Serum protein, albumin (A), globulin (G) and albumin/globulin (A/G) ratio:

A significant difference (P < 0.05) in the serum total protein, globulin and A/G ratio was found among the various treatment groups in the pre- and post-challenge periods (Table 1), whereas no significant difference (P > 0.05)

Table1.Effects of experimental diets on the serum protein and lipid profile of *Catla catla* after challenged with *Aeromonas hydrophila*

		T1 (0:100)[a]	**T2(20:80)[a]**	**T3 (40:60)[a]**	**T4 (60:40)[a]**	**T5 (80:20)[a]**	**T6 (100:0)[a]**
Total protein (gm %)	Before	4.07± 0.03	4.71± 0.06	3.73± 0.13	3.65± 0.05	3.37± 0.01	2.93± 0.05
	After	3.37± 0.07	3.86± 0.09	2.98± 0.08	3.05± 0.05	2.96± 0.09	2.53± 0.04
Albumin (A) (gm %)	Before	1.12 ±0.05	1.14± 0.02	1.16± 0.02	1.15± 0.01	1.15± 0.03	1.17± 0.01
	After	1.12 ±0.05	1.14 ±0.02	1.16± 0.02	1.1±50.01	1.15± 0.03	1.17±0.01
Globulin (G) (gm %)	Before	2.95± 0.02	3.58 ±0.08	2.57 ±0.15	2.49±0.04	2.22± 0.04	1.76 ± 0.06
	After	2.22 ±0.10	2.67± 0.16	1.87 ±0.01	2.67± 0.16	1.87± 0.01	1.32± 0.01

- a is the ratio in the parenthesis indicate G:NG
- Mean values in the same row with different superscript (a, b, c) differ significantly (P < 0.05).

was observed in the serum albumin content in any of the treatment groups in both the pre- and post-challenge periods Serum total protein and globulin content of the post-challenge period was significantly lower ($P < 0.05$) than the pre-challenged fish of all the treatments.
Highest serum total protein and globulin content was recorded in the T2 group and lowest in T6 group (100% G) and reverse trend was observed for A/G ratio during both the pre- and post-challenge periods. A/G ratio in the post-challenge fish was significantly higher ($P < 0.05$) than the pre-challenge period.

3.2 Protection upon challenge:

The survivability was found highest (100%) in the group fed with curcumin whereas the control group showed a survival rate of 40%.
Interest in the application of immunostimulants as an alternative method to chemicals and antimicrobials currently being utilized to control fish diseases is growing. The use of medicinal plant products as potential therapeutic measures for modulating the immune mechanisms as well as to control fish diseases have been focused on (Chakrabarti et al., 2014).
Results demonstrated that peroxidase content was increased significantly in the group of fish fed with curcumin supplemented diet in comparison with control fish fed basal diet ($p<0.05$). The peroxidase produced by fish macrophages are alleged to play significant roles in innate immune - defenses mechanisms against various fish pathogens (Stosik et al., 2001) as well as has been considered as a reliable measure for neutrophilic antimicrobial activity particularly the primary granule exocytosis (Quade and Roth, 1997).Numerous herbs are presently used in commercial aquaculture as potent antimicrobial agents, nutrients as well as growth-promoting substances (Vaseeharan and Thaya, 2014;4).
The bactericidal activity has been viewed as an important tool to analyse the innate immune system. It evaluates the presence of protective proteins in fish blood. Augmented serum bactericidal activities indicate elevated humoral factors involved in the innate and/or adaptive immune mechanisms (Wang et al., 2010). The serum bactericidal activity was significantly increased in fish group fed with curcumin supplemented diet compared to the control group including the post challenge period ($p<0.05$).
Similar results were detected in Indian major carp, *Catla catla*, on dietary administration of Zingiber officinale (Arulvasu et al., 2013) as well as in *Labeo rohita* fed with Magnifera indica kernel (Sahu et al., 2007).

4. Conclusion

Fish survival in the aquatic environment requires a competent immune system to overcome the constant challenge with pathogens. Results showed that curcumin can be a promising candidate for immunostimulant as well as a rival substitute for many antimicrobials currently used in *Catla* fish farming. This prima facie report may be useful for the nutritionists to recast their feed formulation for different species.

References

[1] Alishahi, M. and A.H.J. Nejad, 2012. Effects of propolis, a honeybee product, on growth performance and immune responses of *Barbus barbulus*. Iran. J. Vet. Med., 6: 249-257.
[2] Arulvasu, C., K. Mani, D. Chandhirasekar, D. Prabhu and S. Sivagnanam, 2013. Effect of dietary administration of *Zingiber officinale* on growth, survival and immune response of Indian major carp, *Catla catla (Ham.)*. Int. Pharm. Pharmaceut. Sci., 5: 108-115.
[3] Chakrabarti, R., P.K. Srivastava, N. Verma and J. Sharma, 2014. Effect of seeds of *Achyranthes aspera* on the immune responses and expression of some immune-related genes in carp *Catla catla*. Fish Shellfish Immunol., 41: 64-69.
[4] Doumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta, 31: 87-96.
[5] Dugenci, S.K., N. Arda and A. Candan, 2003. Some medicinal plants as immunostimulant for fish. J. Ethnopharmacol., 88: 99-106.
[6] Elgendy, M.Y., 2007. Epizootiological studies on some bacterial infections in marine fishes. M.Sc. Thesis, Cairo University, Egypt.
[7] Elgendy, M.Y., M. Abdelsalam, M. Moustafa, A.M. Kenawy and A. Seida, 2015. *Caligus elongatus* and Photobacterium *damselae subsp piscicida concomitant* infections affecting broodstock European seabass, *Dicentrarchus labrax*, with special reference to histopathological responses. J. Aquacult. Res. Dev., Vol. 6. 10.4172/2155-9546.1000346
[8] Elgendy, M.Y., M. Moustafa, A.Y. Gaafar and T.B. Ibrahim, 2015. Impacts of extreme cold water conditions and some bacterial infections on earthen-pond cultured Nile tilapia, *Oreochromis niloticus*. Res. J. Pharm. Biol. Chem. Sci., 6: 136-145.
[9] Elgendy, M.Y., W.S. Soliman, H.A. Hassan, A.M. Kenawy and A.M. Liala, 2015. Effect of abrupt environmental deterioration on the eruption of vibriosis in mari-cultured shrimp, *Penaeus indicus*, in Egypt. J. Fish. Aquatic Sci., 10: 146-158.
[10] Galina, J., G. Yin, L. Ardo and Z. Jeney, 2009. The use of immunostimulating herbs in fish: An overview of research. Fish Physiol. Biochem., 35: 669-676.

[11] Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

[12] Moustafa, M., A.E. Eissa, A.M. Laila, A.Y. Gaafar, I.M.K. Abumourad and M.Y. Elgendy, 2015. Investigations into the potential causes of mass kills in mari-cultured Gilthead sea bream, (*Sparus aurata*) at Northern Egypt. Res. J. Pharm. Biol. Chem. Sci., 6: 466-477.

[13] Quade, M.J. and J.A. Roth, 1997. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. Vet. Immunol. Immunopathol., 58: 239-248.

[14] Sahu, S., B.K. Das, B.K. Mishra, J. Pradhan and N. Sarangi, 2007. Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. J. Applied Ichthyol., 23: 80-86.

[15] Stosik, M., W. Deptula and M. Travnicek, 2001. Resistance in carps (*Cyprinus Carpio L.*) affected by a natural bacterial infection. Veterinarni Medicina, 46: 6-11.

[16] Vaseeharan, B. and R. Thaya, 2014. Medicinal plant derivatives as immunostimulants: an alternative to chemotherapeutics and antibiotics in aquaculture. Aquacult. Int., 22: 1079-1091.

[17] Wang, G.X., Y.T. Liu, F.Y. Li, H.T. Gao, Y. Lei and X.T. Liu, 2010. Immunostimulatory activities of bacillus simplex dr-834 to carp (*Cyprinus carpio*). Fish Shellfish Immunol., 29: 378-387.

[18] Yang, J., R. Zhang, G. Lu, Y. Shen and L. Peng et al., 2013. T cell-derived inducible nitric oxide synthase switches off TH17 cell differentiation. J. Exp. Med., 210: 1447-1462.

[19] Yilmaz, S. and S. Ergun, 2014. Dietary supplementation with allspice Pimenta dioica reduces the occurrence of s*treptococcal* disease during first feeding of mozambique tilapia fry. J. Aquat. Anim. Health, 26: 144-148.

[20] Zaidi, S.F., K. Yamada, M. Kadowaki, K. Usmanghani and T. Sugiyama, 2009. Bactericidal activity of medicinal plants, employed for the treatment of gastrointestinal ailments, against *Helicobacter pylori*. J. Ethnophamacol., 121: 286-291.

[21] Zvizdic, M.Z., E. Licek and C. Lam, 2012. Muramyl dipeptide, a stimulator of nonspecific immunity, induces the production of nitric oxide by Rainbow trout (*Oncorhynchus mykiss*) spleen and head kidney cells in vitro. Vet. Arhiv., 82: 617-627.