Effect of immunostimulants on health status of striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878)

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Maharashtra State, India
November, 2019

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THESIS

Submitted to the

Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli

in partial fulfilment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY (FISHERIES)

IN

FISH NUTRITION AND FEED TECHNOLOGY

BY

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With regard to the thesis entitled **"Effect of immunostimulants on health status of striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878)"** submitted by **Miss. Sagarika Swain** (Reg. No. FRRTD0150027) for the degree of this university, I certify that:

1. She has carried out the research work under my direct supervision and guidance in academic years 2015-2018 and that the manuscript of the thesis has been scrutinized by me.
2. The entire thesis comprises the candidate's own work and it is her own achievement. It has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of recognition.
3. The thesis does not contain any conjoint research work with me or anyone else.
4. She has completed her research work to my entire satisfaction.
5. The final typed copy of this thesis, which is being submitted to the university office, has been carefully read by me for its material and language and it is to my entire satisfaction.

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CANDIDATE'S DECLARATION

I hereby declare that this thesis or part thereof has not been submitted by me or any other person to any other university or institute for a degree or diploma.

Date: (Sagarika Swain)

Place: Ratnagiri

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1.0 INTRODUCTION

Freshwater aquaculture sector of India is mainly dominated by Indian major carps. However, with the initiative from the Government of India, pond and cage culture practices of Nile tilapia and striped catfish are being spread rapidly in the recent past (Laxmappa, 2016). With respect to striped catfish, *Pangasianodon hypophthalmus*, Asian continent had a maximum production of 0.51 mmt for the species in the year 2016 (FAO, 2018a). Though there is a lack of production data, it is being popularly used as one of the candidate species for freshwater aquaculture in India. Thus, the species has been selected as an experimental fish in the present study. The species is also well known for its faster growth, high disease resistance and tolerance of a wide range of environmental parameters (Bardach et al., 1972; Stickney, 1979; Begum et al., 2012).

High density fish rearing systems invariably pose an adverse effect on the environment and the species itself causing severe stock loss (Schreck, 1996). In the efforts for increasing production, higher density rearing systems would continue to dominate. With this, there would be an increased need to improve health status of the fishes under the high density systems. Conventionally, antibiotics, vaccines and chemicals are used to prevent or treat fish diseases. With the use of vaccines and chemicals have several drawbacks; the antibiotics have several problems associated with their use including toxicity, cost and government restrictions etc (Talpur et al., 2013). Furthermore, the antibiotic residues can be transferred to the aquaculture environment, to fish pathogens and could be accumulated in the fish bodies posing danger to human health (Wu et al., 2013). The control of diseases that occur in intensive aquaculture has also resulted in the increase of antibiotic-resistant pathogens, bioaccumulation, pollution and immune suppression (Biswas et al., 2012; Babu et al., 2013). Recently, therefore, an increasing emphasis has been given to the use of immunostimulants as adjuvants to vaccines and as potential alternative to use of antibiotics (Burrels et al., 2001).

Immunostimulants have become one of the key areas of applied medical research. Immunostimulants have ability to activate the immune system and a capacity to increase the disease resistance to infectious diseases by enhancing defence mechanisms (Raa, 2000). Immunostimulants also stimulate the natural killer cells, complements and lysozyme antibody responses of fish (Sakai, 1999; Tewary and Patra, 2007). Currently, numerous organisms and natural products have been used as immunostimulant sources to prevent and control fish diseases; such as polysaccharides, microorganisms, vitamins, plant derivatives (Awad and Austin, 2010; Bilen et al., 2011; Tewary and Patra, 2011; Kumar et al., 2013), yeast derivatives etc (Li et al., 2006; Reyes-Becerril et al., 2008; Sarlin and Philip, 2011; Babu et al., 2013; Ma et al., 2013).

Thus, the present study is attempted to find out the most suitable immunostimulant for the striped catfish juveniles using some of the most common and easily available natural immunostimulants such as vitamin C, vitamin E, brewer's yeast and ginger powder in the experimental diets of the striped catfish based on the following information.

Vitamin C (ascorbic acid, AA) is one of the most common immunostimulants used in aquaculture industry. It is useful in improving fish immunity (Hardie et al., 1991; Blazer, 1992), fish growth (Boonyaratpalin and Phromkunthong, 2001; Wang et al., 2003; Zhou et al., 2003), good health, feed conversion, survival (Khajarem and Khajarem,1997), resisting stress (Henrique et al., 1998) and preventing feed oxidation (Shiau ad Hsu, 2002). Most fish species are not capable of vitamin C biosynthesis (Chatterjee et al., 1975) due to the absence of the enzyme L-gulonolactone oxidase necessary for ascorbic acid synthesis (Wilson, 1973). As such, a synthesizable form, i.e. L-ascorbic acid is used in the present study.

Vitamin E is also one of the most important nutrients influencing the fish immune system. The most common form − alpha-tocopherol acetate (ATA) is used as a vitamin E source in fish feed in aquaculture for improving fish growth (Hamre et al., 1998; Kaushik et al., 1998). It is a potent antioxidant and offers protection against oxidative damage to various fish tissues (Adham et al., 2000); enhances the rigidity of red blood cell membranes (Kiron et

al., 2004) and protects leukocyte functions (Sahoo and Mukherjee, 2002b). It is an essential dietary nutrient for fish (Bai and Lee, 1998; Montero et al., 1999) in improving fish health performance, while increasing specific and non-specific immune responses (Wahli et al., 1998; Ortuno et al., 2001; Shiau and Hsu, 2002; Puangkaew et al., 2004). Thus, it is used as second immunostimulant in the present study.

The third immunostimulant used in the study was brewer's yeast. It is a type of fungus formally known as *Saccharomyces cerevisiae*. It is a natural product from the brewing industry that contains various immunostimulating compounds such as ß-glucan, nucleic acids as well as mannan oligosaccharides (MOS) which is used as a diet additive in aquaculture. It is capable of enhancing immune responses (Siwicki et al., 1994) as well as growth of various fish species and thus may serve as an excellent health promoter for fish culture (Cabib et al., 1982; Rumsey et al., 1992; Li and Gatlin, 2003).

One more immunostimulant in the form of ginger, *Zingiber officinale* Roscoe, is selected in the present study. Ginger as a natural antibiotic is the earliest known medicinal plant. It has shown to be effective in treating diseases in humans, poultry and aquaculture owing to its antimicrobial, antioxidant, growth promoter and immunostimulant properties (Shakya, 2015). Ginger is effective for the control of a range of bacterial, fungal and parasitic conditions (Martins et al., 2001). It has been reported to have anti-inflammatory and anti-oxidative properties (Ernst and Pittler, 2000; Chrubasik et al., 2005; Grzanna et al., 2005; Kim et al., 2007) and as an immune modulatory agent in animals, including fish (Benny et al., 2004; Ali et al., 2008; Harikrishnan et al., 2011). Ginger contains natural organic materials that facilitate growth, anti-stress, environmentally friendly and antimicrobial properties in fish (Maqsood et al., 2011).

Effects of the immunostimulants on the striped catfish are assessed on several health indices, which are used as proxies for the general health status of the experimental fish. Health indices based on haematological, biochemical and immunological assays are used to

determine the effect of immunostimulants on the striped catfish, *Pangasiandon hypophthalmus* with the following objectives:

1. To evaluate the growth performance of fish,
2. To study the histological, biochemical and haematological changes of striped cat fishes fed different immunostimulants incorporated diet and
3. To study the health status of fish.

2.0 REVIEW OF LITERATURE

Significance of immunostimulants as one of the prophylactic measures in aquaculture has been elaborated by Anderson (1992) and Secombes (1994). Several studies, as reviewed by Sakai (1999), have been conducted on the modulation of fish immune system in order to address infectious disease problems. Some of the important works are given as below:

2.1 Effect of immunostimulants on growth and survival of fishes

Immunostimulants comprise a group of substances such as polysaccharides, vitamins, different components of bacteria and Chinese herbs (Baulny et al., 1996; Mulero et al., 1998; Verlhac et al., 1998; Esteban et al., 2001). Chemical compounds, bacterial derivatives, yeast derivatives and animal or plant extracts have been studied as prospective immunostimulants for fish (Sakai, 1999) and some have been reported to confer a degree of protection against several disease causing pathogens such as *Aeromonas hydrophila, Vibrio anguillarum* and *Aeromonas salmonicida,* which are commonly found in farmed fish species (Kajita et al., 1990; Raa et al., 1992; Baulny et al., 1996; Mulero et al., 1998; Maqsood et al., 2009). The efficacy of immunostimulants on aquatic organisms has been demonstrated by Raa (2000); Bricknell and Dalmo (2005); Maqsood et al. (2011); Ringo et al. (2012); Barman et al. (2013); Mehana et al. (2015); Mankar (2016) and Wang et al. (2016) in their review articles.

2.1.1 Polysaccharides

2.1.1.1 Chitin and chitosan

One of the sources of polysaccharides, chitin, which is an insoluble linear Beta-1, 4-linked polymer of N-acetyl-D-glucosamine has been found to be effective in freshwater finfishes such as carps and trouts. In carp species, *Labeo rohita* juveniles fed with supplementation of chitin showed reduced mortality rate, enhanced phagocytic and respiratory burst activity (Choudhury et al., 2005). Among trout species, Stickney (2000)

observed an increased protection against *Aeromonas salmonicida*, increased phagocytic and natural killer cell activity, when the brook trout, *Salvelinus fontinalis* were injected with abalone extract and chitin. Vahedi and Ghodratizadeh (2011) observed enhanced complement activity and leucocyte respiratory burst activity in rainbow trout, *Oncorhynchus mykiss* when chitin was incorporated in the diet at a rate of 10, 25 and 50 mg kg^{-1}. Mohan et al. (2009) suggested that dietary supplementation of 2 % chitin in the diet was advantageous for growth of snow trout, *Schizothorax richardsonii* but golden mahseer, *Tor putitora* did not show any significant growth response.

Marine fishes, such as yellowtail, *Seriola quinqueradiata* (Kawakami et al.,1998) and gilthead seabream, *Sparus aurata* (Esteban et al., 2000, Cuesta et al., 2003) have shown immune responses after administering chitin in the diets. Combined effect of chitin and chitosan supplemented through feeds has been evaluated by Gopalakannan and Venkatesan (2006) in common carps, *Cyprinus carpio*; Mari et al. (2014) on mrigala, *Cirrhinus mrigala* and kelp grouper, *Epinephelus bruneus* (Harikrishnan et al., 2012). The studies showed increased survival rates and enhanced immune response against pathogenic agents.

Chitosan, a de-acetyl chitin, which is a type of alkaline polysaccharide found in the shells of aquatic animals such as shrimps-crabs and shellfishes, has been also tried in aquatic organisms. Dietary effect of chitosan has been also tested in a number of studies. Bullock et al. (2000) noticed increased protection against bacterial diseases in rainbow trout, *Oncorhynchus mykiss*. Alishahi et al. (2011) evaluated chitosan nano-particles to carry vitamin C in the digestive system and observed increased lysozyme and complement activity in the rainbow trouts, *Oncorhynchus mykiss.*

In carp species, Sahoo and Mukherjee (1999) and Aathi et al. (2013) studied the influence of chitosan on immune response of rohu, *Labeo rohita* and concluded that chitosan treated fish had significantly higher non-specific immunity. Chitosan incorporated studies on common carps, *Cyprinus carpio* (Dautremepuits et al., 2004; Gopalakannan and Venkatesan, 2006) and Koi, *Cyprinus carpio* (Maqsood et al., 2010; Lin et al., 2011; Lin et al., 2012;

Alishahi et al., 2014; Mustafa et al., 2014) resulted in higher growth, survival, better immune response and improved disease resistance against pathogenic organisms. Chen et al. (2014) and Abu-Elala et al. (2015) observed better survival and increased protection against *Aeromonas hydrophila*, when gibel carp, *Carrasius auratus gibelio* and Nile tilapia, *Oreochromis niloticus* respectively were fed with chitosan incorporated diets. Yan et al. (2017) observed improved growth performance, antioxidant status and immunological responses using chitosan supplementation to Caspian kutum, *Rutilus frisii kutum*.

Effect of chitosan through dietary incorporation on some marine fishes has been evaluated. Cha et al. (2008) positively evidenced non-specific immune response and improved water quality in chitosan incorporated diet in Olive flounder, *Paralichthys olivaceus*. Geng et al. (2011) demonstrated synergistic effect of 1 g kg^{-1} *Bacillus substalis* and 6 g kg^{-1} chitosan in better growth, innate immunity and disease resistance in cobia, *Rachycentron canadon*. In Asian seabass, *Lates calcarifer*, Ranjan et al. (2012) observed enhanced protection against *Vibrio anguillarum* fed with chitosan at 1 g kg^{-1} diet. European seabass, *Dicentrachus labrax* fed with chitosan incorporated diets showed enhanced growth performance, higher survival rates as observed by Zaki et al. (2015) and El- sayed and Barakat (2016).

2.1.1.2 ß-glucans

Brewer's yeast (*Saccharomyces cerevisiae)* is a natural product from brewing industry categorized of various immunostimulating compounds such as ß-glucans, nucleic acids as well as mannan oligosaccharides (MOS). It has been efficient in improving the growth performance and feed efficiency rate in sea bass fry, *Dicentrarchus labrax* (Li and Gatlin, 2003 and 2004), Nile tilapia, *Oreochromis niloticus* (Lara-Flores et al., 2003; He et al., 2009), gilthead seabream, *Sparus aurata* (Esteban et al., 2004; Salnur et al., 2009) and rainbow trout fry, *Oncorhynchus mykiss* (Molook et al., 2009).

Among carp species, β-glucan administered in common carp, *Cyprinus carpio* (Kwak et al., 2003; Selvaraj et al., 2005); rohu, *Labeo rohita* (Misra et al., 2006a); grass carp,

Ctenopharyngodon idella (Kim et al., 2009) and mirror carp, *Cyprinus carpio* (Kuhlwein et al., 2013) was found to be effective in enhanced growth, immune response by the fishes to bacterial infections and increased survival rates. In trout species, Siwicki et al. (2004) observed an increased effectiveness of anti-*Yersinia rukeri* vaccine; whereas Sealey et al. (2008) and Molook et al. (2009) recorded higher growth rates and survival in rainbow trouts, *Oncorhynchus mykiss* fed on β-glucan supplemented diets.

In marine areas, Japanese flounder, *Paralichthys olivaceus* (Ashida et al., 1999); snapper, *Pagrus auratus* (Cook et al., 2003) and yellow croaker, *Larimichthys polyactis* (Ai et al., 2007) have showed enhanced survival rates and increasing resistance against *Edwardsiella tarda* when fed diets containing of 0.09 % β-glucans.

2.1.2 Plant derivatives

A number of herbs and/or their products have been used in aquaculture, animal husbandry and even in human medicines since ages. Some of the herbs used in aquaculture are given below:

2.1.2.1 Tulsi*, Ocimum sanctum*

In tilapias, Arenal et al. (2012) observed decreased serum glucose level in hyperglycemic Nile tilapia, *Oreochromis niloticus* after addition of aqueous extract of tulsi in rearing medium. Panprommin et al. (2016) noticed that tulsi extract at 200 mg kg^{-1} to fish feed enhanced growth, immune response and disease resistance against *Streptococcus agalactiae* in Nile tilapia.

Extract of tulsi has been also tried on cyprinid group of ornamental as well as food fishes. In gold fish, *Carassius auratus*, triherbal extract from *Azadirachta indica* (neem), *Ocimum sanctum* (tulsi) and *Curcuma longa* (turmeric) added at 2.5g kg^{-1} each through feed significantly enhanced phagocytosis activity, respiratory burst activity, alternative complement activity, lysozyme activity and disease resistance against *Aeromonas hydrophila*

(Harikrishnan et al., 2009). Das et al. (2013) demonstrated that tulsi leaf extract added through feed at 0.2% stimulated the immunity and resistance against *Aeromonas hydrophila* in rohu, *Labeo rohita*.

In catfish varieties, leaf extract of tulsi fed to fishes at 2.5 and 5% in feed of *Clarias batrachus* exhibited significant increase in RBC, WBC, serum protein and globulin content (Nahak and Sahu, 2014). In aquarium catfish species, *Mystus keletius,* a combination of plant leaf extract of *Solanum trilobatum* (purple fruited pea eggplant) and *Ocimum sanctum* (tulsi) enhanced the non-specific immunity (Subeenabegum and Navaraj, 2016).

2.1.2.2 Ginger, *Zingiber officinale*

Ginger, either in powdered or in extract form has been found to be useful in aquaculture. Dugenci et al. (2003) observed that rainbow trout, *Oncorhynchus mykiss* fed with a diet containing 1% aqueous extract of powdered ginger roots for three weeks exhibited a significant non-specific immune response. In cyprinids, Arulvasu et al. (2013) reported that ginger powder fed to fishes at 1% level in feed enhanced the non-specific immunity and disease resistance of *Catla catla* to *A. hydrophila* infection. Chanu et al. (2014) studied that ginger acetone extract in fish diet at 0.5 % reduced the stress of *Labeo calbasu* fingerlings. Sukumaran et al. (2016) observed promoted growth performance, enhanced immunity in rohu, *L. rohita* when given dietary supplementation of ginger at 0.8% level.

In Nile tilapias, El-Sayed et al. (2014) observed supplementation of ginger at 1% in fish diet enhanced growth performance and immunity of the fishes. Indian catfish, *Mystus montanus* fed with herbs – tulsi, ginger and garlic each at 0.5% level had significantly higher WBC and RBC counts, whereas growth and relative percentage survival were significantly higher in 1% inclusion level of herbs (Vijaya Kumar et al., 2014). Vahedi et al. (2017) noticed improved haematological parameters and immune functions of juvenile beluga, *Huso huso* after dietary supplementation of ginger at a dose of 1.5 % level.

2.1.2.3 Prickly chaff flower, *Achyranthes aspera*

A variety of carp species have been tested using prickly chaff flower. Rao et al. (2006) observed that prickly chaff flower at 0.5% through feed stimulated immunity and resistance to infection in *rohu* fishes. In rohu fry, Srivastava and Chakrabarti (2012) noticed immunostimulatory property of prickly chaff flower seed when incorporated at a dose of 1.0 % in feed. Dietary supplementation of prickly chaff flower extract has also shown reduced mortality and increased disease resistance in rohu against *Pseudomonas flurorescens* infection as reported by Jaman et al. (2016).

In common carps, *Cyprinus carpio,* Chakrabarti and Rao (2012) reported that a diet incorporated with prickly chaff flower significantly enhanced the specific antibody response. Thangamani et al. (2014) concluded that lower doses of leaf extracts of prickly chaff flower significantly stimulated the antibody response of tilapia, *Oreochromis mossambicus*. In *Pangasius* species, Alam et al. (2016) observed that 1.5 % of prickly chaff flower supplemented feeds altered haematological parameters and triggered the innate immune system against *Pseudomonas flurorescens*.

2.1.2.4 Bhringraj, *Eclipta alba*

Christybapita et al. (2007) demonstrated that the aqueous extract of 1% bhringraj administered as feed supplement significantly enhanced the non-specific immune responses and disease resistance in Mozambique tilapia, *Oreochromis mossambicus* against *Aeromonas hydrophila* infection. In Asian catfish, *Clarias batrachus*, Mishra and Gupta (2017) observed that alcoholic stem extract of bhringraj performed significantly better followed by its root and leaf extract in enhancing packed cell volume and WBC values.

2.1.2.5 Garlic, *Allium sativum*

Garlic is considered as natural antibiotic and extensively used in a variety of fish and shellfish species in aquaculture. In Thai silver barb, *Barbodes gonionotus* fed with extract of garlic at 3% level showed significantly high therapeutic effect recovering the fishes infected with *A. hydrophila* and *P. fluorescens* ($p<0.01$) (Muniruzzaman and Chowdhury, 2008). Nya and Austin (2009b) used garlic to control *A. hydrophila* infection in rainbow trouts, *Oncorhynchus mykiss*. Garlic has also shown to be effective against *A. hydrophila* infection in Nile tilapia, *Oreochromis niloticus* as reported by Aly and Mohamed (2010). In cyprinids, Nargis et al. (2011) observed that aqueous extracts of nishinda, *Vitex nigundo* and garlic, *Allium sativum* enhanced immunity and survival in rohu, *L. rohita*. Garlic was also found to be effective in goldfish; *Carrasius auratus* fed at 1.5 % level resulting in promoted growth, reduced total bacterial viable count and enhanced relative percentage survival (Kalyankar et al., 2013). Manoppo et al. (2016) observed the highest significant weight gain in the common carps, *Cyprinus carpio* when fed with garlic-supplemented pellets at 15 g kg^{-1} pellet ($p<0.05$).

Lee and Gao (2012) elaborated effectiveness of garlic in treating fish diseases in their review work. Shakya and Labh (2014) have explained some the attributes of garlic for use in aquaculture. Saleh et al. (2015) demonstrated that garlic administrated at a level of 10 g kg^{-1} improved survival, growth and feed utilization of sea bass fry. Erguig et al. (2015) described various properties of garlic as antibacterial, antiviral and antiprotozoal for its effectiveness in aquaculture. In *Mugil cephalus* larvae, Fereidouni et al. (2015) and Akbary et al. (2016) reported that garlic inclusion in fish diet at 3% concentration improved the general health of fishes.

2.1.2.6 Aloe, *Aloe vera*

In cyprinids, Ahilan et al. (2010) observed that addition of *Aloe vera* as feed additive enhanced the growth performance of goldfish, *Carassius auratus* as well as its resistance to *A. hydrophila* infections. In common carps, *Cyprinus carpio*, supplementation of feed with 0.5 and 1 % *Aloe vera* crude extract demonstrated enhanced growth, resistance against bacterial infection and reduced stress, improved growth (Alishahi and Abdy, 2013; Mahadavi et al., 2013). In rainbow trouts, *Oncorhynchus mykiss*, *Aloe vera* at 0.1 and 1 % administration levels in feed effectively enhanced the growth performance, skin morphology and resistance against *Streptococcus agalactiae* (Heidarieh et al., 2013). Supplementation of *Aloe vera* extract at a rate of 1% enhanced the non-specific immunity responses in (Haghighi et al., 2014). Gabriel et al. (2015) studied that dietary *Aloe vera* supplementation improved growth, feed utilization and haemato-biochemical parameters of GIFT tilapia. Bazari et al. (2017) noticed that *Aloe vera* extract at a level of 1.5 % enhanced non-specific immunity responses in Siberian sturgeon, *Acipenser baerii*. Adegbesan et al. (2018) observed that 1 % *Aloe vera* leaves paste improved growth performance, nutrient utilization and survival of cultured African catfish, *Clarias gariepinus*.

2.1.2.7. Turmeric, *Curcuma longa*

Sahu et al. (2008) demonstrated that a turmeric dose of 1.0 g kg^{-1} feed provided the greatest protection against *Aeromonas hydrophila* in rohu, *Labeo rohita*, whereas Behera et al. (2011) observed significantly increased respiratory burst activity, myeloperoxidase, haemagglutination, haemolytic activity in rohu after injected intra-peritoneally with a dose of 15 µg of turmeric. In Nile tilapia, *Oreochromis niloticus*, Mahmoud et al. (2014) observed that turmeric supplementation at 0.5% level improved growth performance and protection against *P. fluorescens*. Nan et al. (2015) recommended the use of 0.5 g kg^{-1} of turmeric or 1 – 2.5 g kg^{-1} ginger extract in diets of grouper, *Epinephelus coioides* to achieve some of the non-specific immune responses. Turmeric root powder at 7.5 % inclusion level improved the

growth performance and the immunity of African catfish, *Clarias gariepinus* (Sodamola et al., 2016). Addition of turmeric into commercial feeds improved the growth rate and increased survival rate of common carps, when challenged with *Flexibacter columnaris* (Al-Faragi and Hassan, 2017). Adeshina et al. (2017) concluded that African catfish fed with turmeric at a 2.5 % inclusion levels into the diets had better growth and immunity against *Aeromonas hydrophila*. Hafiz et al. (2017) suggested that dietary inclusion of turmeric enhanced growth performance of channel catfish, *Ictalurus punctatus*. Mooraki et al. (2018) observed that turmeric powder at a level of 0.3 % of the basal diet improved the growth performance and enhanced WBC count in the Green terror, *Andinocara rivulatus*.

2.1.2.8 Peppermint, *Mentha piperita*

Talpur (2014) reported reduced mortalities and significantly improved survival, weight gain and feed conversion ratio using peppermint as a feed additive at 5g kg^{-1} of feed in Asian sea bass, *Lates calcarifer*. Adel et al. (2015) observed that dietary administration of peppermint at 3% level promoted growth performance, increased haematological and immunological parameters in fry of Caspian white fish, *Rutilus frisii kutum*.

2.1.2.9 Guduchi/Amrita, *Tinospora cordifolia*

In Mozambique tilapia, *Oreochromis mossambicus*, Sudhakaran et al. (2006) observed enhanced secondary antibody response after supplementation of petroleum ether or ethanol extract of amrita at a dose of 8mg kg^{-1} of diet. Similarly, Alexander et al. (2010) reported enhanced serum lysozyme, anti-protease, natural haemolytic complement activities and disease resistance in Mozambique tilapia using amrita extract at 6 – 600 mg kg^{-1} of diet. Sharma et al. (2017b) used amrita stem methanolic extract that resulted in increased specific, non-specific immunity and enhanced disease resistance in *L. rohita* fingerlings against *Aeromonas hydrophila* infection.

2.1.2.10 Ashwagandha, *Withania somnifera*

Sharma et al. (2010) observed stimulatory effect of dietary doses at 3 g kg $^{-1}$ of ashwagandha root on immunity and disease resistance against *A. hydrophila* infection in Indian major carp species rohu, *Labeo rohita* fingerlings. In murrel species, *Channa punctatus*, Borkar et al. (2014) concluded that a mixture of ashwagandha and shatavari powder each at 20, 30 and 40 g kg $^{-1}$ diet significantly enhanced growth rates of the fishes. Sharma et al. (2017a) observed a dose of dietary supplement of ashwagandha at 2 g kg $^{-1}$ of feed could be used as to improve the growth, haemato-biochemical response and disease resistance against *A. hydrophila* in *L. rohita* fingerlings. Engy et al. (2017) noticed that ashwagandha supplementation at dose of 2 and/or 4 % improved the body weight and significantly elevated the respiratory burst, phagocytic, lysozyme and bactericidal activities in Nile tilapia.

2.1.2.11 Cinnamon, *Cinnamomum verum*

Alsaid et al. (2010) used cinnamon extract at 3:26 (w/w) for feeding red hybrid tilapia fingerlings. The results revealed that the supplementation of cinnamon could be used as prophylactic herb against the infection of *Streptococcus agalactiae.* In Nile tilapias, *Oreochromis niloticus*, Pongsak and Parichat (2010) observed that cinnamon extract had potential to control *Streptococcus iniae* infection. Similarly, Ahmad et al. (2011) evaluated cinnamon extract's antibacterial activity antagonistic to *Aeromonas hydrophila* infection in Nile tilapias. Sivagurunathan and Innocent (2014) incorporated cinnamon at 1% level that exhibited higher growth and significant increase in total leucocyte counts (TLC) and lymphocyte counts in infected Nile tilapias.

2.1.2.12 Other herbs

Sahu et al. (2007) observed that dietary supplementation of 5 g and 10 g kg^{-1} mango, *Mangifera indica* kernel increased immune response and enhanced disease resistance against *Aeromonas hydrophila* in rohu, *Labeo rohita.* Yilmaz et al. (2012) reported that dietary thyme, *Thymus vulgaris*, rosemary, *Rosmarinus officinalis* and fenugreek, *Trigonella foenum graecum* at 1 % did not change significantly the serum urea, uric acid and creatinine levels of seabass, *Dicentrarchus labrax*. Dhayanithi et al. (2013) reported that mangrove leaf extract of *Avicennia marina* at the concentrations of 220, 200, 175 and 150 µg ml^{-1} enhanced disease resistance against pathogens in clownfish. Gültepe et al. (2014) studied that tilapia, *Oreochromis mossambicus* fed diets supplemented with thyme, rosemary and fenugreek at level of 1 % improved immune response and disease resistance of tilapia against *Streptococcus iniae*. Pratheepa and Sukumaran (2014) observed stimulated antibody production up to 5th day, when fed with higher concentrations (25 g and 50 g kg^{-1} diet) and significantly higher survival percentage in common carps when *Euphorbia hirta* plant leaf extract was given at a concentration 50 g kg^{-1} diet than that of the control diet.

Asimi et al. (2015) studied that dietary inclusion of 0.5 % of clove extract increased growth, enhanced antioxidant activity and increased immunity of rohu against *Aeromonas hydrophila*. Sahan et al. (2015) reported that addition of 5.0 g kg^{-1} spirulina in the diet increased the immune response of Nile tilapia, *Oreochromis niloticus*. Effect of Lapsi - Nepali hog plum, *Choerospondias axillaris* on striped catfish, was tested by Labh et al. (2017). It was concluded that 400mg kg^{-1} diet enhanced growth and immunity in fishes. One more herb variety, commonly called ‘tears of the virgin’ plant, *Eleutherine bulbosa* incorporated at 30 g kg^{-1} in the diets of striped catfish evinced increased leucocyte count and phagocytosis activity in fishes.

2.1.3 Vitamins

2.1.3.1 Vitamin C

Vitamin C, also known as ascorbic acid, is considered as one of the essential nutrients required for growth and immunity in fish (Lim and Lovell, 1978; Anbarasu and Chandran, 2001; Pimpimol et al., 2012). Gouillou-Coustans et al. (1998) reported that dietary incorporation of vitamin C at 279 mg kg^{-1} increased weight gain, length gain and survival of common carp, *Cyprinus carpio*. Sobhana et al. (2002) studied that dietary inclusion of vitamin C at 1000 mg kg^{-1} enhanced resistance against *Aeromonas hydrophila* in mrigal, *Cirrhinus mrigala*. Sahoo and Mukherjee (2003) reported that dietary inclusion of vitamin C at 500 ppm enhanced protection against *Aeromonas hydrophila* infection. Misra et al. (2007) studied that dietary incorporation of vitamin C at 200 mg kg^{-1} enhanced immune response, growth and survival of rohu, *Labeo rohita*. Nayak et al. (2007) observed that dietary inclusion of vitamin C at 100 mg kg^{-1} and *Bacillus subtilis* at 10^8 CFU g^{-1} enhanced the immune response of rohu. Tewary and Patra (2008) observed that rohu fed 1000 mg kg^{-1} vitamin C supplemented diet showed higher specific growth rate and immune response than that of control diet. Innocent et al. (2011) reported that feed supplemented with 100 mg $100g^{-1}$ increased resistance against infection in mrigal. Zehra and Khan (2012) reported that mrigal fed diet with 35 mg kg^{-1} vitamin C had significantly higher weight gain, feed conversion ratio than that of the control diet.

Dietary supplementation of vitamin C have been found to be useful in enhanced growth and immune response in channel catfish, *Ictalurus punctatus* (Li et al., 1998; Yildirim-Aksoy et al., 2008); in catfish, *Mystus gulio* (Anbarasu and Chandran, 2001); in *Heterobranchus longifilis* (Lenient et al., 2006); in stinging catfish, *Heteropneustes fossilis (*Alam et al., 2009); in yellow catfish, *Pelteobagrus fulvidraco* Richardson (Liang et al., 2015) and in *Pangasianodon hypophthalmus* (Daniel et al., 2018a).

2.1.3.2 Vitamin E

With regard to Vitamin E, Sinha and Sinha (1994) reported that carp, *Catla catla* fed with vitamin E at 150 mg kg^{-1} diet increased weight gain and enhanced survival rate of the fish. Sahoo and Mukherjee (2002b) observed that dietary incorporation of DL-α-tocopherol at 1000 mg kg^{-1} enhanced disease resistance against *Aeromonas hydrophila* and enhanced immune response of rohu. Paul et al. (2004) reported that vitamin E at 120 mg kg^{-1} enhanced weight gain, specific growth rate, protein efficiency ratio and lower feed conversion ratio of mrigal fry. Sau et al. (2004) reported that vitamin E at 131.91 mg kg^{-1} increased growth rate of rohu fry.

Huang et al. (2003) reported that there was no significant difference in weight gain, feed conversion ratio and protein efficiency ratio among hybrid tilapia, *Oreochromis niloticus* × *Orechromis aureus* fed vitamin E fed test diets. Kim et al. (2003) studied that dietary supplementation of ascorbic acid and α-tocopheryl acetate had positive effects on growth performance of Nile tilapia. Lim et al. (2009) reported that dietary lipid and vitamin E had no effect on the resistance of Nile tilapia to *Streoptococcus iniae* infection. Ispir et al. (2011) reported that Nile tilapia, fed with 80 mg vitamin E kg^{-1} increased leucocyte count; whereas there was no significant difference in weight gain in fishes.

Clerton et al. (2001) reported that dietary levels of vitamin E 28 and 295 mg kg^{-1} modulated the phagocytic functions of gut leucocytes in rainbow trouts. Pearce et al. (2003) observed that dietary incorporation of vitamin E at 100 and 1000 mg kg^{-1} enhanced the immune response of rainbow trouts. Huang et al. (2004) noticed no significant difference in growth, whole body proximate composition or erythrocyte fragility in coho salmon, *Oncorhynchus kisutch* fed diets containing > 50 IU of vitamin E kg^{-1} diet. Kiron et al. (2004) studied that dietary incorporation of vitamin E at 100 and 1000 mg α-tocopheryl acetate kg^{-1} diet enhanced immune response of rainbow trout. Puangkaew et al. (2004) reported that dietary vitamin E at 1000 mg kg^{-1} enhanced immune response of rainbow trouts.

Ortuno et al. (2000) showed that dietary inclusion of vitamin E at 1200 mg kg^{-1} stimulated immune response in gilthead seabream, *Sparus aurata*. Cuesta et al. (2001) noticed significantly enhanced natural cytotoxic activity at 1800 mg kg^{-1} vitamin E supplementation level in seabream, *Sparus aurata*. Tocher et al. (2002) observed no significant differences in survival or growth rates between the dietary groups of vitamin E at 100 and 1000 mg kg^{-1} in juvenile turbot, *Scophthalmus maximus*; halibut, *Hippoglossus hippoglossus* and sea bream, *Sparus aurata*. Lin and Shiau (2005) reported significantly highest weight gain in grouper, *Epinephelus malabaricus* fed diets with 100 mg kg^{-1} of vitamin E. Wang et al. (2006) observed enhanced immune response and improved disease resistance against *Edwardsiella tarda* in Japanese flounder, *Paralichthys olivaceus* in diets containing vitamin E at different concentrations. Peng et al. (2009) noticed that dietary supplementation of vitamin E at 150 mg kg^{-1} improved growth performance of black sea bream, *Acanthopagrus schlegeli*. Galaz et al. (2010) showed that dietary α-tocopherol acetate at 500 mg kg^{-1} improved resistance of parrot fish, *Oplegnathus fasciatus* against *Vibrio anguillarum*. Amlashi et al. (2012) reported that dietary vitamin E at different concentrations (25, 50, 100 and 200 mg kg^{-1}) enhanced weight gain, specific growth rate of beluga, *Huso huso*. Gao et al. (2012) demonstrated that dietary oxidized fish oil increased the oxidative stress condition of red sea bream, *Pagrus major*, but supplement of more than 100 mg kg^{-1} vitamin E prevented tissues from lipid oxidation and improved growth of red sea bream. Dietary supplementation with 60 mg kg^{-1} emodin and 500 mg kg^{-1} vitamin E increased growth of Wuchang bream, *Megalobrama amblycephala* and resistance to crowding stress (Liu et al. 2014).

2.1.4 Microorganisms

2.1.4.1. Probiotics

Probiotics are non-pathogenic and non-toxic microorganisms mainly belonging to *Lactobacillus* and *Bacillus* bacteria. Application of probiotics has been found to be useful in a number of species in aquaculture ranging from fish eggs, fish larvae, crustaceans, molluscs, live food organisms etc as evidenced from the studies reviewed by Verschuere et al. (2000) and Kesarcodi-Watson et al.(2008). Similarly, Mohapatra et al. (2012), Padmavathi et al. (2012), Pandiyan et al. (2013), Tuan et al. (2013), Rico et al. (2013), Michael et al. (2014), Ibrahem (2015), Raja et al. (2015), Hai (2015), Zorriehzahra et al. (2016), Das et al. (2017), Allameh et al. (2017) and Jahangiri and Esteban (2018) have elucidated usefulness of probiotics in aquaculture. With regard to studies on striped catfish, Krishna et al. (2015) used water and soil probiotics and feed probiotics in polyculture systems of fishes viz. catla, rohu, mrigal and striped catfish. The results showed higher growth and survival rates of fishes in probiotics treated ponds.

The probiotic effect on growth, immune and antioxidant response in striped catfish tested by Gobi et al. (2016) revealed *Bacillus licheniformis* Dahb1 as a potential probiotic to protect *Vibrio parahaemolyticus* infection enhancing growth immune and antioxidant responses in the fishes. Biswas et al. (2016) noticed higher survival rates, disease resistance against *Pseudomonas fluorescens* in the striped catfishes after incorporation of probiotics in the diets. Thy et al. (2017) evaluated potential of *Bacillus* sp. isolates for the effective use of probiotics in aquaculture of striped catfish.

2.1.4.2 Prebiotics and synbiotics

Prebiotics are non-digestible food ingredients that stimulate the growth of gut microflora in the organisms (Flickinger et al., 2003), whereas synbiotics show synergistic effect of pro- and prebiotics in the intestinal tract for improving the health status of the organisms (Guerreiro et al., 2016). The effects of prebiotics for enhancing immune system in

aquatic organisms have been reviewed by Gatlin et al. (2006), Yousefian and Amiri (2009), Denev et al. (2009), Ringo et al. (2010), Merrifield and Zhou (2011), Padmavathi et al. (2012), Song et al. (2014) and Das et al. (2017). However, the studies with regard to prebiotics or synbiotics effects on striped catfish are scantily available. Akter et al. (2015) evaluated positive influence of Mannan oligosaccharides (MOS) in the diets to analyse growth, digestive enzymes, gut morphology, microbiota in juvenile striped catfish. In one more study, Tamamdusturi et al. (2016) observed synbiotic effect of microencapsulated probiotic *Bacillus* sp. NPS and MOS to prevent *Aeromonas hydrophila* infection in the striped catfishes.

2.1.4.3 Lipopolysaccharides (LPS)

Lipopolysaccharide (LPS), a cell wall component of gram negative bacteria, has been used in a variety of fish species and crustaceans. Among freshwater fish species lipopolysaccharide was found to be useful in channel catfish, *Ictalurus punctatus* (Salati et al., 1987, Santander et al., 2014); gold fish, *Carassius auratus* (Neumann et al., 1995); tilapia, *Oreochromis mossambicus* (Pepels et al., 2004); brook trouts, *Salvelinus fontinalis* (Mackenzie et al., 2006); rainbow trout, *Oncorhynchus mykiss* (Nya and Austin, 2010) and grass carp, *Ctenopharyngodon idella* (Sun et al., 2011). The studies have shown enhanced immunity and phagocytic activity against bacterial pathogens.

2.1.5 Levamisole

Levamisole, a levo-isomer of tetramisole, has been widely used as an antihelminthic drug in humans and other animals. It is known for exerting stimulatory effects on various functions of the immune system. In carp species, levamisole has been used in common carp, *Cyprinus carpio* (Cuesta et al., 2002; Gopalakannan and Arul, 2006; Maqsood et al., 2009; in rohu, *Labeo rohita* (Wijendra and Pathiratne, 2007); in catla, *Catla catla* (Perera and Pathiratne, 2008) and in grass carps, *Ctenopharyngodon idella* (Sahidi et al., 2011). Majority of the studies showed increased immune response by the fish species. The response was either in the form of enhanced growth, enhanced phagocytic activity, increased lysozyme

activity, or in the form of increased resistance against bacterial pathogens, increased leucocyte count etc. Other than carp species, levamisole has been also tested in rainbow trout, *Oncorhynchus mykiss* (Kajita et al., 1990), pacu, *Piaractus brachypomus* (Biller-Takahashi et al., 2016) and Nile tilapia, *Oreochromis niloticus* (Bedasso, 2017) that showed increased resistance against bacterial pathogens. Administration of levamisole has been also found to be effective in growth of marine fish species such as gilthead seabream, *Sparus aurata* (Baba et al., 1993); Japanese flounder, *Paralichthys alivaceus* (Choi, 2004); Atlantic salmon, *Salmo salar* (Findlay and Munday, 2000); Turbot, *Scophthalmus maximus* (Alvarez-Pellitero et al., 2006).

2.2 Effects of immunostimulants on haematological parameters

Study of haematological parameters plays a significant role in assessing the health status of fishes (Satheeshkumar et al. 2011). A number of cyprinid species have been dealt in detail. In rohu, *Labeo rohita*, Sahoo and Mukherjee (2002b) observed that either α-tocopherol at 1000 mg kg^{-1} had no effect on the white blood cells (WBC) and haematocrit (Hct) values in the fish. Choudhury et al. (2005) concluded that the haemoglobin (Hb) content and red blood cells (RBC) were not influenced by dietary supplementation of chitin at 25 mg kg^{-1} diet. Misra et al. (2006a and 2007) reported that 10 mg β-glucan kg^{-1} body weight and dietary incorporation of vitamin C at 200 mg kg^{-1} diet increased WBC count in rohu. Tewary and Patra (2008) observed the highest RBC, WBC, Hb and Hct at 1000 mg kg^{-1} of vitamin C in the diets of rohu. Wijendra and Pathiratne (2007) noticed that the total leucocytes, neutrophils, monocytes and lymphocytes were increased significantly on 14 and 21 days post-levamisole at 0.05 % treatment in rohu. Incorporation of turmeric in various concentrations (0.1, 0.5, 1.0 and 5.0 g kg^{-1}) in feed showed increased RBC, WBC and Hb content as compared to control group of rohu (Sahu et al., 2008). Das et al. (2009) reported that dietary inclusion of protozoa, *Euglena viridis* at 0.5 and 1 % in rohu, increased RBC and WBC; whereas Hb content remained unaffected in the treatments. Das et al. (2013) demonstrated that dietary administration of different concentrations of tulsi (0.05, 0.1, 0.2, 0.5 and 1.0 %) increased the total RBC, WBC and haemoglobin contents of rohu. Aathi et al. (2013)

observed increase in RBC, WBC and Hb content after a supplementation of 1 % chitosan in the diet of rohu, *Labeo rohita*.

In one more species of Indian Major Carps, *Catla catla*, Perera and Pathiratne (2008) observed that the total WBC counts were increased in levamisole at 1.25 mg L^{-1} treated fishes in comparison to the control treatments. Arulvasu et al. (2013) reported that a dietary inclusion of ginger, *Zingiber officinale* (0.1, 0.5 and 1.0%) enhanced the total RBC, WBC and Hb content in catla.

In mrigal, *Cirrhinus mrigala*, Innocent et al. (2011) observed that the basophils and eosinophils increased significantly in vitamin C at a dose of 100 mg $100g^{-1}$ supplemented diet. Zehra and Khan (2012) recorded the highest values for Hb, Hct and RBC in the fish group fed with vitamin C at 35 mg kg^{-1} in the diet. Mari et al. (2014) noticed that RBC, WBC, Hct, lymphocytes, monocytes and neutrophils significantly increased in fish fed with 1% of chitin and chitosan enriched diets; whereas mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and the mean corpuscular haemoglobin concentration (MCHC) did not evince any effect on the fish haematology.

In common carps, *Cyprinus carpio*, Selvaraj et al. (2005 and 2006) observed that glucan had a significant effect on WBC, neutrophils and monocytes of the fishes. Gopalakannan and Arul (2006) studied the effect of chitin (1%), chitosan (1%) and levamisole (250 mg kg^{-1}) in common carps and observed the WBC count to increase significantly in chitin treated fish as compared to that of control diet. Maqsood et al. (2009) observed that the total RBC, Hb content and Hct values were significantly enhanced in levamisole at 250 mg kg^{-1} supplemented diet in common carps. Lin et al. (2011) studied that diet supplemented with three immunostimulants such as β-1, 3-glucan (0.5%), chitosan (0.2 %) and raffinose (0.2%) showed higher WBC count than the control group in Koi, *Cyprinus carpio*. Lin et al. (2012) reported the increase in WBC in koi, *Cyprinus carpio* fed 0.1 % *Bacillus coagulans*, 0.2 % of chitosan oligosaccharides and a combination of the chitosan, *Bacillus coagulans* in comparison to the control groups. Alishahi et al. (2014) suggested that

WBC value and blood leucocyte ratio showed no significant difference in chitosan (2.5, 5 and 10 mg kg^{-1}) treated groups in common carps. Pratheepa and Sukumaran (2014) concluded that a leaf extract of Asthma-plant, *Euphorbia hitra* at a dose of 25 g kg^{-1} increased the RBC and WBC count; whereas 50g kg^{-1} of the leaf extract increased the Hb content in common carps. Al-Faragi and Hassan (2017) observed that dietary incorporation of 1.25 % turmeric in fish diet increased RBC, WBC, Hb and PCV in common carp, *Cyprinus carpio.*

In grass carp, *Ctenopharyngodon idella*, Shahidi et al. (2011) detected that dietary levamisole at 3 mg L^{-1} increased the monocyte and neutrophils in the fishes. Adel et al. (2015) studied that dietary administration of peppermint at 1, 2 and 3 % increased the RBC, WBC, Hb and Hct value of Caspian white fish, *Rutilus frisii kutum.* Najafabad et al. (2016) reported that the lymphocytes, eosinophils and neutrophils did not show any significant change among the different levels of chitosan (0.25, 0.5, 1 and 2 g kg^{-1}) treated Caspian white fish, *Rutilus frisii kutum.*

With regard to tilapias, Lim et al. (2009) demonstrated that RBC, WBC, Hb, Hct, MCV, MCH and MCHC were not affected by dietary levels of lipid (6, 10 and 14 %); vitamin E (50, 100 and 200 mg kg^{-1}) or their interactions in Nile tilapia, *Oreochromis niloticus.* Nsonga et al. (2009) observed increased RBC, WBC and Hct values due to dietary ascorbic acid at 60 mg kg^{-1} incorporation in tilapia, *Oreochromis karongae*. The values in the erythrocyte count and haemoglobin concentration did not differ statistically among the brewer's yeast at 2 % treated group and the control group in Nile tilapia (Reque et al., 2010). Ahmad et al. (2011) studied that the values of Hb, RBC and PCV were the highest in Nile tilapia fed at 1 % cinnamon incorporated diet. Ispir et al. (2011) noticed that dietary inclusion of vitamin E at a dose of 80 and 160 mg kg^{-1} increased the RBC and Hb content; whereas vitamin E at 240 mg kg^{-1} increased MCH and MCV in Nile tilapia. Abu-Elala et al. (2013) studied that dietary inclusion of brewer's yeast at a dose of 2g kg^{-1} increased the RBC, WBC, PCV and Hb concentration in Nile tilapia. Sivagurunathan and Innocent (2014) reported that dietary incorporation of cinnamon at 0.5% and 1% increased the Hb, RBC and WBC content of Nile tilapia. *Aloe vera* supplemented fish showed a significant increase in RBC, Hb, Hct

value and WBC in GIFT tilapia (Gabriel et al., 2015). Sahan et al. (2016) studied that dietary incorporation of ginger at 0.5 and 1.0 % levels enhanced values of RBC, Hb and Hct in Nile tilapia. Bedasso (2017) reported that fish fed diets containing 500, 750, 1000, 1250 and 1500 mg levamisole kg^{-1} diet enhanced the RBC, WBC count; whereas the diet containing 750 to1500 mg levamisole kg^{-1}diet enhanced the lymphocytes in Nile tilapia.

Nya and Austin (2009) observed that dietary incorporation of *Allium sativum* (0.1, 0.5 and 1.0 g) increased the RBC, WBC, Hct, MCV, MCH and MCHC of rainbow trout. Farahi et al. (2010) reported that diets containing 20 and 30 g kg^{-1} diet of *Alium sativum* increased RBC, Hb, Hct, MCV, MCH and MCHC content of rainbow trout. Haghighi and Rohani (2013) noticed that rainbow trout with the supplement of powdered ginger rhizome at 1 % showed significantly increased in WBC, Hct and RBC values. Miar et al. (2013) reported that rainbow trout fed diets containing 50 mg kg^{-1} vitamin E and vitamin C had the highest WBC and RBC counts; whereas, the Hct percentage did not differ significantly in fish fed different diets. Haghighi et al. (2014) concluded that the dietary *Aloe vera* extract at 1 % incorporated test diets had no significant effect on RBC, WBC, Hct value, Hb, MCH, MCV and MCHC content of rainbow trouts. Khan et al. (2015) observed significantly higher Hb and Hct values in the diet fed 300 mg kg^{-1} of L-ascorbyl-2- phosphate as compared to the control diet in mahaseer, *Tor putitora*.

Among pangasius fish group, Pimpimol et al. (2012) reported that Mekong giant catfish, *Pangasianodon gigas* fed 500 and 750 mg vitamin C kg^{-1} supplemented diet increased Hct and RBC count. Sirimanapong et al. (2015) noticed that striped catfish, *Pangasianodon hypophthalmus* fed 0.2 % β-glucan increased WBC and RBC count; whereas there was no significant difference in Hct value among brewer's yeast treated group and the control group of fishes. Daniel et al. (2018a) demonstrated that the Hb, RBC, WBC and Hct values increased significantly in vitamin C (170 and 350 mg kg^{-1}) treated group than that of the control group in striped catfish.

In other silurid species, Lim et al. (2000) reported that dietary ascorbic acid at 3000 mg kg $^{-1}$ increased the RBC content of channel catfish, *Ictalurus punctatus*. Nahak and Sahu (2014) reported that aqueous leaf extract of tulsi at 2.5 % and 5 % concentrations fed to *Clarias batrachus* exhibited significant increase in RBC and WBC content. Dietary supplementation of vitamin C at 50 mg kg $^{-1}$ increased PCV and Hb content of *Heterobranchus longifilis* (Ibiyo et al., 2006). Yildirim-Aksoy et al. (2008) observed that fish fed with combination of 500 mg kg $^{-1}$ of vitamin E diet supplemented with 2000 mg kg $^{-1}$ of vitamin C increased the RBC, WBC and Hct values of channel catfish. The African catfish, *Clarias gariepinus* showed no significant differences in the RBC and the WBC count between the levamisole (150 mg kg $^{-1}$) treated group and the control group (Aly et al., 2010). Similarly, in yellow catfish, *Pelteobagrus fluvidraco*, Liang et al. (2015) noticed that the RBC, WBC and Hct values were not significantly influenced by the dietary vitamin C levels at 20, 40, 80, 160 and 320 mg kg $^{-1}$of diet. Sodamola et al. (2016) reported that African catfish fed with graded level of turmeric root powder (2.5,5, 7.5 and 10 %) did not affect the PCV, Hb, lymphocyte, heterophils, eosinophils and basophils. Higher level of WBC count was confirmed when the fresh water catfish, *Mystus keleticus* injected with the mixed extract of 1 :1 ratio of *Solanum trilobactum* (Purple fruited pea eggplant) and *Ocimum sanctum* (tulsi) each at 30 mg kg $^{-1}$ (Subeenabegum and Navaraj, 2016). Mishra and Gupta (2017) noticed that aqueous extract of bhringraj at 10 and 20 ppm had significant role in enhancing the RBC, Hb, PCV and WBC count in *Clarias batrachus*. Adegbesan et al. (2018) reported that dietary supplementation of *Aloe barbadensis* leaves at 3 % level enhanced the Hb, RBC and Hct value of African catfish, *Clarias gariepinus*.

In some marine water fish species, Li and Galin (2003) studied that Hct of fish fed various levels of brewer's yeast (1, 2 and 4%) incorporated diets were not significantly affected in hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). Lin and Shiau (2005) observed higher WBC count in grouper, *Epinephelus malabarichus* fed diets supplemented with vitamin E at 25, 50, 100, 200, 400 and 800 mg kg $^{-1}$ diet. Alvarez-Pellitero et al. (2006) reported that there was no significant difference in Hct values between levamisole (250 and

500 mg kg $^{-1}$) treated fish and control group of turbot, *Scophthalmus maximus*. Falahatkar et al. (2006) studied that dietary vitamin C (100, 200, 400, 800 and 1600 mg kg^{-1}) supplementation had no significant effect on Hct values of great sturgeon, *Huso huso*. Ranjan et al. (2012) reported that RBC and WBC were significantly increased in chitosan incorporation at 10 g kg $^{-1}$ fed to Asian seabass, *Lates calcarifer* as compared to the control fish group. Harikrishnan et al. (2012) observed that the RBC, WBC, Hb, lymphocytes, monocytes and neutrophils significantly increased in kelp grouper, *Epinephelus bruneus* fed with 1 % chitin and chitosan enriched diets; whereas MCV, MCH and MCHC did not show any significant change in the blood parameters of fishes. Zhou et al (2012) reported that RBC and Hct values were significantly influenced by the dietary vitamin C levels (13.6, 27.2, 54.4, 96.6, 193.4 and 386.5 mg kg $^{-1}$) in cobia, *Ranchycentron canadum*. Saleh et al. (2015) reported that dietary incorporation of garlic (10, 20 and 30 g kg $^{-1}$) as well as onion powder (5, 10 and 20 g kg $^{-1}$) significantly enhanced the Hb, Hct, MCV, MCH and WBC count of European seabass, *Dicentrarchus labrax*. Shahkar et al. (2015) noticed that Hb, RBC and MCHC values were not significantly influenced by dietary ascorbic acid levels at 200, 400, 800 and 1600 mg kg $^{-1}$ of diet; whereas, PCV and WBC values were significantly higher in 1686 mg vitamin C kg^{-1} diet in Japanese eel, *Anguilla japonica.* Vahedi et al. (2017) observed no significant difference in RBC, WBC, Hct, monocyte, lymphocyte and neutrophils values between the ginger treated group at 0.5, 1 and 1.5% diet in the juveniles belunga, *Huso huso*.

2.3 Effects of immunostimulants on biochemical parameters

Some of the significant contributions with regard to changes in biochemical parameters in cyprinids are cited as follows. Misra et al. (2006a) observed enhanced the total protein, albumin and globulin content after administration of 15 mg β-glucan kg^{-1} diet in rohu. Similarly, Misra et al. (2007) demonstrated that values of total serum protein, albumin, globulin and A:G ratio in rohu fed with levels of vitamin C at 100, 200 and 500 mg kg^{-1}diet did not differ significantly from the control fish group. Nayak et al. (2007) evaluated that incorporation of *Bacillus subtilis* @ 10^8 CFU g^{-1} and vitamin C at 100 mg kg^{-1} had significant

effect on total protein, albumin and globulin content of rohu. Wijendra and Pathiratne (2007) reported no significant difference in total protein content of rohu after incorporation of levamisole at 0.05 % in the diets. Sahu et al. (2008) noticed that a dietary incorporation of turmeric, *Curcuma longa* at 5.0 g kg $^{-1}$ of rohu diet had significant effect on total serum protein; whereas albumin and globulin contents were higher in different concentrations of turmeric at 0.1, 0.5, 1.0 and 5.0 g kg $^{-1}$ of diet. Srivastava and Chakrabarti (2012) observed that dietary incorporation of prickly chaff flower, *Achyranthes aspera* at 0.5 % and 1 % enhanced the total serum protein and albumin level in fishes; whereas globulin level was found to be the highest in 1 % incorporation level of rohu diet. Aathi et al. (2013) reported that dietary incorporation of chitosan at 1 % enhanced the total serum protein content in rohu. Das et al. (2009) demonstrated that rohu fed with *Euglena viridis* with different concentrations (0.1, 0.5 and 1.0 g kg $^{-1}$) showed no significant difference in total serum protein and albumin levels. Rao et al. (2006) studied that dietary inclusion of prickly chaff flower, *Achyranthes aspera* at 0.5 % level enhanced the albumin content of the fish; whereas there was no significant difference in total serum protein and globulin content in treated group and the control group in rohu. Similarly, Hasan-Uj-Jaman et al. (2017) reported that diet containing root extract of prickly chaff flower at 0.5 % enhanced the serum globulin level in rohu fishes.

In common carps, Maqsood et al. (2009) studied that total serum protein, albumin and globulin levels were significantly enhanced in the levamisole supplemented group at 250 mg levamisole kg^{-1} of diets. Alishahi and Abdy (2013) noticed that dietary supplementation of 0.5 % and 1 % *Aloe vera* extract enhanced the total serum protein and globulin content in common carps. Alishahi et al. (2014) observed no significant difference in total serum protein, albumin and globulin content in common carps, when fed chitosan at 0.5 and 1 % level in the diets. In *Catla catla*, Arulvasu et al. (2013) observed that a dietary supplementation of ginger at 0.5 % and 1 % enhanced the total serum protein in the fishes. Similarly, Mari et al. (2014) noticed that dietary incorporation of combination of 1 % chitin

and chitosan each did not show any significant change on total serum protein and albumin content in mrigal, *Cirrhinus mrigala*.

In salmonids, Nya and Austin (2009) studied that total serum protein and globulin content were significantly higher in fish fed with 15 mg, 3.75 mg and 7.5 mg lipopolysaccharide per 100g of feed; whereas the albumin content did not vary significantly in any of the treated and the control group of rainbow trouts, *Oncorhynchus mykiss*. Farahi et al. (2010) observed significant increase in total plasma protein and decrease in plasma glucose when rainbow trouts were fed with garlic, *Allium sativum* at 30 g kg^{-1} in the diets. Haghighi et al. (2014) reported that dietary supplementation of *Aloe vera* extract at 1 % enhanced the total serum protein, albumin and globulin level of rainbow trouts.

Some of the important contributions in tilapias are as follows. Mousa et al. (2008) observed that dietary supplementation of neem leaf extract at 4 and 11 g L^{-1} increased the total serum protein, albumin, globulin and glucose level of Nile tilapia, *Oreochromis niloticus* and African catfish, *Clarias gariepinus.* Serum protein was significantly increased in Nile tilapia fed 14 % lipid diets but was not affected by supplemented levels of 50, 100 and 200 mg kg^{-1} of vitamin E (Lim et al., 2009). Ahmad et al. (2011) reported that dietary incorporation of cinnamon at 1 % level increased the total protein and glucose content of Nile tilapia. Gabriel et al. (2015) observed that dietary supplementation of *Aloe vera* at different concentrations (0.5, 1 and 2 %) significantly increased the total serum protein content of GIFT tilapia.

Among catfishes, Yildirim-Aksoy et al. (2008) noticed that there was no significant difference in total serum protein content of channel catfish, *Ictalurus punctatus* when supplemented with different concentrations (100, 2000 mg kg^{-1}) of vitamin C and vitamin E (50 and 500 mg kg^{-1}) in the diets. A significant increase in the total protein and globulin was noticed in the levamisole vaccinated groups at 150 mg kg^{-1} of catfish, *Clarias gariepenus* (Aly et al., 2010). Nahak and Sahu (2014) evaluated that dietary inclusion of tulsi, *Ocimum basilicum* at 2.5 % and 5 % concentrations increased total serum protein and globulin content

of *Clarias batrachus*; whereas serum glucose content was higher in 2.5 % incorporated tulsi in the fishes. In yellow catfish, *Pelteobagrus fulvidraco*, Liang et al. (2015) observed no significant influence on total serum protein with the different concentrations of vitamin C (20, 40, 80, 160 and 320 mg kg $^{-1}$) in the diets; however, glucose content was significantly affected by the dietary vitamin C levels. Sodamola et al. (2016) noticed increased total serum protein and serum glucose in African catfish, *Clarias gariepenus* after dietary inclusion of turmeric, *Curcuma longa* at 5 % in the diets.

In pangasius species, Pimpimol et al. (2012) reported that dietary supplementation of vitamin C at 500 and 750 mg vitamin kg^{-1} increased the serum protein and serum glucose of Mekong giant catfish, *Pangasianodon gigas*. Soltanian et al. (2014) studied that there was no significant difference in glucose levels of the control fish group and different concentrations of β-glucan (0.5, 1 and 2 %) treated striped catfish, *Pangasianodon hypophthalmus*. Adloo et al. (2015) noticed that dietary supplementation of β-glucan at 0.5, 1 and 2 % enhanced the total protein content of striped catfish. Daniel et al. (2018a) concluded that dietary supplementation of vitamin C at 175 mg kg^{-1} of diet increased the total serum protein, albumin and globulin content; whereas serum glucose was found to be the highest in 700 mg kg $^{-1}$ diet in striped catfishes.

In other freshwater fish groups, Affonso et al. (2007) noticed that dietary inclusion of vitamin C at 800 mg kg $^{-1}$ increased the total protein; whereas, vitamin C at 400 mg kg $^{-1}$ of diet increased glucose levels of matrinxa, *Brycon amazonicus*. Total serum protein level in wuchang bream, *Megalobrama amblycephala* fed diet with 133.7 mg kg^{-1} vitamin C was significantly higher than the control group (Wan et al., 2014). Belo et al. (2005) reported that the blood glucose concentrations did not vary substantially with dietary supplementation of vitamin E at 100 and 450 mg kg $^{-1}$ in pacu, *Piaractus mesopotamicus*.

A study of Cha et al. (2008) showed that dietary supplementation of 1 % chitosan increased total serum protein content of olive flounder, *Paralichthys olivaceus*; whereas glucose content was higher in the control fish groups. Harikrishnan et al. (2012) observed

significantly increased total serum protein and albumin in grouper, *Epinephelus bruneus* with supplementation of 1 % and 2 % of chitosan diet but not in the chitin diets; however, the globulin content did not show any significant change in both the diets. Ranjan et al. (2012) reported that total serum protein, albumin and globulin contents significantly increased in Asian seabass, *Lates calcarifer* fed chitosan at 5, 10 and 20 g kg $^{-1}$ incorporated diets. Fereidouni et al. (2015) observed that the administration of garlic, *Allium sativum* at 0.5,1 and 3% in diet significantly decreased serum glucose in mullet, *Mugil cephalus*; whereas the total protein, albumin and globulin levels had significant increase in all the groups fed garlic extract. Shahkar et al. (2015) studied that dietary L-ascorbic acid up to 840 mg kg^{-1} ascorbic acid in diet increased plasma glucose concentration in Japanese eel, *Anguilla japonica*. Vahedi et al. (2017) observed that dietary supplementation of ginger at 0.5, 1 and 1.5 % did not show any significant difference in the total serum protein, albumin, globulin and glucose content of the juveniles of beluga, *Huso huso*.

2.4 Effects of immunostimulants on immune parameters

Cyprinids have been studied relatively extensively than many of the other varieties of fishes. In rohu, *Labeo rohita*, Sahoo and Mukherjee (2002b) reported that dietary incorporation of α-tocopherol at 1000 mg kg $^{-1}$ enhanced the phagocytic activity in fishes; whereas Sahoo and Mukherjee (2003) observed that dietary incorporation of vitamin C at 500 mg kg $^{-1}$ diet enhanced phagocytic ratio and serum lysozyme activity in the fishes. Misra et al. (2006a) observed that dietary supplementation of β-glucan at 10 mg kg $^{-1}$ enhanced the phagocytic ratio, phagocytic index, lysozyme activity, complement activity and serum bactericidal activity in rohu. Misra et al. (2007) studied that dietary vitamin C at 200 mg kg $^{-1}$ enhanced phagocytic ratio; whereas 500 mg kg $^{-1}$ vitamin C supplementation enhanced lysozyme activity of the fish. Nayak et al. (2007) reported that dietary supplementation of vitamin C at 100 mg kg $^{-1}$ diet increased respiratory burst activity of blood neutrophils in rohu. Wijendra and Pathiratne (2007) noticed enhanced phagocytic activity, phagocytic index and lysozyme activity in rohu treated with levamisole at 0.05% in diet; whereas no significant differences were found between NBT activities in the blood of levamisole treated fishes.

Sahu et al. (2008) reported that dietary supplementation of turmeric at a dose of 1.0 g kg $^{-1}$ diet enhanced NBT, lysozyme and serum bactericidal activity in rohu. Tewary and Patra (2008) observed enhanced phagocytic activity and respiratory burst activity in rohu given the vitamin C supplementation at 1000 mg kg $^{-1}$ diet. Das et al. (2009) demonstrated that rohu fed with *Euglena viridis* at 0.1- 1, 0.5 and 0.5 - 1 g kg $^{-1}$ diet showed increased levels of superoxide anion production, lysozyme activity and serum bactericidal activity, respectively. Behera et al. (2011) observed significant increase in respiratory burst activity, myeloperoxidase, haemagglutination activity, haemolytic and bacterial agglutination activities in rohu injected with 15 and 1.5 µg of curcumin. Aathi et al. (2013) reported that dietary supplementation of 1% chitosan increased lysozyme and agglutination assay in rohu. A plant extract of prickly chaff flower, *Achyranthes aspera* at 6 % level of supplementation showed the highest response in phagocytic activity of rohu (Hasan-Uj-Jaman et al., 2017); whereas Rao et al. (2006) noticed a dietary incorporation of prickly chaff flower at 0.5 % enhanced superoxide anion production and lysozyme activity in rohu, *Labeo rohita*.

In common carps, *Cyprinus carpio*, Dautremepuits et al. (2004) reported that the fish fed with mixture of copper with chitosan at 0.1 and 0.25 mg L^{-1} had significantly high levels of lysozyme activity in blood plasma. Gopalakannan and Arul (2006) noticed that dietary supplementation of chitin (1%), chitosan(1%) and levamisole (250 mg kg $^{-1}$) significantly stimulated the lysozyme activity and NBT reduction in common carps, *Cyprinus carpio*. Selvaraj et al. (2006) studied that the common carps, treated with β-glucan and lipopolysaccharide by intraperitoneal injection at all doses (100 µg β-glucan + 10 µg LPS, 500 µg β-glucan + 50 µg LPS and 1000 µg β-glucan + 100 µg LPS) showed significant increase in superoxide anion production than the control group fishes. Kim et al. (2009) reported that phagocytic activities of leucocytes and serum lysozyme activity enhanced by oral administration of schizophyllan in common carps and flounder, *Paralichthys olivaceus*. Maqsood et al. (2009) observed that dietary inclusion of levamisole at 250 mg kg $^{-1}$ diet enhanced the lysozyme activity and NBT assay in common carps. Lin et al. (2011) showed that dietary supplementation of β-1, 3-glucan at 0.5 % enhanced respiratory burst activity,

phagocytic activity and lysozyme activity of koi, *Cyprinus carpio*. Biswas et al. (2012) observed a significantly increased phagocytic activity and superoxide anion production in kidney cells with fishes fed with brewer's yeast extract at 5 mg fish $^{-1}$ of common carps. Lin et al. (2012) revealed enhanced phagocytic activity, respiratory burst activity and lysozyme activity in common carps fed diets supplemented with a combination of chitosan oligosaccharides at 0.2 % and *Bacillus coagulans* at 0.1% to the fishes. Mustafa et al. (2014) reported that common carps fed on diet containing 2 % chitosan had significantly increased respiratory burst activity, bacteriocidal activity and lysozyme activity. Alishahi and Abdy (2013) observed that dietary incorporation of 0.5 and 1 % *Aloe vera* crude extract enhanced lysozyme activity, complement activity, respiratory burst activity and serum bactericidal activity of common carps. Alishahi et al. (2014) studied that oral administration of chitosan at 0.5 % and 1 % significantly enhanced NBT reduction activity; whereas serum lysozyme and bactericidal activity showed no significant change among the treatments in common carps. Pratheepa and Sukumaran (2014) reported that dietary incorporation of *Euphorbia hirta* at 50 g kg^{-1} diet enhanced lysozyme activity, phagocytic ration and NBT assay in common carps, *Cyprinus carpio*.

In tilapia species, Christybapita et al. (2007) noticed enhanced lysozyme activity with dietary incorporation of 1 % aqueous extract of bhringraj in Mozambique tilapia. Lim et al. (2009) studied that lysozyme activity significantly increased in Nile tilapia fed 200 mg kg $^{-1}$ vitamin E diets. Alexander et al. (2010) reported that a dietary supplementation of *Tinospora cordifolia* at 6 mg kg $^{-1}$ showed the highest lysozyme activity in Mozambique tilapia. El-Sayed et al. (2014) studied that a dietary inclusion of 1 % ginger increased the lysozyme activity in Nile tilapia. Abu-Elala et al. (2015) reported that Nile tilapia fed 1 % chitosan enhanced phagocytic activity, NBT activity and lysozyme activity. Bedasso (2017) observed that dietary incorporation of levamisole at 1500 mg kg $^{-1}$ diet enhanced phagocytic activity and serum bactericidal activity of Nile tilapia. Engy et al. (2017) noticed that *Withania somnifera* supplementation at dose of 2 and 4 % improved the respiratory burst activity, phagocytic activity and lysozyme activity in Nile tilapia, *Oreochromis niloticus*.

With regard to salmonids, Findlay and Munday (2000) noticed that a freshwater bath with levamisole added at a rate of 2.5 mg L^{-1} in diet enhanced phagocytic index, phagocytic ratio and superoxide anion production of Atlantic salmon. Clerton et al. (2001) stated that dietary incorporation of vitamin E (28 and 295 mg L^{-1}) enhanced the lysozyme activity, oxidative burst activity and phagocytosis in rainbow trouts. Kiron et al. (2004) observed low phagocytic activity, higher serum alternative complement activity and lysozyme activities in rainbow trouts fed vitamin E at 1000 mg kg^{-1} in diets. Puangkaew et al. (2004) observed enhanced lysozyme activity and phagocytosis in fishes with dietary incorporation of vitamin E at 100 mg kg^{-1} diet; whereas, a dose of 1000 mg kg^{-1} diet enhanced superoxide anion production in rainbow trouts. Nya and Austin (2009 and 2010) observed enhanced phagocytic activity and lysozyme activity in rainbow trouts after dietary incorporation of different concentrations of lipopolysaccharide at 15, 7.5 and 3.75 mg kg^{-1} and *Allium sativum* at 0.5 g kg^{-1} of diet respectively. Vahedi and Ghodratizadeh (2011) reported that administration of chitin diet with different doses such as 10, 25 and 50 mg kg^{-1} enhanced respiratory burst activity and NBT reduction of rainbow trouts. Alishahi et al. (2011) demonstrated that dietary chitosan at 150 mg kg^{-1} enhanced lysozyme activity and complement activity of rainbow trouts. Rainbow trouts fed the supplement of powdered ginger rhizome at 1 % showed significantly increased respiratory burst activity and lysozyme activity (Haghighi and Rohani, 2013). Haghighi et al. (2014) reported that *Aloe vera* at a dose of 1 % enhanced respiratory burst activity, phagocytic activity and serum lysozyme activity of rainbow trouts. Moses et al. (2017) showed that dietary supplementation of vitamin C at 800 mg kg^{-1} and *Achyranthes aspera* seeds at 5 g kg^{-1} increased myeloperoxidase and nitric oxide synthase levels of snow trout, *Schozothorax richardsonii*.

In catfish varieties, Kumari and Sahoo (2005) studied that dietary supplementation of vitamin C at 2000 mg kg^{-1} enhanced the superoxide anion production of Asian catfish, *Clarias batrachus*; whereas, the lysozyme activity was not affected by the dietary ascorbic acid supplemented group. Yildinim et al. (2008) reported that a dietary incorporation of vitamin C (100 and 2000 mg kg^{-1}) and E (50 and 500 mg kg^{-1}) increased superoxide anion

production of channel catfish. Aly et al. (2010) noticed that phagocytic percentage was significantly increased in the levamisole (150 mg kg^{-1}) vaccinated group of catfish, *Clarias gariepinus*. Liang et al. (2015) reported the highest lysozyme, total complement activity, phagocytosis index and respiratory burst activity of head kidney in yellow catfish, *Petteobagrus fulvidraco* fed with a diet containing 156.5 mg kg^{-1} vitamin C. The highest level of phagocytic activity was found, when the freshwater catfish, *Mystus keleticus* was injected with the mixed extract of 1 : 1 ratio of *Solanum trilobactum* and *Ocimum sanctum* at 30 mg kg^{-1} (Subeenabegum and Navaraj, 2016). Adeshina et al. (2017) concluded that dietary inclusion of 2.5 % *Curcuma longa* enhanced NBT, lysozyme activity in Asian catfish.

In some of freshwater fish varieties, Harikrishnan et al. (2009) suggested that a dietary inclusion of tri-herbal extract such as *Azadirachta indica*, *Ocimum sanctum* and *Curcuma longa* each at 2.5 g kg^{-1} enhanced lysozyme activity of gold fish, *Carassius auratus*. Li et al. (2011) reported that phagocytic activity of barbel chub, *Squaliobarbus curriculus* treated with levamisole at 10^{-3} ng ml^{-1} enhanced significantly than the control group fishes. Biller-Takahashi et al. (2016) observed increased serum bacterial activity, lysozyme activity and respiratory burst activity of pacu, *Piaractus mesopotamicus* with levamisole at 500 mg kg^{-1} in diet.

In pangus catfishes, Adloo et al. (2015) demonstrated that a dietary incorporation of 0.5 % of β-glucan enhanced serum lysozyme activity of striped catfish. Sirimanapong et al. (2015) studied that striped catfish fed with 0.2 % β-glucan enhanced respiratory burst activity and lysozyme activity. Dietary supplementation of *Achyranthes aspera* at 1.5 % showed significantly increased serum antibody titer, phagocytic activity of striped catfish (Alam et al., 2016).

In marine finfishes, Cuesta et al. (2003) demonstrated enhanced phagocytic activity when leucocytes of gilthead seabream fishes were incubated with 1000 µg ml^{-1} of chitin. Li and Gatlin (2003) reported that there were no significant differences in superoxide anion production and serum lysozyme activity of hybrid striped bass, *Morone chrysops* × *Morone*

saxatilis, after supplementing brewer's yeast at different concentrations such as 2 and 4 %. Ai et al. (2004) reported that dietary supplementation of 489 mg kg^{-1} of ascorbic acid significantly enhanced lysozyme activity and alternative complement pathway activity of seabass, *Lateolabrax japonicus*. Li and Gatlin (2004) showed that a dietary incorporation of brewer's yeast at 1 % and 2 % enhanced the superoxide anion production of hybrid striped bass, *Morone chrysops* × *Morone saxatilis*. Lin and Shiau (2005) studied that grouper, *Epinephelus malabaricus* fed diets with > 400 mg vitamin E kg^{-1} increased the respiratory burst activity; whereas, diets with > 100 mg kg^{-1} enhanced the lysozyme activity in fishes.

Ai et al. (2006) noticed enhanced the lysozyme activity, phagocytic activity in large yellow croaker, *Pseudosciaena crocea* using dietary supplementation of 489 mg kg^{-1} ascorbic acid. Alvarej-Pellitero et al. (2006) reported that dietary incorporation of levamisole at 500 mg kg^{-1} enhanced the respiratory burst activity of *Scophthalmus maximus*. Japanese flounder, *Paralichthys olivaceus* showed highest lysozyme activity when fed with the diet containing 213 mg α-tocopherol kg^{-1} and 2.0 % n-3 HUFA (Wang et al., 2006). Phagocytosis percentage (PP) and respiratory burst activity were significantly higher in large yellow croaker, *Pseudosciera crocea* when fed with the diet containing 0.09 % glucan than those in fishes fed with the control diet (Ai et al., 2007). Cha et al. (2008) observed enhanced lysozyme activity in olive flounder, *Paralichthys olivaceus* with dietary supplementation of chitosan at 7500 mg kg^{-1}. Eo and Lee (2008) reported that a dietary inclusion of L-ascorbyl-2-monophosphate at 80 and 160 mg kg^{-1} diet increased plasma lysozyme activity of tiger puffer, *Taxifuges rubripes*. Galaz et al. (2010) showed that a dietary supplementation of DL-α-tocopheryl acetate (α-TA) at 87 mg kg^{-1} diet exhibited significantly higher NBT activity in the parrot fish, *Oplegnathus fasciatus*. Geng et al. (2011) observed that dietary inclusion of chitosan at 3 g kg^{-1} increased phagocytic activity and respiratory burst activity of cobia. Harikrishnan et al. (2012) reported that a dietary inclusion of 1 % each of chitin and chitosan enhanced phagocytic activity, respiratory activity, complement activity and lysozyme activity of kelp grouper, *Epinephelus bruneus*. Ranjan et al. (2012) observed that respiratory burst activity and phagocytic ratio were significantly increased in Asian seabass, *Lates calcarifer*

fed chitosan at 10 g kg $^{-1}$ incorporated diets compared with control group of fish. Grouper, *Epinephelus coioides* fed with *Curcuma zedoaria* at 0.5 g kg^{-1} diet and *Zingiber zerumbet* at 1 g kg^{-1} diet enhanced superoxide anion production and phagocytic activity (Nan et al., 2015). Bazari et al. (2017) observed increased lysozyme activity of Siberian sturgeon, *Acipenser baerii* with dietary incorporation of *Aloe vera* at 1.5 % in diet. Vahedi et al. (2017) noticed increased alternative complement activity and lysozyme activity in juveniles of beluga, *Huso huso* using dietary supplementation of 1.5 % ginger in the diets of fish.

2.5 Effects of immunostimulants on histology

Genc et al. (2007) observed no detrimental effects on liver of hybrid tilapia when fed different levels of dietary mannan oligosaccharide (MOS). Yilmaz et al. (2012) noticed longer intestinal villi of fish fed with diets supplemented with 1.5 or 3.0 % mannan oligosaccharide than those of fish fed 4.5 % or no dietary MOS in rainbow trouts. Rodrigues et al. (2009) concluded that striped catfish, *Pseudoplatystoma fasciatum* fed dry diets had normal intestinal histology. The histological studies carried out by Aly et al. (2010) revealed activation of melanomacrophages and tubular necrosis mainly vacuolar degeneration in the kidney, liver congestion and activation of melanomacrophages in the catfish, *Clarias gariepinus* treated with levamisole at 150 mg kg $^{-1}$ of diet. Dimitroglou et al. (2010) evaluated that MOS at 0.4 % had no effect on glycogen deposition in liver and villi morphology in the anterior intestine of gilthead seabream. Merrifield et al. (2011) demonstrated that histology of intestinal tract was not affected by dietary alginic acid at 5g kg $^{-1}$ inclusion in tilapia. Heidarieh et al. (2012) studied that in ergosan at 5 g kg $^{-1}$ treated group, higher percentage of goblet cell was shown in proximal intestine and pyloric caeca. Jarmolowicz et al. (2012) studied that dietary supplement of brewer's yeast diet at 20, 40 and 60 g kg $^{-1}$ had no significant effect on intestinal epithelial cells and hepatocyte nuclei of juvenile pike perch, *Sander lucioperca*. Different levels of dietary mannan oligosaccharide (1.5 and 4.5 g kg $^{-1}$) had no detrimental effects on liver tissue and intestine of common carp (Genc et al., 2013). Heidarieh et al. (2013) studied that *Aloe vera* (0.01, 0.1 and 1 %) treated groups showed improvement in proximal intestine, pyloric caeca and skin epidermis histology in rainbow

trout. Ashade et al. (2014) noticed histopathological changes in the intestine of *Clarias gariepinus* fed with 10, 20, 30 and 50 % of unfermented ginger peel in fish meal. Seabass fed on diets supplemented with different levels of chitosan (0.5, 1, 2, 3 and 4 g kg^{-1}), showed normal villi structure in intestine in all the treatments (Zaki et al., 2015). Meshram et al. (2016) observed that a diet incorporated with 1 g kg^{-1} of β-glucan showed development of microvilli structure and goblet cells. Najafabad et al. (2016) demonstrated that the intestinal villi length increased in fish fed diet containing 1 g kg^{-1} of chitosan compared to the control group.

Apines-Amar et al. (2012) noticed liver necrosis, fatty globule deposition, vacuolation and presence of short rod-shaped bacteria in the control group than that of the fishes fed with experimental diets containing onion (2%), ginger (2%), β-glucan (1%), vitamin C (3%) in marbled grouper, *Epinephelus fuscoguttatus*. These treated fishes also showed no signs of pathological changes in kidney sections. Sarvestani (2017) studied that liver tissue in carnivore fish (*Notopterus chitala*) had larger hepatocytes with clear nucleus and intestinal organelles and many fat cells in comparison to those of herbivore fish (*Pangasius pangasius*). Liver histology of control fish was normal, while fatty degenerations were seen in *Aloe vera* (1, 2 and 3 %) treated fish (Adegbesan et al., 2018). Gelibolu et al. (2018) observed no adverse effects on intestine and liver histological structures in gilthead seabream that were fed on a diet supplemented with mannan oligosaccharide at 0.1, 0.2, 0.3 and 0.4 % levels.

Hedayati and Tarkhani (2014) observed gill damages in striped catfish exposed to 1 ppm diazinon as well as 0.015 and 0.020 ppm deltamethrin. Abdelrazek et al. (2017) revealed slight differences in spleens of tilapia fed 4 g kg^{-1} turmeric than the control group. Pournori et al. (2017) noticed that dietary nucleotide at 0.75 % had no significant difference in histological alternation of gill, liver and kidney of striped catfish, *Pangasianodon hypophthalmus*.

3.0 MATERIAL AND METHODS

A total of two experiments (Experiment 1 and Experiment 2) were carried out in the present work. In Experiment 1, effects of four different immunostimulants were tested and in Experiment 2, the immunostimulant that showed better response in Experiment 1, was incorporated in the diet at different levels concentration to find out the optimum level of incorporation. Each experiment was conducted for a period of 90 days. Details of the experiments conducted along with different treatments are given below.

3.1 Experimental fishes

Juveniles of the striped catfish, *Pangasiandon hypophthalmus* in the weight range of 4.23 to 5.25 g size were used for the experiment. The fishes were disinfected using 10 ppm $KMnO_4$ bath treatment for 30 min (Francis-Floyd and Klinger, 2002) before stocking into circular fibreglass tanks of 500 L capacity. The fishes were acclimatized to laboratory condition for about fifteen days prior to starting up of the experiments. The fishes were fed with a basal diet containing 32 % protein level.

3.2 Experimental diets

In Experiment 1, a total of four practical diets with a crude protein content of 32% were formulated using locally available feed ingredients such as fish meal, soya flour, groundnut oil cake, wheat flour, rice bran, tapioca, fish oil, etc. (Table 1). The ingredients in powdered form were mixed with water to make dough and steamed in a pressure cooker. The dough was then cooled to room temperature by spreading in an enamel tray. After cooling, a required quantity of brewer's yeast @ 10 g (F1), L-ascorbic acid @ 1000 mg (F2), α-tocopherol @ 80 mg (F3) and ginger @ 10 g (F4) were added, mixed and blended per kilogram of basal feed according to Parmar et al. (2012), Ispir et al. (2011), Sobhana et al. (2002) and Sahan et al. (2016), respectively. The dough was extruded through hand pelletizer having 2 mm diameter. Extruded pellets were dried till the moisture level reduced to less than 10%. Commercial feed (F0) was treated as control. Diets were packed and labeled

separately. In Experiment 1, the ginger incorporated diet showed better response than that of the remaining diets. Thus, in Experiment 2, ginger was used in the diets at various levels of concentration. A total of five practical diets with a crude protein of 32% were formulated using locally available feed ingredients such as fish meal, soya flour, groundnut oil cake, wheat flour, rice bran, tapioca and fish oil. Ginger powder in different levels of concentration such as 5 (F1), 10 (F2), 15 (F3) and 20 g kg^{-1}(F4) of feed were added in the diets. F0 was used as control without any immunostimulant in it. The composition of the diets is given in the Table 2.

3.3 Experimental design

The experiment was conducted for a period of 90 days in the Wet Laboratory, Department of Aquaculture, College of Fisheries, Ratnagiri. The setup consisted of 20 fibreglass tanks, each of 400 litre capacity with 50 fishes in each tank. The experiment consisted of five treatments and four replicates following a completely randomized design (CRD). Aeration was provided in each experimental unit. Experimental tanks were cleaned manually by siphoning the water along with faecal matter and left over feed daily. Water exchange of up to 15-20% was carried out daily. Feeding was done twice a day at 0900 and 1700 h.

3.4 Proximate analysis of the diets and carcass tissues

Proximate analysis of the diets and carcass tissue was done by the standard methods (AOAC, 2006).

3.4.1 Moisture

The moisture content of the experimental diets and carcass tissue was determined by taking a known weight of the sample in a petri dish and drying it in a hot air oven at 100-105^{o}C until a constant weight was achieved. The difference in weight of the sample gave the moisture content, which was calculated by using the following formula:

$$\text{Moisture } \% = \frac{(\text{Initial weight of sample} - \text{Final weight of sample})}{\text{Initial weight of sample}} \times 100$$

3.4.2 Crude protein

Nitrogen content (%) of dried samples of the experimental diets and carcass tissue was estimated quantitatively by Kjeltec semi-automated apparatus by titrimetric analysis. The crude protein percentage was obtained by multiplying the nitrogen percentage by a factor of 6.25.

$$\text{Crude protein (\%)} = N_2\,(\%) \times 6.25$$

3.4.3 Crude fat

Crude fat content of dried samples of the experimental diets and carcass tissue was estimated by Socs plus apparatus using diethyl ether (boiling point 55 ± 5^{o}C) as a solvent. The calculation was made as follows:

$$\text{Crude Fat}(\%) = \frac{\text{Weight of the ether extract}}{\text{Weight of the dried sample}} \times 100$$

3.4.4 Total ash

Ash content of the experimental diets and carcass tissue was estimated by taking a known weight of dried samples in a silica crucible and placing it in a muffle furnace at 600˚C for 5 - 6 hours. The calculation was done as follows:

$$\text{Total Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of dried sample}} \times 100$$

3.4.5 Total carbohydrate

Total carbohydrate (TC) of the experimental diets and carcass tissue was calculated by subtracting the percentage of other nutrients from 100.

$$\text{TC (\%)} = 100 - [\text{CP (\%)} + \text{CF (\%)} + \text{Ash (\%)} + \text{Moisture(\%)}]$$

where, CP = Crude protein; CF = Crude fat

3.5 Growth parameters

Sampling was done after every 30 days interval to assess body weight of the fishes. The growth parameters such as weight gain, specific growth rate, feed conversion ratio and fish survival were determined according to standard formulae (FAO, 2008).

3.5.1 Weight Gain

The percentage weight gain was calculated using the following formula:

$$\text{Weight Gain (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

3.5.2 Specific Growth Rate (SGR)

The specific growth rate was calculated by the following formula:

$$\text{SGR} = \frac{\text{Log e (Final weight)} - \text{Log e (Initial weight)}}{\text{Number of days}} \times 100$$

3.5.3 Feed Conversion Ratio (FCR)

The FCR was calculated by the following formula:

$$\text{FCR} = \frac{\text{Total feed consumed by fish (g)}}{\text{Total weight gain by fish (g)}}$$

3.5.4 Survival

The survival of fishes was calculated by the following formula:

$$\text{Survival (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

3.6 Experimental challenge study with *Aeromonas hydrophila*

3.6.1 Collection and growth of *Aeromonas hydrophila*

Aeromonas hydrophila was collected from Microbial Type Culture Collection and Gene Bank (MTCC). The subcultures were maintained at -40°C. Bacteria were inoculated in an incubator overnight at 37°C in tryptone soya broth (HiMedia) and harvested at 0.8 optical density (O.D).

3.6.2 LD_{50} of *Aeromonas hydrophila*

Mean lethal dose (LD_{50}) for striped catfish was estimated according to Reed and Muench (1938). Juveniles were maintained in 60 L capacity tanks (10 fish $tank^{-1}$) with aeration. Experiment was carried out in replicates of two. The isolate of *Aeromonas hydrophila* was grown overnight on tryptone soya broth medium at 37°C and cell suspensions were prepared in phosphate buffered saline (PBS). Each fish was injected intraperitoneally with 0.2 ml of *Aeromonas hydrophila* at a density ranging from 10^2 to 10^8 cfu ml^{-1}. Fishes from the control group were injected at 0.2 ml of phosphate buffered saline (PBS). Fish mortality was recorded daily for 10 days.

3.6.3 Challenge study with *Aeromonas hydrophila*

After immunomodulation trial through feed over 90 days duration, the fishes in various experimental groups were challenged to the pathogenic isolates of *Aeromonas hydrophila*. The pathogenic isolates of *Aeromonas hydrophila* were grown on tryptone soya broth at 37°C in BOD incubator. The cells were resuspended in PBS at a concentration of 10^7 cfu ml^{-1}. The fishes in each experimental group were injected with 0.2 ml of this suspension intraperitoneally. Serum and blood samples of different groups were collected 10 days post-challenge to study the haematological, biochemical, immunological parameters.

3.7 Haematological assays

3.7.1 Collection of blood

A few numbers of randomly selected fishes were anesthetized with 50 ppm clove oil before taking blood from them. Blood was withdrawn from vena caudalis using one ml medical syringe pre-rinsed with 2.7% EDTA solution. Collected blood was then transferred immediately to EDTA coated vials and shook well in order to prevent haemolysis of blood.

3.7.2 Collection of serum

A portion of blood was collected and transferred immediately to the pre-labelled 1.5 ml eppendorf tubes from randomly selected fishes. The tubes were allowed to stand in tilting

position at a room temperature for an hour which allowed the blood to clot. After clotting the blood, the yellow straw color serum was carefully collected and transferred to another set of pre-labelled eppendorf tubes.

3.7.3 Haemoglobin content

The haemoglobin level of blood was analysed following the cyanmethemoglobin method using Drabkins fluid (Make: Qualigens). The blood (20 µl) was mixed with 5 ml of Drabkin's working solution. The absorbance was measured using a spectrophotometer at 540 nm wavelength. The haemoglobin concentration was calculated by using the following formula:

$$\text{Haemoglobin (g/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{60}{1000} \times 251$$

3.7.4 Total Erythrocyte count (TEC)/Red Blood Cells (RBC)

Counting of cells was done using haemocytometer as per the method given by Shah and Altindag (2004). Blood sample was diluted at 1:200 with Hayem's fluid (Mishra et al., 1977). Erythrocytes number was recorded as 10^6 mm^{-3} (Wintrobe, 1967).

A blood sample was drawn into the RBC pipette up to 0.5 mark and immediately the diluting Hayem's fluid was drawn up to 101 mark to achieve dilution of 1: 200 (blood:Hayem's fluid). The solution was added by shaking gently and kept aside for two to three minutes. The counting chamber was cleaned and a cover slip was placed over it followed by gentle mixing of solution. A drop of fluid was allowed to flow under the cover slip by holding the pipette at an angle of 45^0. It was allowed to settle for two to three minutes. The number of RBCs was counted in five squares of the counting chambers under high power magnification and the number of RBCs per cubic mm was calculated by using following formula:

$$\text{Number of RBC} = \frac{\text{N} \times \text{Dilution factor} \times \text{Depth factor}}{\text{Area counted}}$$

where, N is the total number of red blood cells counted in 5 squares of the haemocytometer.

3.7.5 Total Leucocyte Count (TLC)/White Blood Cells (WBC)

Blood collection and processing procedure were the same as described in section 3.6.4. As far as the counting (Neubaur counting chamber) procedure of WBC, each of these four squares millimeter area was subdivided into 16 squares. By using low power objective and adjusting ocular carefully, cells were counted on the Neubaur chamber. The following formula was used for counting of WBC.

$$\text{Number of WBC} = \frac{\text{N} \times \text{Dilution factor} \times \text{Depth factor}}{\text{Area counted}}$$

where, N is the total number of white blood cells counted in 4 squares of the haemocytometer.

3.7.6 Packed Cell Volume (PCV)/ Haematocrit Value (Hct)

Packed cell volume was determined by drawing non-dotted blood by capillary action in to microhaematocrit tubes. One end of the tubes was sealed with synthetic sealant. The sealed tube was centrifuged in a microhaematocrit centrifuge for 5 min at 10500 rpm. The PCV was measured using microhaematocrit reader and expressed as percentage.

3.8 Biochemical assays

3.8.1 Total serum protein

Total serum protein was estimated by Biuret method (Reinhold, 1953) using kit (Autospan ® Arkray Health Pvt. Ltd). Proteins present in the serum bind with copper ions in alkaline medium of the biuret reagent to produce a purple coloured complex, whose absorbance is proportional to the protein concentration. A total of three test tubes labelled as B (blank), S (standard) and T (test) were filled up with 1000 µl of biuret reagent and 2 ml of distilled water. A quantity of 0.05 ml of protein standard was taken in the test tube labelled as 'S' and 0.05 ml of serum was added in to the test tube labelled as 'T'. It was then mixed well

and incubated at 37°C for 10 min. The absorbance of S (standard) and T (test) were measured against the B (blank) in a spectrophotometer at 630 nm. The calculation was done as follows:

$$\text{Total serum protein (g/dl)} = \frac{\text{Absorbance of test (T)}}{\text{Absorbance of standard(S)}} \times 6$$

3.8.2 Serum albumin

Albumin was estimated by bromocresol green binding method (Doumas and Biggs, 1972) using kit (Autospan ® Arkray Health Pvt. Ltd). Albumin binds with bromocresol green forming a green coloured complex, which is proportional to albumin concentration in the sample. A total of three test tubes labelled as B (blank), S (standard) and T (test) were added with Buffered dye reagent of 1.0 ml and 2.0 ml of distilled water. Albumin standard of 0.01 ml was considered as standard. A serum sample of 0.01 ml was added in to the test tube and labeled as 'T'. It was mixed well and incubated at 37°C for 10 min. The absorbance of S (standard) and T (test) were measured immediately against B (blank) in a spectrophotometer at 630 nm. The calculation was done by using following formula:

$$\text{Serum albumin (g/dl)} = \frac{\text{Absorbance of test (T)}}{\text{Absorbance of standard(S)}} \times 5$$

3.8.3 Globulin

Globulin was calculated by subtracting albumin values from total plasma protein.

Globulin (g/dl) = Total serum protein (g/dl) – Serum albumin (g/dl)

3.8.4 Glucose

Glucose was estimated by Trinder's method (Trinder, 1969) using kit (Yucca Diagnostics). Glucose in the sample is oxidized to yield gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The enzyme peroxidase catalyses the oxidative coupling of 4-aminoantipyrine with phenol to yield a coloured quinonemine complex, which is directly proportional to the amount of glucose present in a sample. A total of three test tubes labelled as B (blank), S (standard) and T (test) were added with 1000 µl of glucose reagent. A serum quantity of 10 µl of was added in to the test tube labelled as 'T'. It was then mixed well and

incubated at 37°C for 10 min. The absorbance of S (standard) and T (Test) were measured against blank (B) in a spectrophotometer at 505 nm. The calculation was done as follows:

$$\text{Glucose (mg/dl)} = \frac{(\text{Abs. T}) - (\text{Abs. B})}{(\text{Abs. T}) - (\text{Abs. B}))} \times 100$$

3.8.5 Glycogen

Glycogen content was estimated by treating with anthrone reagent (Hassid and Abraham, 1957). A fish liver was put in 30% potassium hydroxide and boiled in water bath until it was digested. After cooling, 5 ml 95% ethanol was added and centrifuged at 6000 rpm for 10 min. Supernatant was discarded and glycogen was again precipitated as above. The precipitate was dissolved in distilled water and an aliquot was reacted with anthrone and the optical density was read at 620 nm. Glucose was used as a standard and the amount of glycogen was calculated by multiplying by a factor of 0.927.

3.9 Immunological assays

3.9.1 Nitroblue Tetrazolium (NBT) Assay

Blood was collected from vena caudalis and transferred immediately to EDTA coated vials. A quantity of 100 µl of blood was placed into the wells of 'U' bottom microtiter plates and incubated at 37°C for 1 hour to facilitate adhesion of cells. The supernatant was removed and loaded wells were washed three times in PBS. After washing, 100 µl of 0.2% NBT was added and incubated for further 1 hr. The cells were then fixed with 100% methanol for 2-3 minutes and again washed thrice with 30% methanol. The plates were then air dried. A quantity of 60 µl 2N KOH and 70 µl dimethyl sulphoxide were added into each well to dissolve the formazon blue precipitate formed. The OD of the turquoise blue coloured solution was then read in ELISA reader.

3.9.2 Phagocytosis assay

Phagocytic cells were detected using *Staphylococcus aureus* as described by Anderson and Siwicki (1995). A sample of 0.1 ml of blood was placed in a microtitre plate

well. A sample of 0.1 ml of *Staphylococcus aureus* (1×10^7 cells) suspended in phosphate buffered saline pH 7.2 was added and mixed well. The bacteria-blood solution was incubated for 20 minutes at room temperature. Five microliter of this solution was taken on to a glass slide and smear was prepared. The smear was air dried, then fixed with ethanol (95%) for 5 minutes and air dried. The smear was stained with Giesma strain for 10 min. A total of two smears were made from each fish. A total of 100 neutrophils and monocytes from each smear were observed under the light microscope and the number of phagocytizing cells and the number of bacteria engulfed by the phagocyte were counted.

$$\text{Phagocytic activity} = \frac{\text{Number of phagocytizing cells}}{\text{Total number of bacteria engulfed by phagocytes}}$$

3.9.3 Serum lysozyme activity

Serum lysozyme activity was measured using colorimetric method. The method involves measuring the clearing of a suspension of dead bacterial cells as their walls breakdown by enzyme lysozyme. Serum samples were diluted with phosphate buffer (pH 7.4). In a suitable cuvette, 3 ml of *Micrococcus leutus* suspension in phosphate buffer saline (Absorbance at 450 = 0.5 - 0.7) was withdrawn, to which 50 µl of diluted serum sample was added. The content of the cuvette was mixed well for 15 s and read in a spectrophotometer at 450 nm exactly after 60 s of addition of serum sample. Lysozyme activity was expressed as U/ min.

3.9.4 Clotting time

Blood was collected from the experimental fishes. Clotting time of blood was determined by the capillary tube method as used in clinical haematology (Srivastava, 1969).

3.10 Histology

The internal organs such as liver, intestine and gills of the fishes were collected and fixed in 10% neutral buffered formalin for histological studies. The preserved organs were cut into a proper size (1-2 mm) and washed under the gentle flow of tap water. The properly washed tissues were dehydrated with a series of different concentrations of alcohol and at last

by xylene and then were embedded into paraffin. The paraffin embedded tissues were sectioned at 5 µ thickness using microtome (Leica RM 2125RT) and stained with haematoxylin and eosin (H&E). Pathological changes manifested in the tissue sections were noted down and microphotographs were taken.

3.11 Water quality parameters

Water parameters such as temperature, dissolved oxygen, pH, free carbon dioxide, total alkalinity and total hardness were recorded at 30 days interval by using the standard methods as given by APHA (2005).

3.12 Statistical analysis

The experimental data were analysed for One-way ANOVA using SAS (9.3).

4.0 RESULTS

4.1 Experiment 1

Water quality parameters observed during the experiment are given in Table 3. Proximate analysis of experimental diets and carcass composition of experimental fishes fed different immunostimulants incorporated diets is given in Table 4 and Table 5. The levels of crude protein% in the diets were found to be around 32%, whereas carcass protein level was around 64%.

4.1.1 Growth Parameters

i. Weight gain (%)

In the experimental duration of 90 days, the fishes showed weight gain in the range of 219.05 - 287.73 %. Average percentage weight gain in striped catfish observed in the Experiment 1 is given in the Table 6 and Fig 1. The maximum weight gain of 283.73 ± 4.02 % was observed in the treatment F4 followed by F3, F2 and F1; while F0 diet showed the minimum average weight gain of 219.05 ± 1.59 %. ANOVA (Table 7) showed significant difference ($P < 0.05$) in the weight gain (%) between the treatments. Tukey's test indicated that weight gain (%) in F4 was significantly higher ($P < 0.05$) than that of other treatments. There was no significant difference ($P > 0.05$) in weight gain in the other treatments F1, F2 and F3.

ii. Length gain (%)

Average length gain in striped catfish is given in Table 6 and Fig 2. The maximum length gain of 78.45 ± 2.46 % was observed in F4 than other treatments. ANOVA (Table 7) showed significant difference ($P <0.05$) in the average length gain (%) between the treatments. Tukey's test indicated that average length gain (%) in F4 was significantly higher

($P < 0.05$) than other treatments except F3. There was no significant difference ($P > 0.05$) in the treatment F0, F1 and F2.

iii. Specific growth rate (SGR)

Average specific growth rate in striped catfish is given in Table 6 and Fig 3. The maximum specific growth rate of 283.73 ± 4.02 was observed in F4 than other treatment. ANOVA (Table 7) showed significant difference ($P<0.05$) in the specific growth rate between the treatments. Tukey's test indicated that average specific growth rate in F4 was significantly higher ($P < 0.05$) than other treatments.

iv. Feed conversion ratio (FCR)

FCR observed is given in Table 6 and Fig 4. Lower FCR of 1.71 ± 0.01 was found in F4. ANOVA (Table 7) showed significant difference ($P < 0.05$) in the feed conversion ratio (FCR) between the treatments. There was no significant difference ($P > 0.05$) in the treatments F0, F1, F2 and F3.

4.1.2 Haematological parameters

i. Haemoglobin (Hb)

Pre-challenge haemoglobin (Hb) content of different treatments is shown in Table 8 and Fig 5. In pre-challenge group, maximum average Hb content of 9.54 ± 0.18 g dl^{-1} was observed in the treatment F4, followed by F3, F2 and F1, while F0 showed the minimum Hb content of 5.63 ± 0.07 g dl^{-1}. A similar trend was observed in post-challenge test as well (Table 9 and Fig 5). A significant difference ($P < 0.05$) was observed between pre- and post-challenged groups (Table 10).

ii. Red blood cells (RBC)

Red blood cell count in the pre-challenge test for different treatments is given in the Table 8 and Fig 6. The maximum and minimum average RBC count of 2.34 ± 0.03 × 10^6 mm^{-3} and 1.12 ± 0.03× 10^6 mm^{-3} and was observed in the treatment F4 followed by F1, F2, F3, while the treatment F0 showed the minimum RBC count in pre-challenge study. Post-

challenge red blood cell content of different treatments is shown in Table 9 and Fig 6. In post-challenge study, a same trend was observed with maximum RBC count of 1.97 ± 0.04 × 10^6 mm^{-3} in the treatment F4 followed by F1, F2 and F3; while the treatment F0 showed minimum RBC count of 0.89 ± 0.01× 10^6 mm^{-3}. Between pre- and post-challenge groups, a significant difference ($P < 0.05$) was observed for treatments (Table 10).

iii. White blood cells (WBC)

White blood cell count in pre-challenge is given in the Table 8 and Fig 7. In pre-challenge group, the highest WBC count of 83.17 ± 0.38 × 10^3 mm^{-3} found in the treatment F4, while the treatment F0 showed minimum white blood cell count of 65.60 ± 1.04 × 10^3 mm^{-3}. Post-challenge white blood cell content of different treatments is shown in Table 9 and Fig 7. In post-challenge group, the highest white blood cell count of 86.47 ± 0.27 × 10^3 mm^{-3} was found in F4 and F0 showed the lowest white blood cell count of 67.60 ± 0.92× 10^3 mm^{-3}. However, between pre- and post-challenge groups (Table 10), no significant difference ($P > 0.05$) was observed for treatments.

iv. Haematocrit value (Hct)

Haematocrit value of pre-challenge is shown in the Table 8 and Fig 8. The highest pre-challenge Hct value of 24.93 ± 0.59 % was found in treatment in treatment F4; whereas the lowest value of 19.95 ± 0.14 % was found in the control group. Post-challenge haematocrit value (Hct) content of different treatments is shown in Table 9 and Fig 8. In post-challenge group, the maximum Hct values of 22.13 ± 0.31% and 22.05 ± 0.15 % were found in treatments F3 and F4, respectively and the minimum Hct value of 18.57 ± 0.32 % was found in the control group. There was no significant difference ($P > 0.05$) between pre- and post-challenge groups (Table 10) except for F4 and F2 treatments.

v. Mean Corpuscular Volume (MCV)

The pre-challenge, mean corpuscular volume (MCV) of different experimental groups is given in the Table 8 and Fig 9. In pre-challenge group, the highest mean corpuscular volume of 179.82 ± 4.65 µ3 was found in the control group followed by F1, F2,

F3 and the lowest MCV of 106.55 ± 2.37 µ3 was found in F4. Post-challenge mean corpuscular volume (MCV) of different treatments is shown in Table 9 and Fig 9. In post-challenge group, the maximum MCV of 209.80 ± 5.76 µ3, 195.37 ± 7.74 µ3 were found in the F0 and F3, respectively. The minimum MCV of 112.42 ± 2.32 µ3 was found in treatment F4. Between pre- and post-challenge groups, a significant difference ($P<0.05$) was observed for the treatments except for F4 and F2 groups (Table 10).

vi. Mean Corpuscular Haemoglobin (MCH)

MCH of pre-challenge for different experimental groups is given in the Table 8 and Fig 10. The maximum pre-challenge MCH value of 50.69 ± 1.35 pg was found in control groups, whereas the minimum MCH value of 40.75 ± 0.57 pg was found in the treatment F4. Post-challenge mean corpuscular volume (MCV) of different treatments is shown in Table 9 and Fig 10. In post-challenge group, the highest MCH value of 50.97 ± 1.21pg was found in the control group and the lowest MCH value of 39.73 ± 0.65 pg was found in treatment F4. There was no significant difference ($P > 0.05$) between pre- and post-challenged test (Table 10).

vii. Mean Corpuscular Haemoglobin Concentration (MCHC)

Pre-challenge mean corpuscular haemoglobin concentration (MCHC) is shown in the Table 8 and Fig 11. In the pre-challenge group, the highest MCHC of 38.45 ± 1.02 g dl^{-1} was found in the treatment F4 and the lowest MCHC of 28.19 ± 0.20 g dl^{-1} was found in the treatment F0. A same trend was observed in post-challenge as well (Table 9 and Fig 11). ANOVA (Table 10) showed a significant difference ($P<0.05$) between pre- and post-challenged groups except for F2 and F4 groups. ANOVA model for pre-challenge, post-challenge and between pre- and post-challenge tests for haematological parameters are given in the Table 17, 18 and 19 respectively.

4.1.3 Biochemical Parameters

i. Total serum protein

Total serum protein content of pre-challenge test of different experimental groups is given in the Table 11 and Fig 12. The highest pre-challenge total protein value of 3.20 ± 0.02 g dl^{-1} was found in treatment F4 followed by F2, F1 and F3 and the lowest total protein content of 1.60 ± 0.02 g dl^{-1} was found in the treatment F0. A similar trend was observed in post-challenge test (Table 12 and Fig 12). There was a significant difference ($P< 0.05$) between pre- and post-challenged fish groups (Table 13).

ii. Serum albumin

The pre-challenge, serum albumin level of different experimental groups is given in Table 11 and Fig 13. In pre-challenged group, the maximum serum albumin level of 1.08 ± 0.04 g dl^{-1} was found in the treatment F4 and the minimum serum albumin level of 0.66 ± 0.01 g dl^{-1}, 0.68 ± 0.03 g dl^{-1}, and 0.71 ± 0.02 g dl^{-1} was found in treatment F0, F3 and F1, respectively. Post-challenge serum albumin content of different treatments is shown in Table 12 and Fig 13. In post-challenged group, the highest serum albumin level of 1.01 ± 0.01 g dl^{-1} was found in treatment F4 and the lowest serum albumin level of 0.47 ± 0.02 g dl^{-1} was found in treatment F0. There was no significant difference ($P>0.05$) between pre- and post-challenge tests except for F0 group (Table 13).

iii. Serum globulin

Serum globulin level of pre-challenge test for the treatments is presented in the Table 11 and Fig 14. The treatment F4 showed the highest serum globulin level of 2.11 ± 0.04 g dl^{-1} in pre-challenge test followed by F2, F1 and F3 and a lowest serum globulin level of 0.93 ± 0.01 g dl^{-1} was observed in F0. Serum globulin level of post-challenge test for the treatments is presented in the Table 12 and Fig 14. In post-challenge test, the maximum serum globulin level of 1.96 ± 0.03 g dl^{-1} was found in treatment F4 followed by F2, F1 and F3, while the minimum serum globulin level of 0.82 ± 0.01 g dl^{-1} was found in the treatment F0. A significant difference ($P<0.05$) was observed between pre- and post-challenged groups (Table 13) in F1, F2 and F4 except F0 and F3 treatments.

iv. Serum glucose

Serum glucose in pre-challenge group of different treatments is shown in the Table 11 and Fig 15. The maximum serum glucose level of 135.88 ± 0.80 mg dl^{-1} was found in the treatment F2; while there was no significant difference in F0, F1, F3 and F4 in the pre-challenge test. Serum glucose in post-challenge group of different treatments is shown in the Table 12 and Fig 15. In the post-challenge test, the highest serum glucose level of 133.83 ± 0.53 mg dl^{-1} was observed in the treatment F2 and the lowest serum glucose level of 125.58 ± 0.38 mg dl^{-1} was found in the treatment F4. There was no significant difference ($P > 0.05$) between pre- and post-challenged groups (Table 13) for all the treatments.

v. Glycogen

Glycogen content of pre-challenge challenged groups of different treatments is shown in the Table 11 and Fig 16. ANOVA showed no significant difference ($P > 0.05$) among the treatments in pre-challenge group. A similar trend was observed in post-challenge tests (Table 12 and Fig 16). There was a significant difference ($P < 0.05$) between pre- and post-challenged groups (Table 13) for all the treatments. ANOVA model for pre-challenge, post-challenge and between pre- and post-challenge tests for biochemical parameters are given in the Table 17, 18 and 19 respectively.

4.1.4 Immunological parameters

i. Nitroblue tetrazolium (NBT) assay

Pre-challenge NBT assays for the fishes of different experimental groups are presented in the Table 14 and Fig 17. The highest NBT assay of 3.25 ± 0.08 (OD/540 nm) was found in the treatment F4 in pre-challenge test followed by F1, F2 and F3, respectively; while the lowest NBT assay of 0.15 ± 0.01 (OD/540 nm) was noticed in the treatment F0 in pre-challenge test. Similar results were found in post-challenge study (Table 15 and Fig 17). There was a significant difference ($P < 0.05$) between pre- and post-challenged groups (Table 16) in all the treatments except for the control group.

ii. Serum lysozyme activity

Serum lysozyme activity in pre-challenge tests for the treatments is shown in Table 14 and Fig 18. In pre-challenge test, the highest lysozyme activity of 1.43 ± 0.03 U min^{-1} was found in the treatment F4 followed by F1, F2 and F3. No significant difference ($P > 0.05$) was observed between the treatments F2 and F3. The lowest lysozyme activity of 0.32 ± 0.02 U min^{-1} was found in the treatment F0. Similar trend was observed in post-challenge study (Table 15 and Fig 18). ANOVA showed a significant difference ($P<0.05$) between pre- and post-challenged groups except for F3 and F1 groups (Table 16).

iii. Phagocytic activity

Phagocytic activity in pre-challenge group of fishes is presented in Table 14 and Fig 19. The highest phagocytic activity of 19.89 ± 0.29 % was found in the treatment F4 followed by F1, F2 and F3 in pre-challenged condition. The lowest phagocytic activity of 11.42 ± 0.10 % was found in the control group. Similar results were found in post-challenged condition as well (Table 15 and Fig 19). ANOVA showed a significant difference ($P<0.05$) between pre- and post-challenged groups (Table 16).

iv. Clotting time

Clotting time in pre-challenge group is shown in Table 14 and Fig 20. There was no significant difference ($P > 0.05$) in all the treatments in pre-challenge test. Clotting time in post-challenge group is shown in Table 15 and Fig 20. In post-challenge test, the maximum clotting time of 32.33 ± 0.21 s was observed in the treatment F4 and the minimum clotting time of 30.67 ± 0.33 s was observed in the control group. ANOVA showed a significant difference ($P < 0.05$) between pre- and post-challenged groups (Table 16). ANOVA model for pre-challenge, post-challenge and between pre- and post-challenge tests for immunological parameters are given in the Table 17, 18 and 19 respectively.

4.1.5 Relative Percentage Survival

Relative percentage of survival of striped catfish after challenge study with *Aeromonas hydrophila* in different experimental groups is represented in Table 20 and Fig 21. The highest relative percentage of survival of 93.75 ± 0.00 % was found in the treatment F4

and the lowest relative percentage of survival of 48.75 ± 2.34 % was observed in the control group. A significant difference ($P < 0.05$) in relative percentage survival was seen in the different treatments.

4.1.6 Histology

Histological changes were studied in gill, liver and intestine in both control and treated groups. The general histological examination indicated incidence of damage in the fish species after post challenged condition.

i. Gill

Pre-challenge study, gill histology (Plate 1) showed a typical structural arrangement of primary and secondary lamellae with uniform inter-lamellar space in the fishes treated with immunostimulant incorporated diets. The control group of pre-challenge fishes showed histological changes in gills. Changes include breakdown of secondary gill lamellae, epithelial necrosis, shortening of secondary gill lamellae and severe damage of lamellar region.

Post-challenge gill histology (Plate 2) showed normal arrangement of primary and secondary lamellae with uniform inter-lamellar space in immunostimulant treated fish groups The control group of fishes showed degeneration of secondary gill lamellae, lamellar fusion and distorted blood capillaries (Plate 2; A). Epithelial necrosis and epithelial hyperplasia were seen in the brewer's yeast, vitamin C and vitamin E incorporated diets (Plate 2; B, C and D). Relatively better structural arrangements were noticed in ginger treated fish groups when compared to the other fish groups.

ii. Liver

Pre-challenge histological changes in liver of the control group fishes showed aggregation of melanomacrophages, hepatocyte degeneration and medium cytoplasmic vacuolation (Plate 3; A). The structure of liver revealed polygonal hepatocytes with densely stained nucleus without any lesions and surrounded with sinusoidal portal blood in the fish

groups treated with brewer's yeast, vitamin C, vitamin E and ginger incorporated diet (Plate 3; B, C, D and E).

In post-challenge studies, series of changes were noticed as cytoplasmic vacuolation, hepatocyte degeneration, aggregation of melanomacrophages, haemorrhages in the fishes of control group. The histopathological damages were found to be relatively severe in the fish fed the control diet; whereas the pathological changes seen in liver fed brewer's yeast, vitamin C and vitamin E incorporated group (Plate 4; A, B, C and D) were found to be milder the than the control group of fishes. The fish groups given ginger incorporated diet showed normal hepatocyte and sinusoids structures (Plate 4; E).

iii. Intestine

Pre-challenge intestine histology of the control fish group showed fusion of villi forming a network with intact muculosa. Close- up view of the section at higher magnification (X400) showed thick villi with distinct lamina propria. Villi vacuolation was seen between lamina propria and epithelial cells of brush border (Plate 5; A2), whereas brewer's yeast, vitamin C, vitamin E and ginger incorporated diet fish groups showed normal cellular architecture of villi with intact submucosa, muscularis and serosa (Plate 5; B1 and C1, Plate 6; D1 and E1).

Post-challenge intestine histology of the control, brewer's yeast and vitamin E incorporated diet fish groups showed short and stout villi. Villi vacuolation was seen between lamina propria and epithelial cells of brush border (Plate 7; A2, B2 and Plate 8; D2). Vitamin C incorporated diet fish groups showed villi fusion to form a network. Close-up view of the section at higher magnification (X400) showed distorted and vacuolated villi (Plate 7; C2); whereas ginger incorporated diet fishes showed typically normal epithelial cells and villi structure of intestine (Plate 8; E1 and E2).

4.2 Experiment 2

Water quality parameters observed in the Experiment 2 are given in Table 21. Proximate analysis of experimental diets and carcass composition of experimental fishes fed

different concentration of ginger is given in Table 22 and Table 23. A level of crude protein in the diets was found to be between 32.01 and 32.45 %, whereas carcass protein level was between 63.91 and 64.13 %.

4.2.1 Growth parameters

i. Weight gain (%)

Average percentage weight gain of striped catfish observed in the Experiment 2 is shown in the Table 24 and Fig 22. The maximum average percentage weight gain of 352.20 ± 5.44 % was observed in the treatment F2, while F0 diet group showed the minimum average weight gain of 279.71 ± 4.38. ANOVA (Table 25) showed a significant difference ($P < 0.05$) in the average weight gain (%) between the treatments. There was no significant difference ($P > 0.05$) between the treatments F1, F3 and F4.

ii. Length gain (%)

Average percentage length gain in fishes is given in Table 24 and Fig 23. ANOVA showed significant difference ($P < 0.05$) in the average length gain (%) between the treatments (Table 25). Tukey's test indicated that average length gain (%) in F2, F3 and F1 was significantly higher ($P < 0.05$) than other treatments and the lowest length gain of 88.44 ± 13.32 and 91.68 ± 3.13 was found in the treatments F0 and F4, respectively.

iii. Specific growth rate (SGR)

Specific growth rate of striped catfish is given in Table 24 and Fig 24. ANOVA (Table 25) showed significant difference ($P < 0.05$) in the specific growth rate between the treatments. Tukey's test indicated that specific growth rate in F2 was significantly higher ($P < 0.05$) than that of the other treatments. There was no significant difference ($P > 0.05$) between the treatments F1, F3 and F4.

iv. Feed conversion ratio (FCR)

Feed conversion ratio (FCR) of striped catfish is given in Table 24 and Fig 25. ANOVA (Table 25) showed significant difference ($P < 0.05$) in the feed conversion ratio

between the treatments. A lower FCR was found in F2 treatment and a higher FCR was recorded in treatment F0.

4.2.2 Haematological parameters

i. Haemoglobin (Hb)

Haemoglobin (Hb) content of pre-challenge group is shown in the Table 26 and Fig 26. In pre-challenge group, the highest Hb content of 8.05 ± 0.05 g dl^{-1} was found in treatment F2 followed by F4, F3 and F1, while the lowest Hb content of 6.11 ± 0.07 g dl^{-1} was found in the treatment F0. A similar trend was observed in post-challenge study (Table 27 and Fig 26). ANOVA showed a significant difference ($P < 0.05$) between pre- and post-challenged groups (Table 28).

ii. Red blood cells (RBC)

Pre-challenge red blood cell (RBC) count is given in the Table 26 and Fig 27. The highest red blood cell count of 2.01 ± 0.07 $\times 10^6$ mm^{-3} was found in treatment F2 and the lowest RBC count of 1.04 ± 0.03 $\times 10^6$ mm^{-3} was found in treatment F0 in pre-challenge test. Post-challenge red blood cell (RBC) count is given in the Table 27 and Fig 27. In post-challenge test, the highest red RBC of 1.84 ± 0.03 $\times 10^6$ mm^{-3} was found in treatment F2 and the lowest RBC count of 0.89 ± 0.03 $\times 10^6$ mm^{-3} was found in treatment F0. There was no significant difference ($P > 0.05$) between the pre- and post-challenge groups except F3 (Table 28).

iii. White blood cells (WBC)

The white blood cell (WBC) count of pre-challenge tests is given in the Table 26 and Fig 28. In pre-challenge test, the maximum WBC count of 84.53 ± 0.36 $\times 10^3$ mm^{-3} was found in treatment F2 and the lowest WBC count of 66.28 ± 1.32 $\times 10^3$ mm^{-3} was observed in treatment F0. More or less similar trend was noticed in post-challenge as well (Table 27 and Fig 28). There was no significant difference ($P > 0.05$) was seen between pre- and post-challenge treatment groups (Table 28).

iv. Haematocrit value (Hct)

Hct values in pre-challenge are given in the Table 26 and Fig 29. There was a significant difference ($P < 0.05$) among the various treatment groups in both pre-and post-challenge treatments. The highest Hct value of 26.94 ± 0.34 % was found in the treatment F2 and the lowest value of 20.93 ± 0.14 % was found in the control group in pre-challenge test. A similar trend was observed in post-challenge test as well (Table 27 and Fig 29). ANOVA (Table 28) showed no significant difference ($P > 0.05$) within pre- and post-challenged groups except in the F0 and F2 group.

v. Mean Corpuscular Volume (MCV)

Pre-challenge mean corpuscular volume (MCV) is shown in the Table 26 and Fig 30. In pre-challenge test, the highest MCV of 203.36 ± 5.71 µ3 and 203.01 ± 5.17 µ3 were found in the treatments F0 and F1 respectively; while the lowest MCV of 135.99 ± 5.29 µ3 was noticed in the treatment F2. Post-challenge mean corpuscular volume (MCV) is shown in the Table 27 and Fig 30. In post-challenge test, there was no significant difference ($P > 0.05$) between the treatments F1, F3, F0 and F4. The lowest MCV of 133.76 ± 2.61 µ3 was found in treatment F2. With the exception of treatment F3, no significant difference ($P > 0.05$) was found between pre- and post-challenged groups (Table 28).

vi. Mean Corpuscular Haemoglobin (MCH)

MCH content of pre-challenge test is represented in the Table 26 and Fig 31. In pre-challenged group, the lowest MCH of 40.57 ± 1.36 g dl^{-1} was found in the treatment F2 compared to other treatments. A similar trend was observed in post-challenge tests (Table 27 and Fig 31). ANOVA (Table 28) showed no significant difference ($P > 0.05$) between pre- and post-challenged groups except for F3.

vii. Mean Corpuscular Haemoglobin Concentration (MCHC)

The mean corpuscular haemoglobin concentration (MCHC) of pre-challenge is shown in the Table 26 and Fig 32. There was no significant difference ($P > 0.05$) between the

treatments in pre-challenged group. In post-challenge study, the highest MCHC of 31.80 ± 0.52 pg and 31.25 ± 0.36 pg was observed in treatment F0 and F2, respectively (Table 27 and Fig 32). There was no significant difference (P > 0.05) between pre- and post-challenged treatment groups (Table 28). ANOVA model for pre-challenge, post-challenge and between pre- and post-challenge tests for haematological parameters are given in the Table 35, 36 and 37 respectively.

4.2.3 Biochemical parameters

i. Total serum protein

Pre-challenge total protein content of fish groups is shown in the Table 29 and Fig 33. In pre-challenge test, the highest total protein content of 3.06 ± 0.04 g dl $^{-1}$ was found in the treatment 2 and the lowest total protein content of 1.55 ± 0.02 g dl $^{-1}$ was found in the control group. Post-challenge total protein content of fish groups is shown in the Table 30 and Fig 33. In post-challenge test, the highest total protein content of 2.03 ± 0.02 g dl^{-1} was found in treatment F2 and the lowest total protein content of 1.09 ± 0.03 g dl^{-1} and 1.19 ± 0.01 g dl^{-1} were found in treatment F0 and F1 groups, respectively. A significant difference (P < 0.05) was observed between pre- and post-challenge tests (Table 31).

ii. Serum albumin

Serum albumin level of pre-challenge group is shown in the Table 29 and Fig 34. The highest serum albumin level of 1.12 ± 0.04 g dl $^{-1}$ was found in treatment F2 and the lowest of 0.62 ± 0.02 g dl $^{-1}$ in the treatment F0 in pre-challenged group. There was no significant difference (P>0.05) in the treatment F1, F3 and F4. Serum albumin level of post-challenge group is shown in the Table 30 and Fig 34. In post-challenge test, the highest serum albumin level of 0.85 ± 0.03 g dl^{-1} was found in the treatment F2 and the lowest serum albumin level of 0.50 ± 0.01 g dl^{-1} was found in the treatment F0. There was a significant difference (P < 0.05) between pre- and post-challenge tests (Table 31).

iii. Serum globulin

Serum globulin level of pre-challenge study is given in the Table 29 and Fig 35. In pre-challenge test, the maximum serum globulin level of 1.95 ± 0.04 g dl^{-1} was found in the treatment F2 and the minimum serum globulin level of 0.93 ± 0.03 g dl^{-1} was found in the treatment F0. Serum globulin level of post-challenge study is given in the Table 30 and Fig 35. In post-challenge test, the maximum serum globulin level of 1.18 ± 0.04 g dl^{-1} was found in the treatment F2 and minimum serum globulin level of 0.60 ± 0.02 g dl^{-1} and 0.67 ± 0.01 g dl^{-1} were found in the treatments F0 and F1, respectively. Statistically, a significant difference ($P < 0.05$) was observed between pre- and post-challenged groups (Table 31).

iv. Serum glucose

Pre-challenge, serum glucose content is given in the Table 29 and Fig 36. The maximum serum glucose of 130.54 ± 0.38 mg dl^{-1} and 130.28 ± 0.27 mg dl^{-1} were found in the treatments F0 and F1 in pre-challenge test; while the lowest serum glucose of 124.35 ± 0.40 mg dl^{-1} and 124.48 ± 0.27 mg dl^{-1} were found in the treatment F3 and F4, respectively. Similar result was found in post-challenge groups (Table 30 and Fig 36). ANOVA showed significant difference ($P < 0.05$) between (Table 31) for pre- and post-challenge groups.

v. Glycogen

Glycogen content of pre-challenge and post-challenge is shown in Table 29, Table 30 and Fig 37. In both pre- and post-challenge tests, there was no significant difference ($P > 0.05$) in all the treatments. Statistically, a significant difference ($P < 0.05$) was observed between pre- and post-challenge group of fishes (Table 31). ANOVA model for pre-challenge, post-challenge and between pre- and post-challenge tests for biochemical parameters are given in the Table 35, 36 and 37 respectively.

4.2.4 Immunological parameters

i. Nitroblue tetrazolium (NBT) assay

Pre-challenge NBT value is represented in Table 32 and Fig 38. In pre-challenge test, the highest NBT value of 2.12 ± 0.11 (OD/540nm) was found in the treatment F2, while the

lowest NBT assay of 0.20 ± 0.01 (OD/540nm) was found in the treatment F0. There was no significant difference (P > 0.05) between the treatments F1, F3 and F4. Post-challenge NBT value is represented in Table 33 and Fig 38. In post-challenge study, the highest NBT value of 1.67 ± 0.03 (OD/540nm) was found in the treatment F2 and the lowest of 0.11 ± 0.00 in the treatment F0. A significant difference (P < 0.05) between pre- and post-challenged groups was seen except for the control group (Table 34).

ii. Serum lysozyme activity

Lysozyme activity of pre-challenge lysozyme activity is shown in Table 32 and Fig 39. There was a significant difference (P < 0.05) among the different treatments in pre- and post-challenged groups. In pre-challenge test, the maximum lysozyme activity of 1.44 ± 0.02 U min^{-1} was found in the treatment F2 and minimum lysozyme activity of 0.24 ± 0.01 U min^{-1} was found in the control group. Lysozyme activity of post-challenge lysozyme activity is shown in Table 33 and Fig 39. In post-challenge test, the highest lysozyme activity of 1.00 ± 0.03 min^{-1} was found in treatment F2 followed by F3, F4 and F1, while the lowest lysozyme activity of 0.11 ± 0.00 min^{-1} was found in the control group. A significant difference (P < 0.05) was observed between pre- and post-challenge group except for control group (Table 34).

iii. Phagocytic activity

Phagocytic activity of pre-challenge is represented in Table 32 and Fig 40. There was a significant difference (P < 0.05) among the different experimental groups in pre- and post-challenge tests. In pre-challenge test, the highest phagocytic activity of 19.57 ± 0.37 % was observed in the treatment F2 followed by F3, F4 and F1, while F0 showed the lowest phagocytic activity of 11.23 ± 0.11%. There was no significant difference (P > 0.05) in the treatments F4 and F1. Phagocytic activity of post-challenge is represented in Table 33 and Fig 40. In post-challenge test, the highest phagocytic activity of 21.38 ± 0.21 % was observed in the treatment F2 followed by F3, F4 and F1 and the lowest phagocytic activity of 12.94 ± 0.10 % was found in the control group. There was no significant difference (P >

0.05) in phagocytic activity between pre- and post-challenged group except F0 and F2 (Table 34).

iv. Clotting time

Clotting time of pre-challenge study is shown in the Table 32 and Fig 41. There was no significant difference ($P > 0.05$) between in all the treatments in pre-challenged condition. In post-challenge test, the highest clotting time of 33.00 ± 0.26 s was found in the treatment F2 compared to other treatments (Table 33 and Fig 41). ANOVA showed a significant difference in clotting time ($P < 0.05$) between pre- and post-challenged conditions (Table 34). ANOVA model for pre-challenge, post-challenge and between pre- and post-challenge tests for immunological parameters are given in the Table 35, 36 and 37 respectively.

4.2.5 Relative Percentage Survival

The relative percentage survival of striped catfish after challenge with *Aeromonas hydrophila* in different experimental groups is given in Table 38 and Fig 42. A significant difference ($P < 0.05$) in relative percentage survival was observed among the different treatments. The highest relative percentage of survival of 95.00 ± 1.25 % was found in treatment F2 and least survival of 50.00 ± 1.98 % was recorded in control group.

4.2.6 Histology

i. Gill

In pre-challenge control group, the gill histology showed epithelial hyperplasia and epithelial lifting (Plate 9A); whereas there was regular arrangement of primary lamellae, secondary lamellae in the fish groups of 0.5 %, 1 % and 1.5 % and 2 % ginger incorporated diet (Plate 9; B, C, D and E).

Post-challenge gill histology showed severe necrosis in secondary lamellae, severe damage of lamellar region and fusion of secondary gill lamellae in control group (Plate 10; A); while there was normal gill structure in the fish groups of 0.5 %, 1 % and 1.5 % ginger

incorporated diet (Plate 10; B, C and D). The gill histology of fishes treated with 2 % ginger incorporated diet showed epithelial necrosis in primary gill lamellae (Plate 10; E).

ii. Liver

In pre-challenge control group and 0.5 % ginger incorporated group, liver sections showed cytoplasmic vacuolation, aggregation of melanomacrophages, slightly haemorrhage and cytoplasmic vacuolation (Plate 11; A and B); while fish groups given 1 %, 1.5 % and 2 % ginger incorporated diet showed normal hepatic cell and sinusoids in liver sections (Plate 11; C, D and E).

In post-challenge, aggregation of melanomacrophages, cytoplasmic vacuolation, slightly haemorrahages were seen in the liver histology of the control group, 0.5 %, 1.5 % and 2 % ginger incorporated diet groups Plate 12; A, B, D and E; whereas 1 % ginger incorporated diet liver structure showed normal appearance of hepatocytes without any lesions (Plate 12; C).

iii. Intestine

Pre-challenge intestinal histology of control diet group showed distorted villi with loss of normal cellular architecture of musculosa (Plate 13; A1 and A2); whereas the intestine structure of the fishes fed ginger incorporated diet at 0.5 %, 1 %, 1.5 % and 2 % showed a normal architecture of villi with intact submucosa, muscularis and serosa (Plate 13; B1 and C1, Plate 14; D1 and E1).

In post-challenge control group and 0.5 % ginger incorporated diet groups the intestine structure showed distorted villi with loss of normal cellular architecture of muscularis, submucosa and villi vacuolation between lamina propria and epithelial cells of brush border (Plate 15; A1, A2, B1 and B2); whereas intestine of fishes with ginger treatment at 1 %, 1.5 % and 2 % showed normal appearance of villi, submucosa, muscularis and lamina propria (Plate 15; C1, C2, Plate 16; D1, D2, E1 and E2).

5.0 DISCUSSION

Striped catfish, *Pangasianodon hypophthalmus* though not a native species, has gained tremendous popularity as one of the candidate species for high density rearing in India. Government of India has aptly included its culture under future thrust areas (GOI, 2018). It is being widely cultured in various parts of India (Lakra and Singh, 2010) but due to distantly located farming units and relatively unorganised domestic marketing system, India is yet to develop an effective data collection system for the species like the striped catfish. Presently, this has resulted in lack of production data for the species at national level. With the inception of 'Blue Revolution' scheme by Indian government, the farming practices are likely to expand for the species. Though the species possesses wide ranges of tolerance to the environmental parameters; it is also susceptible to some of the infectious diseases (Faruk, 2008; Lakra and Singh, 2010; FAO, 2018b).

Considering a susceptibility of the species to diseases under high density rearing, the culture practices need to be modified giving due the species, needs to be developed with due consideration to the carrying capacity of water body in particular and aquatic environment in general. Jana and Jana (2003) have pointed out some of the key factors for the development and cautions for utilization of the water bodies for culture of the striped catfish in India. The culture needs to be further enhanced by complementing the innate immunity of the species which is expected to increase the survival and growth rates. Immunostimulants have proved to be capable feed additives in strengthening the non-specific defence mechanisms and conferring protection against diseases (Jeney and Anderson, 1993). Based on successful models of *Penaeus monodon* and *Litopenaeus vannamei* shrimp aquaculture, effective management practices are required to be developed for the striped fish for attaining sustainability. The striped catfish, being an air-breathing fish, is often reared in high stocking densities. Thus, there is a looming danger of being prone to contagious diseases. Since, immunostimulants are known to add immunity to the fishes, present study was attempted to

formulate a suitable feed using easily available and affordable immunostimulants for the striped catfish.

Nutritional studies of striped catfish show that the fish requires crude protein between 25 to 45% in the diets (Jayant et al., 2017). Fingerlings in grow-out practices require slightly lower level of crude protein between 27.07 to 32.5% (Kader et al., 2003). Based on most of the studies, around 32% crude protein level is maintained in commercial diets (Ahmed et al., 2013). Thus, in the present experiments, the practical diets were prepared keeping a crude protein level of diets around 32%. Studies carried out on proximate composition of carcass showed that the species has 65-75 % percent crude protein level (Halver and Hardy, 2002). In the present study, carcass composition had a protein of about 64%. The level of protein in practical diets used in the present study is very much within the advocated ranges of protein requirement of the species (FAO, 2018b).

Mixing of feed additives is an important process in the feed preparation. Additives in the form of immunostimulants were mixed after cooling the dough, in order to maintain efficacy of the added immunostimulants. This was done in accordance with the process followed by Parmar et al. (2012).

Capacity and nature of rearing containers play a vital role in well-being of the fishes while rearing aquatic organisms under captive laboratory conditions. The striped catfish has a habit of swimming at the bottom of the tank and often makes vigorous movements with slight disturbance that results into turbid water conditions. Thus, a small air-lift filtration system was fitted to each tank to maintain clear water quality. The fiberglass rearing tanks of 400 litres capacity each were used considering stocking density of 50 fishes per tank. Stocking density of fingerlings in net cages generally ranges between 42 - 150 m^{-3} (Rahman et al., 2006); under pond rearing conditions, the stocking density is found to be between 3 to 7 fingerlings m^{-3} (Kader, et al. 2003; Kumar et al., 2017). Although, the stocking density maintained in the present study was relatively higher, cent percent survival of fishes was observed in both the experiments. Relatively higher density used in a number of studies on

silurid catfish was found to exhibit no negative signs on these air-breathing fishes. Similar findings were observed on the striped catfish by Haroon and Hossain (2001) in which a survival rate of about 100% was achieved in cemented tanks. Maniruzzaman (2001) also reported that the survival rate was about 94-96% in earthen ponds. Water quality parameters observed in the present study were found to be within optimum ranges of the species (FAO, 2018b).

Experiment 1

Growth parameters:

Weight gain was significantly improved in all the immune-stimulant-supplemented diet fed fish compared to the control. However, the highest gain was found in ginger supplemented group. Thus, the study demonstrated the growth promoting effect of ginger as an effective dietary supplement in the fish. The results of the study are in agreement with the findings of Arulvasu et al. (2013), Sukumaran et al. (2016) and Payung et al. (2017) who reported that the incorporation of ginger in diets ranging between 1.0 to 8.5 g kg^{-1} exhibited growth-promoting effects in catla, Nile tilapia, and rohu, respectively. Talpur and Ikhwanuddin (2012) also reported significantly higher growth rates after feeding ginger incorporated feed to rainbow trout.

Overall growth with respect to length gain is also found to the highest with ginger supplemented diets showing significantly higher response than that of the remaining treatments. Feed conversion ratio (FCR) provides a measure of aquaculture production efficiency by comparing the amount of feed used for unit weight gain of the species being grown. It also indicates the environmental performance, since it provides an indication of the undesirable outputs and loss of nutrients to the environment, with potential consequences such as accelerated eutrophication, loss of biodiversity and other ecosystem services (Waite et al., 2014). As such, it reflects on the species growth, environment and economics of the enterprise. In the present study, lower FCR of 1.82 to 1.88 was found in ginger treated group as compared to the control. In striped catfish cage culture, Azimuddin et al. (1999) found the

FCR value to range between 1.73 – 2.04 in three months rearing period. Halder and Jahan (2001) reported an FCR of 2.64 to 3.64 in the culture period of 12 months in cemented cisterns. Thus, feed used in the study showed comparatively better assimilation efficiency and digestibility in the species.

Haematological parameters:

Since, immunostimulants boost up the defense mechanism of fish, a few of the fishes were randomly selected for the challenge study from both the experiments to assess their impacts at haematological, biochemical, immunological and histological levels in fish organs like gills, liver and intestine. After challenging the fishes with pathogenic bacteria, *Aeromonas hydrophila*, immunomodulated fishes were expected to react variously at organ level. As practiced commonly in veterinary and in human medicine, it is found to be suitable for fish health assessment in aquaculture too. Haematological parameters are used to provide information about the health and physiological status of fish, feeding condition and habitat water quality (Fazio et al., 2013). Under stressful conditions, hemoglobin (Hb) and red blood cells (RBC) tend to reduce in number; whereas white blood cells (WBC) show an increase in number (Talpur et al. 2013). Thus, Hb, RBC, WBC, (Hematocrit) Hct checks are particularly recommended on a routine basis to monitor the health of the stock in fishes farms (Satheeshkumar et al., 2011).

The hemoglobin content in the blood plays a vital role and serves as transportation element of oxygen to body tissues. Overall range of Hb content in the present work was very much within the range observed by Daniel et al. (2018a) with vitamin C incorporated diet for the striped catfish. However, the range of Hb content in ginger group fishes was significantly higher; probably due to the presence of considerable iron content in ginger (Kulkarni et al., 2012).

The range of RBC count except ginger fed group of fishes observed in the present study is very much similar to the studies carried by Sirimanapong et al. (2014) for the striped catfish. The RBC count was significantly higher in ginger treated groups, exhibiting positive

health effects on fish. Patrick-Iwuanyanwu et al. (2007) reported that ginger might possess constituents that would trigger the erythropoietic system to produce red cells. Studies of Talpur et al. (2013) and Nya and Austin (2009a) also reported significantly higher RBC in rainbow trout after feeding ginger diet. With regard to white blood cells (WBC), they serve as one of the important factors in defense mechanism. The increase in WBC count, neutrophils, following feeding of ginger diet, demonstrates the immunostimulatory effects and anti-infection properties of ginger which is in line with the works of Sirimanapong et al. (2014) and Nya and Austin (2009a) who obtained increased WBC after feeding striped catfish and rainbow trout with ginger diet. Increase in RBC and WBC counts suggests that the stressor – *A. hydrophila* did not impair the hematopoietic system and in turn retained the normal blood functions in the fishes.

In the present study, there was increase in Hct % in ginger fed groups compared to other groups, attributed as a result of immunostimulatory effect of ginger. There was a slight decrease in Hct % in post-challenge, which might be due to impairment of osmoregulation in fishes due to pathogenic stress. The relative decrease in Hct in post-challenge studies has also been observed by Nya and Austin (2009) in rainbow trout and Daniel et al. (2018b) in striped catfish. The levels of MCV and MCH were found to be significantly higher after exposing the fishes to pathogenic bacteria. The values of MCHC were on the lower side during post-challenge study. The blood parameter profiles were found to be in accordance with the natural immune defence exhibited by the fishes in general.

Biochemical parameters:

Serum protein is the most important indicator of the biochemical, nutritional and health status of fish (Patriche et al., 2009). In the present study, the highest serum protein was found in ginger treated group as compared to other groups. The use of diet supplemented with ginger powder showed an increase of total serum protein in the Asian Seabass (Talpur et al., 2013) and juveniles of European sturgeon, *Huso huso* (Gholipour et al., 2014). The decreased

serum protein in post-challenge tests might be due to the loss of protein either due to reduced protein synthesis or increased proteolytic activity or degradation processes (Labh et al., 2017).

It has been recognized that albumin and globulin are the vital elements for maintaining a healthy immune system (Jha et al., 2007). In the present study, the albumin and globulin contents were the highest in ginger treated group compared to other groups. The results are parallel to the work of Nya and Austin (2009a) relating to fish fed with ginger diets. Wiegenties et al. (1996) reported that an increase in the serum protein level via albumin and globulin are associated with stronger innate immune response of fish and are vital fractions for sustaining healthy immune system. Therefore, the improved serum albumin and serum globulin content might be due to the ginger eliciting a positive immune response in the fish juveniles. As a natural defence mechanism, the albumin and globin values were found to be on the lower side in post-challenge study.

Plasma glucose is a good indicator of stress in fishes (Menezes et al., 2006). Differences in physiological and metabolic status of fish results in the variation of glucose concentration of fish serum (El-Khaldi, 2010). Zhou et al. (2006) stated that an increase in glucose content of the fish was related to the healthy physiological status of fish. In the present study, the higher serum glucose content was found in vitamin C treated group than that of the other groups. Similar finding were noticed by Zhou et al. (2012) and Pimpimol et al. (2012) using vitamin C diets in giant catfish, *Pangasianodon gigas* and in juvenile cobia**,** *Rachycentron canadum*, respectively. Conversely, Soltanian et al. (2014) noticed no significant difference in serum glucose levels of striped catfishes in control and β-glucan administered diets.

With regard to glycogen, no significant difference in glycogen content was observed within the pre- and post-challenge tests in the present work. But relative decrease in post-challenge was higher as compared to pre-challenge test. The loss of glycogen or lipid can occur as a direct effect of intoxication or it may occur secondary to decreased body condition caused by emaciation, stress or concurrent disease (Wolfe and Wolfe, 2005). Shivashni et al.

(2013) observed that the higher doses of acetaminophen depleted the hepatic level of glycogen in *Pangasius sutchi* indicating impairment in the lipid and carbohydrate metabolism.

Immunological parameters:

Phagocytosis and respiratory burst response by phagocytes in blood and tissues exhibit a major anti-bacterial defence mechanism in fishes (Secombes et al., 1996). Phagocytosis is considered as an important defense mechanism against pathogenic bacteria (Nya and Austin, 2009a, Sahu et al., 2007, Rao et al., 2006). The results of this study demonstrated higher phagocytic activity was found in ginger treated groups than other groups. This is in agreement with the studies carried out by Sirimanapong et al. (2014), where increased phagocytic activity was noticed after infection of *A. hydrophila* in the striped catfishes.

Respiratory burst activity measured by NBT is one of the most important bactericidal mechanisms in fish (Secombes and Fletcher, 1992). In present study, the NBT activity was found to be the highest in ginger treated group as compared to other groups. This increased NBT activity might be due to the potent anti-oxidant properties of ginger that act as effective forager of superoxide radicals regarded as a promising protective mechanism against stress. Nya and Austin (2009a) and Talpur et al. (2013) also noticed higher NBT activities in the fishes fed ginger incorporated diets.

Lysozyme activity is relevant as one of the barriers in defense system of an organism resulting in the diminution of disease by preventing bacterial pathogens (Misra, 2004). In the present study, increased lysozyme activity was found in ginger treated group as compared to the control. Supplementation of ginger might be helping the fishes to show increased lysozyme activity in pre-challenge study. This finding is analogous with the studies conducted by Nya and Austin (2009a) and Talpur and Ikhwanuddin (2012), where increased serum lysozyme activity was recorded in rainbow trout and in Asian seabass fed with the ginger and garlic incorporated diets, respectively.

Srivastava (1969) and Casillas and Smith (1977) demonstrated that blood coagulation system is a potential system capable of serving as an indicator of environmental stress in several teleostean fishes. In the present study, there was no significant difference in blood clotting time within the treatments in pre- and post-challenge group of fishes. However, a significant difference in clotting time was noticed between pre- and post-challenge studies in the fish groups ($p<0.05$). The results are comparable with the findings of Doolittle and Surgenor (1962), who recorded decreased blood clotting time in anchovies after exposure to cobalt that might have resulted from greater abundance of circulating thrombocytes. It appeared that acute stress evoked a rapid mobilization of the system in preparation for any eventual blood loss (Cnowell and Read, 1955).

Challenge Study:

One of the major bacterial pathogens in aquaculture, *Aeromonas hydrophila*, is known to cause a variety of diseases in fish such as haemorrhagic septicaemia, infectious dropsy, tropical ulcerative disease and fin rot leading to heavy mortalities in aquaculture farms (Kumar and Dey, 1988; Rath, 1993). *Aeromonas hydrophila* was therefore used in the challenge studies in the striped catfish. In the present study, the highest relative percentage survival was found in ginger treated group. This can be supported by the results of Talpur et al. (2013), where reduction in the mortality rate was noticed in Asian sea bass fed ginger containing diet (Immanuel et al., 2009 and Nya and Austin, 2009a).

Experiment 2

Growth parameters:

The growth parameters were found to be dose dependant; suggesting supplementation of ginger @ 10g kg $^{-1}$diet was the most favourable for the growth of striped catfish juveniles. Out of the five diets tested, ginger diet with the incorporation level of 10g kg $^{-1}$diet showed the highest weight gain, FCR and survival percent. A higher weight gain and significantly better FCR was observed by Nya and Austin (2009a) and El-Desouky et al. (2012) using ginger diet in the rainbow trouts and giant freshwater prawn, respectively. The herbal diets are reported to improve the animal performance by stimulating secretion of the digestive enzyme that could result in improvement in digestibility, stimulating the appetite and increasing food consumption (Elabd et al., 2012). Moreover, better FCR demonstrates better feed acceptability and assimilation efficiency by the fishes, which might be due to the appetizing properties of ginger. Thus, the digestibility was increased and in turn, the energy benefits resulted in enhanced the growth rates of the fishes.

Haematological parameters:

Haemoglobin content was found to be higher among the different ginger treatment groups as compared to the control group. This indicates higher immunity of fish in treated groups. Among the various doses of ginger, higher Hb was found in 10g kg $^{-1}$. Hb content was found to be decreasing in the fishes treated with higher ginger concentration. The excessive doses would be probably resulting in immunosuppression (Sakai, 1999). The decreased Hb was observed in post-challenge study, which might be due to severe bacterial infection affecting the haematopoiesis mechanism of the fishes. These findings can be also supported by the results of Nya and Austin (2009a), Talpur et al. (2013) and Aurlvasu et al. (2013). The higher RBC count in treated group than that of the control might be due to incorporation of ginger in the diets. Herbal immunostimulants are known to increase immune functions by stimulating the production of the blood cells (Sahu et al., 2007; Talpur and Ikhwanuddin, 2012). The decrease in RBC in post-challenge study suggests induced

leukocytosis following erythroblastosis in the fishes as a result of and/or stress developed by pathogenic bacteria. The WBCs are well known as one of the prime factors of body defence increasing quickly with the outbreak of infection. In the present study, the WBC count of treated group was higher than the control group fishes and there was an increase in count after the challenge test. Changes in WBC count indicate the response to infections in fish and the increase in WBC denotes activation of defence mechanism to combat pathogenic attack of *Aeromonas hydrophila*.

In the present study, there was consistent increase in Hct % of treated group compared to the control in pre-challenge test. However, there was a decrease in Hct in post-challenge, which would be due to impaired osmoregulation as well as tissue damage by the pathogen. No significant change in MCV, MCH and MCHC values was seen in post-challenge study after challenging the fish with pathogenic bacteria. Sirimanapong et al. (2014) also noticed no significant difference in Hct and MCV values between pre- and post-challenge studies in striped catfish. As such, obvious differences were not observed in these parameters due to pathogen exposure to fishes.

Biochemical parameters:

The increase in serum protein, albumin and globulin contents are considered to reflect strong innate immunity (Jha et al. 2007; Nya and Austin, 2009a). In the present study, total serum protein was higher in treated group that of control group. The total serum protein was slightly lower in post-challenge. Globulin contents are essential for maintaining a healthy immune system and immune functions in the blood, whereas albumin is vital for sustaining the osmotic pressure needed for proper distribution of body fluids and act as plasma carrier (Jha et al., 2007). In the present study, the higher albumin and globulin contents were found in treated group compared to control. However, in post-challenge, there was a slight decrease in albumin and globulin content that might be reflecting in some degrees of physiological stress acting upon the fishes due to bacterial challenge.

The use of ginger had clearly showed its hypoglycemic potential by decreasing the blood glucose (Ahmed and Sharma, 1997). Results of the present study revealed that, except for low concentration of ginger i.e. 5 g kg^{-1} diet and control group, plasma glucose concentration reduced significantly in rest of the groups fed ginger diet. The bioactive compounds such as polyphenols, flavonoid, and saponins present in ginger might have sptimulated the insulin activity, thus reducing the glucose levels in fishes. In the post-challenge test, a slight reduction in glucose that could be attributed to pathogenic stress. The results of the study are in agreement with the findings of Elshater et al. (2009) and Talpur et al. (2013).

Glycogen, commonly called as the animal starch, is the storage polysaccharide. Metabolic breakdown of glycogen assumes considerable importance involving liver and muscle glycogen. In the present study, there was no significant difference in glycogen content within the treatments. In post-challenge study, a slight reduction in glycogen content might be due to stressed pathogenic condition. Thus, there might be decrease in the synthesis rate or might be utilized by the animal for its metabolic activities.

Immunological parameters:

NBT test is one of the methods used for monitoring the non-specific response through the metabolic activity of neutrophils. The assay focuses on the ability of neutrophils to produce oxygen radicals (O_2^- and OH^-) and subsequently reduce a soluble nitrobluetetrazolium dye to insoluble formazon (Anderson et al., 1992). In the present study, the highest NBT activity was found in treated groups compared to the control. In post-challenge study, there was a slight decrease in NBT activity. This activity is inversely proportional to the stress. As such, due to challenge study, there would be reduction in activity of the fishes.

Lysozymes catalyze the hydrolysis of peptidoglycans of bacterial cell walls and act as non-specific innate immunity molecules against the inclusion of detrimental bacteria (Saurabh and Sahoo, 2008). The serum lysozyme activity is considered as a defence barrier against bacterial pathogens, thus, resulting in the reduction of disease (Misra et al., 2006a). In the present study, higher lysozyme activity was observed as general stress response induced by pathogenic bacteria. The findings are comparable with the results of Sirimanapong et al. (2014) and Hang et al. (2014) who also noticed increased lysozyme activity in the striped fishes given exposure to pathogenic bacteria.

Ginger is believed to activate the phagocytosis, which is an important component of the non-specific immune system of fish (MacArthur and Fletcher, 1985). In the present study, increase in phagocytic activity in treated groups was as a result of ginger's bioactive constituents (Benny et al., 2004). Nya and Austin (2009a) also reported enhanced phagocytic activity after feeding ginger incorporated diets to the rainbow trout.

Challenge test:

In the present study, the highest percentage survival was found in the treated groups as compared to the control group. These results are supported by Talpur et al. (2013) who reported ginger induced beneficial effect of disease protection due to improved immune

response in Asian seabass after challenging with *Vibrio harveyi*. Better survival rate could be explained as a result of bioactive compounds, polyphenols, flavonoids, tannins and saponins found in ginger triggering the immune mechanism in fishes (Shirin and Prakash, 2010; and Talpur et al., 2013) or the immunostimulatory effects of ginger in group presumably attributed to a better coordination of its stimulatory and antioxidant properties (Apines-Amar et al., 2012). Polyphenols and flavonoids are recognised to have antioxidant properties with suggested roles in the prevention of infections and hypoglycemic potential (Scalbert et al., 2005). Saponins have been demonstrated to have cholesterol-lowering effects, hypoglycaemic activity and antimicrobial properties to stop attacks by foreign pathogens and tannins have been reported to hasten the healing of wounds (Otunola et al., 2010).

The use of ginger in the diet and subsequent response evinced in the form of growth of striped fish suggests that ginger is a better immunostimulants for incorporation in the prepared diet of the striped catfish. The response shown by the fishes with respect to hematological, biochemical, immunological parameters and histological studies seemed to outpace the other immunostimulants tested. The health status as revealed by histological sections was distinctly different than that of the fishes fed other immunostimulants. The increase in structure of villi in intestine, normal haepatocytes in liver and regular arrangement of primary and secondary gill lamellae were seen in immunostimulant incorporated diets in the present study. More or less similar observations were made by Zhou et al. (2012) and Zaki et al. (2015) for channel catfish and European seabass intestine with the incorporation of yeast polysaccharides and chitosan in fish diets, respectively. Apines-Amar et al. (2012) also revealed positive effect on liver histology when marbled groupers were fed with ginger incorporated diet. The extent of damage seen in fishes fed ginger incorporated diet was the minimal at tissue levels such as gill, liver and intestine. It suggests that ginger as an immunostimulant is also benefitting the fishes at systemic level. The gross result of the wellbeing of the fish was also reflected in better survival and the growth rates exhibited by the fishes in ginger treated groups. Since all the feed additives are dose dependent, in the present study too, a ginger powder with a dose of 10 g kg^{-1} was found to be the most

effective in maintaining healthy physiological status of the fishes. Higher dosages might be causing supply of unwanted compounds like saponins resulting in reduced growth and affecting other parameters. The findings of the present study would certainly help the striped catfish famers and the feed manufacturers alike in making use of ginger as an effective immunostimulant to enhance fish production. However, the studies are needed to be confirmed by undertaking the experimental trials at field level before advocating fish farmers.

6.0 SUMMARY

Aquaculture industry is presently facing problem of frequent disease outbreaks as a result of high density rearing in order to increase fish production. Fish diseases were being controlled earlier times using antibiotics. The use of antibiotics evidenced to be deleterious effects on human beings; simultaneously producing disease resistant pathogenic microorganisms. Immunostimulants have proved to be one of the effective alternatives to antibiotics to control fish diseases by enhancing immunity of the aquatic organisms. In the present study, a few of the easily available immunostimulants were tested to assess the health status of one of the popular freshwater catfish species, *Pangasianodon hypophthalmus*, commonly known as striped catfish. This omnivorous air breathing fish variety is well known for its hardy nature, faster growth rate and its acceptance to prepared diets.

The present work was carried out in two separate experiments of 90 days each. Both the experiments were conducted using 20 fiberglass tanks of 400 l capacity with 50 fishes in each tank. The experiments consisted of five treatments and four replicates adopting completely randomised design (CRD). The hatchery reared striped catfish juveniles with a weight range between 4.23 and 5.25 g were used for the experiments. In Experiment 1, effect of four different immunostimulants such as brewer's yeast (F1), vitamin C (F2), vitamin E (F3) and ginger powder (F4) was compared with commercial diet (F0). In Experiment 2, an immunostimulant that resulted in better response, i.e. ginger incorporated diet was tested in various levels of incorporation in the experimental diets. A level of crude protein of around 32 % was maintained in the diets. The health status of the fishes after completion of experimental duration of 90 days was assessed on the basis of growth, hematological, biochemical, immunological and histological studies. A few of the experimental fishes were challenged to pathogenic bacteria, *Aeromonas hydrophila* to assess efficacy of the tested immunostimulants.

Results of Experiment 1 showed that growth parameters such as percentage weight gain, feed conversion ratio, survival rates were significantly affected by experimental diets.

The ginger incorporated diet showed significantly higher percentage weight gain, survival and feed conversion ratio ($P<0.05$). Water quality parameters such as pH, temperature, dissolved oxygen, and alkalinity were periodically analyzed and found to be within the optimum ranges.

Haematological parameters such as haemoglobin, RBC, WBC, Hct, MCV, MCH and MCHC were analyzed at pre- and post-challenge studies. Among all the immunostimulants, the ginger incorporated diet fish groups resulted in significant increase ($P<0.05$) of Hb, RBC, WBC and Hct contents in pre- as well as post-challenge study. Analysis of biochemical parameters such as albumin, globulin, total serum protein, glycogen and glucose was carried out during pre- and post-challenge study. The fishes groups fed ginger incorporated diet revealed significantly ($P<0.05$) higher content of albumin, globulin and the serum protein than that of the other treatments. Immune parameters such as NBT assay, serum lysozyme activity and phagocytic activity were found to be significantly higher ($P<0.05$) in the fishes given ginger diet in pre- and post-challenge tests. Histopathological assessment showed that the fish groups fed ginger diet had relatively lower tissue damage of gills, liver and intestine even after challenge study. The relative percentage of survival was also found to be the highest in the fishes treated with ginger diet. Thus, in the Experiment 1, a ginger incorporated diet showed better growth promoter and immunomodulator property for the juveniles of catfish.

In Experiment 2, a total of five experimental diets with a crude protein level of around 32 % and different concentrations of ginger such as 5g kg^{-1} (F1), 10 g kg^{-1} (F2), 15g kg^{-1} (F3), 20 g kg^{-1} (F4) and F0 as the control without any immunostimulant in the diet. The experiment was conducted in 400 l capacity circular fiberglass tanks with four replicates and five treatments following CRD for a period of 90 days. The results showed that percentage weight gain, specific growth rate and feed conversion ratio were found to be significantly higher ($P<0.05$) in the fish groups fed diet with ginger concentration of 10 g kg^{-1}. Water quality parameters such as pH, water temperature, dissolved oxygen and alkalinity were observed to be within the optimal ranges of environmental parameters.

Haematological studies revealed significantly higher Hb, RBC, WBC and Hct levels in the fishes fed with 10g kg^{-1} ginger diet in pre- and post-challenge tests ($P<0.05$). Biochemical parameters such as albumin, globulin and total serum protein were also found to be significantly higher in the fishes of 10g kg^{-1} ginger incorporated diet in pre- and post-challenge studies ($P<0.05$). The glucose concentration was significantly higher ($P<0.05$) in the control group than that of the remaining groups. No significant difference was observed in glycogen content of liver among the various treatments ($P>0.05$). Immunological parameters such as NBT, lysozyme activity and phagocytic activity in pre- and post-challenge study were found to be significantly higher in the fishes fed with a ginger concentration of 10g kg^{-1} diet ($P<0.05$). There was no significant difference in blood clotting time among the different fish groups ($P>0.05$). The highest percentage survival was also recorded in the fish groups fed 10g kg^{-1}ginger incorporated diet during the challenge study. Histopathological study also revealed less tissue damages of sections of gill, liver and intestine reflecting in relatively better immune response by the fishes fed with 10g kg^{-1} ginger diet.

Thus, on the basis of better growth performance observed in Experiment 1 and Experiment 2, a ginger incorporated diet was found to be suitable for the juvenile rearing of striped catfish. A relatively higher percentage of the observations made on haematological, biochemical and immunological parameters showed significantly better health status of fishes with ginger incorporation of 10 g kg^{-1} diet. At histological assessment, overall fish health was also found to be better in the fish groups fed ginger at 10 g $kg^{-1}$of diet. The findings of the study are expected to help the aquaculturists and the feed manufacturers to make the use of ginger as a natural immunostimulant in feed to enhance the immunity and production potential of striped catfish.

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सारांश

पंगस, स्ट्राइप्ड कॅटफिश, पँगॅसियानोडॉन हायपोथ्यालमस माशांच्या पिल्लांवर त्यांच्या खाद्यांत रोगप्रतिकारक प्रेरके वापरुन त्यांच्या आरोग्यावर होणारा परिणाम दोन विविध प्रयोगातून अभ्यासण्यात आला. सदर अभ्यास प्रयोगशाळेत ४०० लिटर आकारमानाच्या २० फायबरग्लास टाक्या पाच विविध खाद्य आणि चार प्रतित सी. आर. डी. प्रायोगीक संरचना वापरुन प्रत्येकी ९० दिवसांसाठी करण्यात आला. दोन्ही प्रयोगाअंती पंगस माशांची पिल्ले एरोमोनास हायड्रोफिला या रोगकारक जिवाणूच्या संपर्कात आणून माश्यांवरील रोग होण्याच्या प्रभावाची तिव्रता अभ्यासण्यात आली. प्रयोग क्र. १ मध्ये चार विविध रोगप्रतिकारक प्रेरके समाविष्ठ खाद्य ज्यामध्ये ब्रुअर्स यीस्ट, विटॅमीन सी, विटॅमीन इ, सुंठ पावडर आणि व्यवसाय प्रचलित खाद्य हे प्रमाणखाद्य म्हणून वापरले गेले. प्रयोगाअंती, अभ्यासलेल्या खाद्यांपैकी, सुंठमिश्रीत खाद्याने माशांच्या वाढीवर आणि फिड कन्व्हर्जन रेशो मध्ये सांख्यिकी दृष्ट्या ($p<0.05$) वाढीव फरक दर्शविला. त्याच प्रमाणे सदर खाद्याने माशांच्या एचबी, आरबीसी, डब्ल्युबीसी, एचसीटी, सिरम अल्ब्युमीन, ग्लोब्युलीन इ. मध्ये आणि फॅगोसायटीक, लायसोझाइम आणि एनबीटी कार्यक्षमतेत रोगकारक जिवाणूंच्या संपर्कात येण्यापूर्वी आणि आल्यानंतर सांख्यिकी दृष्ट्या ($p<0.05$) लाक्षणिक वाढ दर्शविली. म्हणून प्रयोग क्र. २ साठी सुंठ पावडर विविध चार मात्रेमध्ये (५, १०, १५ आणि २० ग्रॅम प्रति १ किलो खाद्य) माशांच्या खाद्यात समाविष्ठ करुन वापरली गेली आणि प्रमाणखाद्य म्हणून सुंठपावडर विरहित खाद्य अशी पाच खाद्ये चार प्रतित सी. आर. डी. प्रायोगिक संरचेत अभ्यासली गेली. प्रयोगाअंती, माशांचे वजन प्रत्यक्ष वाढीचा दर आणि फिड कन्व्हर्जन रेशो यामध्ये १० ग्रॅम प्रति किलो सुंठमिश्रीत खाद्य वापरलेल्या माशांत सांख्यिकी दृष्ट्या ($p<0.05$) परिणाम कारक जास्त वाढ दिसून आली. माशांचे एचबी, आरबीसी, डब्ल्युबीसी, एचसीटी, सिरम अल्ब्यूमीन, ग्लोब्यूलीन, एनबीटी, फॅगोसायटीक आणि लायसोझाइम कार्यक्षमतेवर जिवाणू संपर्कपूर्व आणि जिवाणूसंपर्क पश्चात रोगप्रतिकारक क्षमतेत १० ग्रॅम सुंठ पावडर प्रति किलो खाद्य दिलेल्या पंगस पिल्लांवर सांख्यिकी दृष्ट्या ($p<0.05$) वाढीव फरक निदर्शनास आला. दोन्ही प्रयोगातील जिवाणू संपर्कपूर्व काळातील माशांच्या पिल्लांचे जगणूकीचे प्रमाण १००% नोंदविले गेले. शारीरीक पेशींच्या संरचनेतील अभ्यासात देखील १० ग्रॅम सुंठपावडर मिश्रीत खाद्यातून योग्य पेशी संरचना निदर्शनास आली. दोन्ही प्रयोगाअंती असे सूचित केले गेले की, १० ग्रॅम प्रतिकिलो सुंठमिश्रीत खाद्य पंगस माशांच्या खाद्यात समाविष्ठ केल्यास त्यांच्या वाढीवर, जगणूकीवर आणि सर्वसाधारण रोगप्रतिकारक क्षमतेत लक्षणीय वाढ होते.

ABSTRACT

Effect of immunostimulants on health status of juvenile striped catfish, *Pangasianodon hypophthalmus* was analysed in two separate experiments followed by challenging the fishes to bacterial pathogen, *Aeromonas hydrophila*. Both the experiments were conducted in 20 fiberglass tanks each of 400 l capacity with 50 fishes per tank. The experiments were subjected to five treatments and four replicates using completely randomised design (CRD). Experimental duration was 90 days in both the experiments. In Experiment 1, effect of four different immunostimulants such as brewer's yeast, vitamin C, vitamin E and ginger powder was compared with commercial diet as control. Results showed that of the five tested diets, ginger incorporated diet showed significantly better percentage weight gain and feed conversion ratio ($P<0.05$) as compared to other tested diets. However, the survival percentage was higher in all the tested diets. Dietary ginger significantly increased Hb, RBC, WBC, Hct, serum protein, albumin, globulin, phagocytic, lysozyme activity and NBT assay in the pre- as well as post-challenge study ($P<0.05$). In Experiment 2, a ginger incorporated diet that resulted in better response in experiment 1 was tested in various levels of incorporation such as 5, 10, 15 and 20 g kg^{-1} in the experimental diets. Control diet was kept without any immunostimulant added in the basal diet. The percentage weight gain and specific growth rate were observed higher in ginger diet with incorporation level of 10 g kg^{-1} diet, while the percentage survival was higher in all levels of incorporation of ginger. Similarly, the diet also showed significant increase ($P<0.05$) feed conversion ratio in the fishes as compared to control. Pre- and post-challenge studies also revealed significantly better Hb, RBC,WBC, Hct, total serum protein, albumin, globulin, NBT assay, phagocytic and lysozyme activity in fishes fed dietary ginger at 10 g kg^{-1} diet ($P<0.05$). Histologocal sections of tissues of the fishes fed ginger incorporated diet also showed better arrangement of the cells both in pre and post-challenge studies. The study suggested that a dietary level of ginger powder of 10 g kg^{-1} diet is beneficial for the growth, survival and to enhance overall immunity of striped catfish juveniles.

Table 3 Water quality parameters of Experiment 1

Sr. No.	Water quality parameters	Range
1.	Temperature (°C)	23 - 25
2.	pH	7.0 – 7.5
3.	Total alkalinity (mg L^{-1} as $CaCO_3$)	75 - 82
4.	Dissolved oxygen (mg L^{-1})	5.6 - 6.2

Table 4 Proximate composition of test diets in the Experiment 1

Parameters	F0	F1	F2	F3	F4
Protein	32.21	32.50	32.25	32.37	32.35
Fat	5.42	5.73	5.56	5.31	5.63
Fibre	14.83	15.01	14.86	14.78	14.56
Ash	6.70	6.63	6.60	6.80	7.09
Moisture	9.1	9.3	10.01	9.8	9.5

Table 5 Carcass composition of striped catfish fed different test diets

Parameters	F0	F1	F2	F3	F4
Protein	64.28	64.96	64.85	63.96	64.97
Fat	8.13	8.16	7.91	8.15	8.17
Fibre	0.81	0.53	0.61	0.58	0.63
Ash	8.43	9.31	8.98	8.86	8.73
Moisture	11.20	10.84	10.82	10.75	10.74

Table 6 Tukey's Studentized Range (HSD) test results showing effect of immunostimulants on growth parameters of striped catfish

Treatments	Weight gain (%)	Length gain (%)	Specific growth rate	Feed conversion ratio
F0	219.05^{a}±1.59	78.45^{a}±2.46	1.28^{a}±0.00	1.89^{b}±0.01
F1	228.58^{ab}±3.57	82.90^{a}±3.53	1.30^{ab}±0.01	1.89^{b}±0.02
F2	234.68^{b}±2.40	83.05^{a}±2.98	1.31^{b}±0.00	1.88^{b}±0.02
F3	235.35^{b}±2.79	83.86^{ab}±3.16	1.32^{b}±0.01	1.87^{b}±0.02
F4	283.73^{c}±4.02	96.35^{b}±2.48	1.41^{c}±0.01	1.71^{a}±0.01

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 7 ANOVA for effect of immunostimulants on growth parameters of striped catfish

Parameters	Source	DF	Type III SS	Mean Square	F-Value	Pr > F
Weight gain	Treatment	4	10123.61	2530.90	70.34	<.0001
Length gain		4	724.46	181.11	5.20	0.0078
Specific growth rate		4	0.04	0.01	80.73	<.0001
Feed conversion ratio		4	0.10	0.02	23.05	<.0001

Table 8 Haematological parameters for striped catfish fed different immunostimulants incorporated diets in pre-challenge study

Parameters	Treatments	Pre-challenge
Hb (g dl^{-1})	F0	5.63^{a}±0.07
	F1	7.51^{c}±0.10
	F2	7.22^{c}±0.09
	F3	6.58^{b}±0.08
	F4	9.54^{d}±0.18
RBC (× 10^{6} $mm^{-3)}$	F0	1.12^{a}±0.03
	F1	1.72^{d}±0.04
	F2	1.60^{c}±0.02
	F3	1.46^{b}±0.02
	F4	2.34^{e}±0.03
WBC (× 10^{3} $mm^{-3)}$	F0	65.60^{a}±1.04
	F1	79.78^{b}±1.38
	F2	80.02^{b}±0.71
	F3	80.83^{b}±0.67
	F4	83.17^{b}±0.38
HCT (%)	F0	19.95^{a}±0.14
	F1	22.28^{b}±0.16
	F2	22.76^{b}±0.23
	F3	22.09^{b}±0.16
	F4	24.93^{c}±0.59
MCV (µ3)	F0	179.82^{d}±4.65
	F1	130.03^{b}±3.37
	F2	142.91^{c}±2.73
	F3	151.22^{c}±2.29
	F4	106.55^{a}±2.37
MCH (pg)	F0	50.69^{c}±1.35
	F1	43.86^{ab}±1.33
	F2	45.25^{b}±0.54
	F3	44.98^{b}±0.63
	F4	40.75^{a}±0.57
MCHC (g dl^{-1})	F0	28.19^{a}±0.20
	F1	33.73^{b}±0.50
	F2	31.75^{ab}±0.52
	F3	29.78^{ab}±0.44
	F4	38.45^{c}±1.02

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 9 Haematological parameters for striped catfish fed different immunostimulants incorporated diets in post-challenge study

Parameters	Treatments	Post-challenge
Hb (g dl^{-1})	F0	4.52^{a} ± 0.09
	F1	6.42^{c}±0.13
	F2	6.15^{c}±0.08
	F3	5.58^{b}±0.15
	F4	7.80^{d}±0.11
RBC (× 10^{6} $mm^{-3)}$	F0	0.89^{a}±0.01
	F1	1.40^{c}±0.03
	F2	1.32^{c}±0.02
	F3	1.14^{b}±0.05
	F4	1.97^{d}±0.040
WBC (× 10^{3} $mm^{-3)}$	F0	67.60^{a}±0.92
	F1	82.30^{b}±0.66
	F2	82.27^{b}±0.29
	F3	82.18^{b}±0.31
	F4	86.47^{c}±0.27
HCT (%)	F0	18.57^{a}±0.32
	F1	20.95^{b}±0.25
	F2	20.48^{b}±0.24
	F3	22.13^{c}±0.31
	F4	22.05^{c}±0.15
MCV (µ3)	F0	209.80^{c}±5.76
	F1	149.43^{b}±1.60
	F2	155.37^{b}±3.38
	F3	195.37^{c}±7.74
	F4	112.42^{a}±2.32
MCH (pg)	F0	50.97^{c}±1.21
	F1	45.77^{b}±0.65
	F2	46.63^{bc}±0.97
	F3	49.23^{bc}±2.04
	F4	39.73^{a}±0.65
MCHC (g dl^{-1})	F0	24.40^{a}±0.84
	F1	30.63^{b}±0.53
	F2	30.02^{b}±0.24
	F3	25.27^{a}±0.74
	F4	35.37^{c}±0.40

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 10 Haematological parameters for striped catfish fed different immunostimulants incorporated diets in pre- and post-challenge study

Parameters	Treatments	Between pre- and post-challenge (P-value)
Hb (g dl^{-1})	F0	<.0001
	F1	<.0001
	F2	<.0001
	F3	<.0001
	F4	<.0001
RBC (× 10^6 $mm^{-3)}$	F0	0.0002
	F1	<.0001
	F2	<.0001
	F3	<.0001
	F4	<.0001
WBC (× 10^3 $mm^{-3)}$	F0	0.8960
	F1	0.6936
	F2	0.8112
	F3	0.9915
	F4	0.3159
HCT (%)	F0	0.1177
	F1	0.1560
	F2	0.0003
	F3	1.0000
	F4	<.0001
MCV (µ3)	F0	<.0001
	F1	0.0291
	F2	0.4573
	F3	<.0001
	F4	0.9883
MCH (pg)	F0	1.0000
	F1	0.9742
	F2	0.9971
	F3	0.2193
	F4	0.9998
MCHC (g dl^{-1})	F0	0.0052
	F1	0.0501
	F2	0.7287
	F3	0.0003
	F4	0.0525

Table 11 Biochemical parameters for striped catfish fed different immunostimulants incorporated diets in pre-challenge study

Parameters	Treatments	Pre-challenge
Total serum protein ($g\ dl^{-1}$)	F0	$1.60^{a}\pm0.02$
	F1	$2.15^{c}\pm0.03$
	F2	$2.29^{d}\pm0.02$
	F3	$1.81^{b}\pm0.01$
	F4	$3.20^{e}\pm0.02$
Serum albumin ($g\ dl^{-1}$)	F0	$0.66^{a}\pm0.01$
	F1	$0.71^{a}\pm0.02$
	F2	$0.85^{b}\pm0.01$
	F3	$0.68^{ab}\pm0.03$
	F4	$1.08^{c}\pm0.04$
Serum globulin ($g\ dl^{-1}$)	F0	$0.93^{a}\pm0.01$
	F1	$1.43^{c}\pm0.04$
	F2	$1.44^{c}\pm0.01$
	F3	$1.14^{b}\pm0.02$
	F4	$2.11^{d}\pm0.04$
Serum glucose ($mg\ dl^{-1}$)	F0	$130.33^{a}\pm0.63$
	F1	$130.36^{a}\pm0.55$
	F2	$135.88^{b}\pm0.80$
	F3	$130.28^{a}\pm0.49$
	F4	$128.08^{a}\pm0.64$
Glycogen ($mg\ g^{-1}$)	F0	$70.74^{a}\pm0.11$
	F1	$70.70^{a}\pm0.17$
	F2	$70.71^{a}\pm0.17$
	F3	$70.73^{a}\pm0.16$
	F4	$70.72^{a}\pm0.22$

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 12 Biochemical parameters for striped catfish fed different immunostimulants incorporated diets in post-challenge study

Parameters	Treatments	Post-challenge
Total serum protein ($g\ dl^{-1}$)	F0	$1.29^{a}\pm0.02$
	F1	$1.83^{c}\pm0.02$
	F2	$2.04^{d}\pm0.03$
	F3	$1.67^{b}\pm0.01$
	F4	$2.97^{e}\pm0.02$
Serum albumin ($g\ dl^{-1}$)	F0	$0.47^{a}\pm0.02$
	F1	$0.68^{b}\pm0.02$
	F2	$0.81^{c}\pm0.01$
	F3	$0.66^{b}\pm0.00$
	F4	$1.01^{d}\pm0.01$
Serum globulin ($g\ dl^{-1}$)	F0	$0.82^{a}\pm0.01$
	F1	$1.14^{c}\pm0.03$
	F2	$1.24^{d}\pm0.02$
	F3	$1.01^{b}\pm0.01$
	F4	$1.96^{e}\pm0.03$
Serum glucose ($mg\ dl^{-1}$)	F0	$128.05^{b}\pm0.36$
	F1	$127.23^{ab}\pm0.74$
	F2	$133.83^{c}\pm0.53$
	F3	$128.20^{b}\pm0.45$
	F4	$125.58^{a}\pm0.38$
Glycogen ($mg\ g^{-1}$)	F0	$69.25^{a}\pm0.11$
	F1	$69.29^{a}\pm0.21$
	F2	$69.21^{a}\pm0.23$
	F3	$69.25^{a}\pm0.26$
	F4	$69.23^{a}\pm0.22$

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 13 Biochemical parameters for striped catfish fed different immunostimulants incorporated diets in pre- and post-challenge study

Parameters	Treatments	Between pre- and post-challenge (P-value)
Total serum protein ($g\ dl^{-1}$)	F0	<.0001
	F1	<.0001
	F2	<.0001
	F3	0.0032
	F4	<.0001
Serum albumin ($g\ dl^{-1}$)	F0	<.0001
	F1	0.9971
	F2	0.9801
	F3	1.0000
	F4	0.7503
Serum globulin ($g\ dl^{-1}$)	F0	0.1730
	F1	<.0001
	F2	0.0003
	F3	0.0634
	F4	0.0172
Serum glucose ($mg\ dl^{-1}$)	F0	0.3670
	F1	0.0546
	F2	0.5295
	F3	0.5061
	F4	0.2496
Glycogen ($mg\ g^{-1}$)	F0	<.0001
	F1	0.0002
	F2	<.0001
	F3	<.0001
	F4	<.0001

Table 14 Immunological parameters for striped catfish fed different immunostimulants incorporated diets in pre-challenge study

Parameters	Treatments	Pre-challenge
NBT (OD/540nm)	F0	0.15^{a}±0.01
	F1	2.36^{d}±0.15
	F2	1.30^{c}±0.04
	F3	0.85^{b}±0.06
	F4	3.25^{e}±0.08
Lysozyme activity ($U\ min^{-1}$)	F0	0.32^{a}±0.02
	F1	1.03^{c}±0.04
	F2	0.89^{b}±0.03
	F3	0.82^{b}±0.02
	F4	1.43^{d}±0.03
Phagocytic activity (%)	F0	11.42^{a}±0.10
	F1	17.29^{c}±0.14
	F2	13.97^{b}±0.16
	F3	13.30^{b}±0.16
	F4	19.89^{d}±0.29
Clotting time (s)	F0	36.58^{a}±0.23
	F1	36.67^{a}±0.22
	F2	36.33^{a}±0.14
	F3	36.92^{a}±0.23
	F4	36.83^{a}±0.21

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 15 Immunological parameters for striped catfish fed different immunostimulants incorporated diets in post-challenge study

Parameters	Treatments	Post-challenge
NBT (OD/540nm)	F0	0.12^{a}±0.00
	F1	0.76^{d}±0.01
	F2	0.61^{c}±0.03
	F3	0.46^{b}±0.01
	F4	2.71^{e}±0.04
Lysozyme activity ($U\ min^{-1}$)	F0	0.13^{a}±0.00
	F1	0.90^{c}±0.02
	F2	0.65^{b}±0.04
	F3	0.73^{b}±0.03
	F4	1.16^{d}±0.03
Phagocytic activity (%)	F0	15.01^{a}±0.06
	F1	18.91^{c}±0.22
	F2	16.98^{b}±0.16
	F3	16.82^{b}±0.14
	F4	22.46^{d}±0.25
Clotting time (s)	F0	30.67^{a}±0.33
	F1	31.50^{ab}±0.34
	F2	31.83^{ab}±0.31
	F3	31.83^{ab}±0.31
	F4	32.33^{b}±0.21

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 16 Immunological parameters for striped catfish fed different immunostimulants incorporated diets in pre- and post-challenge study

Parameters	Treatments	Between pre- and post-challenge (P-value)
NBT (OD/540nm)	F0	1.0000
	F1	<.0001
	F2	<.0001
	F3	0.0500
	F4	0.0008
Lysozyme activity ($U\ min^{-1}$)	F0	0.0047
	F1	0.1391
	F2	0.0001
	F3	0.6993
	F4	<.0001
Phagocytic activity (%)	F0	<.0001
	F1	<.0001
	F2	<.0001
	F3	<.0001
	F4	<.0001
Clotting time (s)	F0	<.0001
	F1	<.0001
	F2	<.0001
	F3	<.0001
	F4	<.0001

Table 17 ANOVA for effect of different immunostimulants on haematological, biochemical and immunological parameters of striped catfish in pre-challenge study

(a) Haematological parameters						
Parameters	Source	DF	Type III SS	Mean Square	F value	Pr > F
Hb	Stimulant	4	100.88	25.22	175.13	<.0001
RBC		4	9.67	2.42	246.72	<.0001
WBC		4	2347.89	586.97	59.99	<.0001
Hct		4	151.91	37.98	33.79	<.0001
MCV		4	34995.52	8748.88	71.08	<.0001
MCH		4	620.76	155.19	14.03	<.0001
MCHC		4	760.89	190.22	43.96	<.0001
(b) Biochemical parameters						
Total protein	Stimulant	4	18.17	4.54	744.72	<.0001
Albumin		4	1.44	0.36	46.80	<.0001
Globulin		4	9.54	2.39	266.21	<.0001
Glucose		4	404.42	101.11	21.21	<.0001
Glycogen		4	0.01	0.00	0.01	0.9999
(c) Immunological parameters						
NBT	Stimulant	4	72.62	18.15	221.67	<.0001
Lysozyme activity		4	7.73	1.93	175.23	<.0001
Phagocytic activity		4	549.29	137.32	349.79	<.0001
Clotting time		4	2.50	0.63	1.19	0.3246

Table 18 ANOVA for effect of different immunostimulants on haematological, biochemical and immunological parameters of striped catfish in post-challenge study

(a) Haematological parameters						
Parameters	Source	DF	Type III SS	Mean Square	F value	Pr > F
Hb	**Stimulant**	4	34.60	8.65	111.46	<.0001
RBC		4	3.84	0.96	157.96	<.0001
WBC		4	1263.83	315.96	171.07	<.0001
Hct		4	50.66	12.67	31.34	<.0001
MCV		4	36167.61	9041.90	67.00	<.0001
MCH		4	442.56	110.64	12.47	<.0001
MCHC		4	475.44	118.86	57.78	<.0001
(b) Biochemical parameters						
Total protein	**Stimulant**	4	9.49	2.37	849.15	<.0001
Albumin		4	0.95	0.24	196.44	<.0001
Globulin		4	4.56	1.14	421.58	<.0001
Glucose		4	232.90	58.22	37.45	<.0001
Glycogen		4	0.02	0.01	0.02	0.9989
(c) Immunological parameters						
NBT	**Stimulant**	4	24.98	6.24	2026.01	<.0001
Lysozyme activity		4	3.50	0.87	183.62	<.0001
Phagocytic activity		4	192.41	48.10	252.63	<.0001
Clotting time		4	9.13	2.28	4.13	0.0106

Table 19 ANOVA for effect of different immunostimulants on haematological, biochemical and immunological parameters of striped catfish in pre- and post-challenge study

(a) Haematological parameters						
Parameters	Source	DF	Type III SS	Mean Square	F value	Pr > F
Hb	Stimulant	9	164.28	18.25	148.10	<.0001
RBC		9	15.37	1.71	197.78	<.0001
WBC		9	3715.10	412.89	56.53	<.0001
Hct		9	251.56	27.95	31.09	<.0001
MCV		9	81154.79	9017.20	71.12	<.0001
MCH		9	1101.01	122.33	11.79	<.0001
MCHC		9	1449.50	161.06	44.50	<.0001
(b) Biochemical parameters						
Total protein	Stimulant	9	28.91	3.21	634.08	<.0001
Albumin		9	14.74	1.64	233.73	<.0001
Globulin		9	2.49	0.28	48.88	<.0001
Glucose		9	752.84	83.65	22.23	<.0001
Glycogen		9	43.51	4.83	14.72	<.0001
(c) Immunological parameters						
NBT	Stimulant	9	106.02	11.78	205.71	<.0001
Lysozyme activity		9	11.91	1.32	145.97	<.0001
Phagocytic activity		9	905.57	100.62	302.59	<.0001
Clotting time		9	517.32	57.59	107.98	<.0001

Table 20 Relative percentage survival of striped catfish in different immunostimulant groups after challenge study

Treatments	Relative percentage of survival (%)
F0	$48.75^{a} \pm 2.34$
F1	$83.75^{c} \pm 1.53$
F2	$77.50^{b} \pm 1.53$
F3	$76.25^{b} \pm 1.25$
F4	$93.75^{d} \pm 0.00$

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 21 Water quality parameters in Experiment 2

Sr. No.	Water quality parameters	Range
1.	Temperature (°C)	22 - 23
2.	pH	7.0 – 7.5
3.	Total alkalinity (mg L^{-1} as $CaCO_3$)	75 - 88
4.	Dissolved oxygen (mg L^{-1})	4.8 – 6.0

Table 22 Proximate composition of test diets in the Experiment 2

Parameters	F0	F1	F2	F3	F4
Protein	32.01	32.35	32.45	32.31	32.26
Fat	5.73	5.89	5.67	5.71	5.58
Fibre	14.86	14.91	14.83	14.87	14.67
Ash	6.63	6.59	6.71	6.78	6.69
Moisture	10.10	10.33	10.21	10.26	9.85

Table 23 Carcass composition of striped catfish fed different doses of ginger

Parameters	F0	F1	F2	F3	F4
Protein	63.91	64.01	64.13	64.08	63.97
Fat	8.16	7.98	8.13	8.19	7.91
Fibre	0.91	0.87	0.93	0.89	0.91
Ash	8.53	8.67	8.59	8.71	8.63
Moisture	10.81	10.67	11.10	10.69	11.01

Table 24 Tukey's Studentized Range (HSD) test results showing effect of different doses of ginger on growth parameters of striped catfish

Treatments	Weight gain (%)	Length gain (%)	Specific growth rate	Feed conversion ratio
F0	279.71^{a}±4.38	88.44^{a}±13.30	3.81^{a}±0.02	1.88^{b}±0.01
F1	320.20^{b}±4.47	95.75^{ab}±1.09	4.01^{b}±0.02	1.86^{ab}±0.01
F2	352.20^{c}±5.44	119.53^{b}±2.50	4.15^{c}±0.02	1.82^{a}±0.02
F3	317.18^{b}±8.56	100.18^{ab}±2.74	3.98^{b}±0.02	1.87^{ab}±0.02
F4	311.00^{b}±5.06	91.68^{a}±3.13	3.96^{b}±0.03	1.83^{ab}±0.03

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 25 ANOVA for effect of different doses of ginger on growth parameters of striped catfish

Parameters	Source	DF	Type III SS	Mean Square	F-Value	Pr > F
Weight gain	Treatment	4	10683.56	2670.89	19.90	<.0001
Length gain		4	2393.82	598.45	3.70	0.0274
Specific growth rate		4	0.25	0.06	34.11	<.0001
Feed conversion rate		4	0.01	0.003	3.61	0.03

Table 26 Haematological parameters for striped catfish fed different doses of ginger in pre-challenge study

Parameters	Treatments	Pre-challenge
Hb (g dl^{-1})	F0	6.11^{a}±0.07
	F1	6.98^{b}±0.04
	F2	8.05^{d}±0.05
	F3	7.58^{c}±0.03
	F4	7.62^{c}±0.05
RBC ($\times 10^{6}$ mm^{-3})	F0	1.04^{a}±0.03
	F1	1.19^{ab}±0.01
	F2	2.01^{c}±0.07
	F3	1.37^{b}±0.02
	F4	1.34^{ab}±0.04
WBC ($\times 10^{3}$ mm^{-3})	F0	66.28^{a}±1.32
	F1	77.65^{b}±0.97
	F2	84.53^{c}±0.36
	F3	78.94^{b}±0.59
	F4	79.03^{b}±0.84
HCT (%)	F0	20.93^{a}±0.14
	F1	24.03^{b}±0.53
	F2	26.94^{c}±0.34
	F3	24.67^{b}±0.47
	F4	24.36^{b}±0.55
MCV (µ3)	F0	203.36^{c}±5.71
	F1	203.01^{c}±5.17
	F2	135.99^{a}±5.29
	F3	179.69^{b}±2.87
	F4	184.36^{bc}±7.14
MCH (pg)	F0	59.32^{b}±1.59
	F1	58.99^{b}±0.62
	F2	40.57^{a}±1.36
	F3	55.26^{b}±0.72
	F4	57.67^{b}±1.94
MCHC (g dl^{-1})	F0	29.21^{a}±0.37
	F1	29.21^{a}±0.62
	F2	29.93^{a}±0.43
	F3	30.82^{a}±0.56
	F4	31.48^{a}±0.86

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 27 Haematological parameters for striped catfish fed different doses of ginger in post-challenge study

Parameters	Treatments	Post-challenge
Hb (g dl^{-1})	F0	5.73^{a}±0.05
	F1	6.65^{b}±0.04
	F2	7.67^{d}±0.03
	F3	7.05^{c}±0.08
	F4	6.95^{c}±0.10
RBC ($\times 10^{6}$ $mm^{-3)}$	F0	0.89^{a}±0.03
	F1	1.04^{b}±0.04
	F2	1.84^{c}±0.03
	F3	1.11^{b}±0.03
	F4	1.16^{b}±0.02
WBC ($\times 10^{3}$ $mm^{-3)}$	F0	69.58^{a}±0.42
	F1	79.63^{b}±0.55
	F2	86.50^{c}±0.46
	F3	79.92^{b}±0.38
	F4	79.63^{b}±0.42
HCT (%)	F0	18.05^{a}±0.30
	F1	22.72^{b}±0.28
	F2	24.55^{c}±0.24
	F3	23.22^{b}±0.18
	F4	22.92^{b}±0.30
MCV (µ3)	F0	203.84^{b}±7.63
	F1	220.05^{b}±7.20
	F2	133.76^{a}±2.61
	F3	209.73^{b}±5.02
	F4	197.21^{b}±3.56
MCH (pg)	F0	64.73^{b}±2.11
	F1	64.49^{b}±2.41
	F2	41.80^{a}±1.00
	F3	63.73^{b}±1.92
	F4	59.84^{b}±1.44
MCHC (g dl^{-1})	F0	31.80^{b}±0.52
	F1	29.30^{a}±0.43
	F2	31.25^{b}±0.36
	F3	30.38^{ab}±0.48
	F4	30.34^{ab}±0.42

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 28 Haematological parameters for striped catfish fed different doses of ginger in Pre- and post-challenge study

Parameters	Treatments	Between pre- and post-challenge (P-value)
Hb (g dl^{-1})	F0	0.0007
	F1	0.0044
	F2	0.0005
	F3	<.0001
	F4	<.0001
RBC ($\times 10^6$ $mm^{-3)}$	F0	0.3355
	F1	0.3355
	F2	0.1528
	F3	0.0017
	F4	0.1393
WBC ($\times 10^3$ $mm^{-3)}$	F0	0.2583
	F1	0.8762
	F2	0.8789
	F3	0.9990
	F4	0.9998
HCT (%)	F0	0.0012
	F1	0.5919
	F2	0.0144
	F3	0.4441
	F4	0.4526
MCV (µ3)	F0	1.0000
	F1	0.6235
	F2	1.0000
	F3	0.0274
	F4	0.8943
MCH (pg)	F0	0.3738
	F1	0.3497
	F2	0.9999
	F3	0.0150
	F4	0.9945
MCHC (g dl^{-1})	F0	0.1307
	F1	1.0000
	F2	0.9066
	F3	1.0000
	F4	0.9597

Table 29 Biochemical parameters for striped catfish fed different doses of ginger in pre-challenge study

Parameters	Treatments	Pre-challenge
Total serum protein ($g\ dl^{-1}$)	F0	$1.55^{a}\pm0.02$
	F1	$1.98^{b}\pm0.02$
	F2	$3.06^{d}\pm0.04$
	F3	$2.16^{c}\pm0.04$
	F4	$2.08^{bc}\pm0.01$
Serum albumin ($g\ dl^{-1}$)	F0	$0.62^{a}\pm0.02$
	F1	$0.85^{b}\pm0.02$
	F2	$1.12^{c}\pm0.04$
	F3	$0.93^{b}\pm0.01$
	F4	$0.90^{b}\pm0.01$
Serum globulin ($g\ dl^{-1}$)	F0	$0.93^{a}\pm0.03$
	F1	$1.13^{b}\pm0.02$
	F2	$1.95^{d}\pm0.04$
	F3	$1.25^{c}\pm0.02$
	F4	$1.18^{bc}\pm0.02$
Serum glucose ($mg\ dl^{-1}$)	F0	$130.54^{c}\pm0.38$
	F1	$130.28^{c}\pm0.27$
	F2	$126.80^{b}\pm0.20$
	F3	$124.48^{a}\pm0.27$
	F4	$124.35^{a}\pm0.40$
Glycogen ($mg\ g^{-1}$)	F0	$70.82^{a}\pm0.14$
	F1	$70.90^{a}\pm0.18$
	F2	$70.98^{a}\pm0.15$
	F3	$70.80^{a}\pm0.16$
	F4	$70.41^{a}\pm0.19$

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 30 Biochemical parameters for striped catfish fed different doses of ginger in post-challenge study

Parameters	Treatments	Post-challenge
Total serum protein ($g\ dl^{-1}$)	F0	$1.09^{a}\pm0.03$
	F1	$1.19^{a}\pm0.01$
	F2	$2.03^{d}\pm0.02$
	F3	$1.71^{c}\pm0.04$
	F4	$1.59^{b}\pm0.02$
Serum albumin ($g\ dl^{-1}$)	F0	$0.50^{a}\pm0.01$
	F1	$0.52^{a}\pm0.02$
	F2	$0.85^{c}\pm0.03$
	F3	$0.67^{b}\pm0.02$
	F4	$0.61^{b}\pm0.01$
Serum globulin ($g\ dl^{-1}$)	F0	$0.60^{a}\pm0.02$
	F1	$0.67^{a}\pm0.01$
	F2	$1.18^{c}\pm0.04$
	F3	$1.04^{b}\pm0.05$
	F4	$0.98^{b}\pm0.03$
Serum glucose ($mg\ dl^{-1}$)	F0	$127.47^{c}\pm0.19$
	F1	$126.92^{c}\pm0.24$
	F2	$123.77^{b}\pm0.29$
	F3	$121.92^{a}\pm0.19$
	F4	$121.75^{a}\pm0.25$
Glycogen ($mg\ g^{-1}$)	F0	$67.95^{a}\pm0.29$
	F1	$68.00^{a}\pm0.08$
	F2	$68.43^{a}\pm0.45$
	F3	$67.32^{a}\pm0.34$
	F4	$67.34^{a}\pm0.37$

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 31 Biochemical parameters for striped catfish fed different doses of ginger in pre-and post-challenge study

Parameters	Treatments	Between pre- and post-challenge (P-value)
Total serum protein ($g\ dl^{-1}$)	F0	<.0001
	F1	<.0001
	F2	<.0001
	F3	<.0001
	F4	<.0001
Serum albumin ($g\ dl^{-1}$)	F0	0.0253
	F1	<.0001
	F2	<.0001
	F3	<.0001
	F4	<.0001
Serum globulin ($g\ dl^{-1}$)	F0	<.0001
	F1	<.0001
	F2	<.0001
	F3	0.0010
	F4	0.0020
Serum glucose ($mg\ dl^{-1}$)	F0	<.0001
	F1	<.0001
	F2	<.0001
	F3	<.0001
	F4	<.0001
Glycogen ($mg\ g^{-1}$)	F0	<.0001
	F1	<.0001
	F2	<.0001
	F3	<.0001
	F4	<.0001

Table 32 Immunological parameters for striped catfish fed different doses of ginger in pre- challenge study

Parameters	Treatments	Pre-challenge
NBT (OD/540nm)	F0	0.20^{a}±0.01
	F1	1.55^{b}±0.04
	F2	2.12^{c}±0.11
	F3	1.74^{b}±0.02
	F4	1.66^{b}±0.01
Lysozyme activity ($U\ min^{-1}$)	F0	0.24^{a}±0.01
	F1	0.79^{b}±0.06
	F2	1.44^{d}±0.02
	F3	0.92^{c}±0.01
	F4	0.81^{bc}±0.01
Phagocytic activity (%)	F0	11.23^{a}±0.11
	F1	14.44^{b}±0.24
	F2	19.57^{d}±0.37
	F3	16.89^{c}±0.19
	F4	15.48^{b}±0.50
Clotting time (s)	F0	35.75^{a}±0.22
	F1	36.00^{a}±0.21
	F2	36.00^{a}±0.28
	F3	36.08^{a}±0.19
	F4	35.83^{a}±0.21

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 33 Immunological parameters for striped catfish fed different doses of ginger in post- challenge study

Parameters	Treatments	Post-challenge
NBT (OD/540nm)	F0	0.11^{a}±0.00
	F1	1.00^{b}±0.03
	F2	1.67^{d}±0.03
	F3	1.45^{c}±0.02
	F4	1.39^{c}±0.01
Lysozyme activity ($U\ min^{-1}$)	F0	0.11^{a}±0.00
	F1	0.58^{b}±0.02
	F2	1.00^{d}±0.03
	F3	0.76^{c}±0.01
	F4	0.63^{b}±0.02
Phagocytic activity (%)	F0	12.94^{a}±0.10
	F1	15.81^{b}±0.13
	F2	21.38^{e}±0.21
	F3	18.03^{d}±0.16
	F4	16.90^{c}±0.37
Clotting time (s)	F0	30.83^{a}±0.31
	F1	31.33^{a}±0.33
	F2	33.00^{b}±0.26
	F3	32.00^{ab}±0.26
	F4	31.67^{a}±0.33

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table 34 Immunological parameters for striped catfish fed different doses of ginger in pre- and post-challenge study

Parameters	Treatments	Between pre- and post-challenge (P-value)
NBT (OD/540nm)	F0	0.9762
	F1	<.0001
	F2	<.0001
	F3	0.0088
	F4	0.0191
Lysozyme activity ($U\ min^{-1}$)	F0	0.1927
	F1	0.0012
	F2	<.0001
	F3	0.0297
	F4	0.0082
Phagocytic activity (%)	F0	0.0194
	F1	0.1308
	F2	0.0107
	F3	0.3436
	F4	0.1029
Clotting time (s)	F0	<.0001
	F1	<.0001
	F2	<.0001
	F3	<.0001
	F4	<.0001

Table 35 ANOVA for effect of different doses of ginger on haematological, biochemical and immunological parameters of striped catfish in pre-challenge study

(a) Haematological parameters						
Parameters	Source	DF	Type III SS	Mean Square	F value	Pr > F
Hb	Stimulant	4	27.04	6.80	239.08	<.0001
RBC		4	6.64	1.66	86.84	<.0001
WBC		4	2154.77	538.69	58.30	<.0001
Hct		4	222.17	55.54	24.50	<.0001
MCV		4	36272.86	9068.21	25.77	<.0001
MCH		4	2974.01	743.50	34.17	<.0001
MCHC		4	48.44	12.11	2.88	0.0307
(b) Biochemical parameters						
Total protein	Stimulant	4	14.68	3.67	485.97	<.0001
Albumin		4	1.55	0.39	65.95	<.0001
Globulin		4	7.23	1.81	195.90	<.0001
Glucose		4	435.53	108.88	92.48	<.0001
Glycogen		4	2.32	0.58	1.78	0.1460
(c) Immunological parameters						
NBT	Stimulant	4	25.93	6.48	202.03	<.0001
Lysozyme activity		4	8.87	2.22	188.90	<.0001
Phagocytic activity		4	455.30	113.82	95.48	<.0001
Clotting time		4	0.90	0.23	0.38	0.82

Table 36 ANOVA for effect of different doses of ginger on haematological, biochemical and immunological parameters of striped catfish in post-challenge study

(a) Haematological parameters						
Parameters	Source	DF	Type III SS	Mean Square	F value	Pr > F
Hb	Stimulant	4	11.98	3.00	122.36	<.0001
RBC		4	3.23	0.81	148.47	<.0001
WBC		4	880.75	220.19	184.24	<.0001
Hct		4	147.11	36.78	88.79	<.0001
MCV		4	27935.41	6983.85	37.61	<.0001
MCH		4	2291.83	572.96	28.08	<.0001
MCHC		4	22.00	5.50	4.60	0.0064
(b) Biochemical parameters						
Total protein	Stimulant	4	3.54	0.88	217.72	<.0001
Albumin		4	0.48	0.12	57.66	<.0001
Globulin		4	1.50	0.37	59.77	<.0001
Glucose		4	175.93	43.98	131.87	<.0001
Glycogen		4	5.38	1.35	2.09	0.1119
(c) Immunological parameters						
NBT	Stimulant	4	9.09	2.27	684.30	<.0001
Lysozyme activity		4	2.57	0.64	322.22	<.0001
Phagocytic activity		4	229.09	57.27	200.97	<.0001
Clotting time		4	15.87	3.97	7.35	0.0005

Table 37 ANOVA for effect of different doses of ginger on haematological, biochemical and immunological parameters of striped catfish in pre- and post-challenge study

(a) Haematological parameters						
Parameters	Source	DF	Type III SS	Mean Square	F value	Pr > F
Hb	Stimulant	9	43.18	4.80	177.17	<.0001
RBC		9	10.52	1.17	78.76	<.0001
WBC		9	3100.71	344.52	51.22	<.0001
Hct		9	440.98	49.00	29.03	<.0001
MCV		9	66916.06	7435.12	24.79	<.0001
MCH		9	5681.38	631.26	29.59	<.0001
MCHC		9	75.09	8.34	2.56	0.0122
(b) Biochemical parameters						
Total protein	Stimulant	9	26.60	2.96	457.48	<.0001
Serum albumin		9	3.35	0.37	79.04	<.0001
Serum globulin		9	11.84	1.32	158.42	<.0001
Serum glucose		9	782.97	87.00	95.22	<.0001
Glycogen		9	184.54	20.50	48.20	<.0001
(c) Immunological parameters						
NBT	Stimulant	9	37.23	4.14	179.11	<.0001
Lysozyme activity		9	12.43	1.38	158.84	<.0001
Phagocytic activity		9	728.62	80.95	89.10	<.0001
Clotting time		9	363.99	40.44	69.83	<.0001

Table 38 Relative percentage survival of striped catfish in different doses of ginger groups after challenge study

Treatments	Relative percentage of survival (%)
F0	$50.00^{a} \pm 1.98$
F1	$75.00^{b} \pm 1.98$
F2	$95.00^{d} \pm 1.25$
F3	$82.50^{c} \pm 1.25$
F4	$82.50^{c} \pm 1.25$

Mean values in the same column with different superscript differ significantly ($P < 0.05$). Data expressed as mean ± SE

Table1. Composition of ingredients for test diets (Experiment 1)

Ingredients	Composition (g kg $^{-1}$)			
	F1	F2	F3	F4
Soyaflour	350.00	350.00	350.00	350.00
Fish meal	280.00	280.00	280.00	280.00
Wheat flour	80.00	80.00	80.00	80.00
Maize flour	70.00	70.00	70.00	70.00
Tapioca	70.00	70.00	70.00	70.00
Groundnut oil cake	80.00	80.00	80.00	80.00
Rice flour	50.00	50.00	50.00	50.00
Fish oil	20.00	20.00	20.00	20.00
Brewer's yeast	10 g	-	-	-
Vitamin C	-	1000 mg	-	-
Vitamin E	-	-	80 mg	-
Ginger	-	-	-	10 g

Table 2. Composition of ingredients for test diets (Experiment 2)

Ingredients	Composition (g kg $^{-1}$)				
	F0	F1	F2	F3	F4
Soyaflour	350.00	350.00	350.00	350.00	350.00
Fish meal	280.00	280.00	280.00	280.00	280.00
Wheat flour	80.00	80.00	80.00	80.00	80.00
Maize flour	70.00	70.00	70.00	70.00	70.00
Tapioca	70.00	70.00	70.00	70.00	70.00
Groundnut oil cake	80.00	80.00	80.00	80.00	80.00
Rice flour	50.00	50.00	50.00	50.00	50.00
Fish oil	20.00	20.00	20.00	20.00	20.00
Ginger	0	5	10	15	20

सारांश

पंगस, स्ट्राइप्ड कॅटफिश, पँगॅसियानोडॉन हायपोप्थालमस माशांच्या पिल्लांवर त्यांच्या खाद्यांत रोगप्रतिकारक प्रेरके वापरुन त्यांच्या आरोग्यावर होणारा परिणाम दोन विविध प्रयोगातून अभ्यासण्यात आला. सदर अभ्यास प्रयोगशाळेत ४०० लिटर आकारमानाच्या २० फायबरग्लास टाक्या पाच विविध खाद्य आणि चार प्रतित सी. आर. डी. प्रायोगीक संरचना वापरुन प्रत्येकी ९० दिवसांसाठी करण्यात आला. दोन्ही प्रयोगाअंती पंगस माशांची पिल्ले एरोमोनास हायड्रोफिला या रोगकारक जिवाणूच्या संपर्कात आणून माश्यांवरील रोग होण्याच्या प्रभावाची तिव्रता अभ्यासण्यात आली. प्रयोग क्र. १ मध्ये चार विविध रोगप्रतिकारक प्रेरके समाविष्ठ खाद्य ज्यामध्ये ब्युअर्स यीस्ट, विटॅमीन सी, विटॅमीन इ, सुंठ पावडर आणि व्यवसाय प्रचलित खाद्य हे प्रमाणखाद्य म्हणून वापरले गेले. प्रयोगाअंती, अभ्यासलेल्या खाद्यांपैकी, सुंठमिश्रीत खाद्याने माशांच्या वाढीवर आणि फिड कन्व्हर्जन रेशो मध्ये सांख्यिकी दृष्ट्या ($p<0.05$) वाढीव फरक दर्शविला. त्याच प्रमाणे सदर खाद्याने माशांच्या एचबी, आरबीसी, डब्ल्युबीसी, एचसीटी, सिरम अल्ब्युमीन, ग्लोब्युलीन इ. मध्ये आणि फॅगोसायटीक, लायसोझाइम आणि एनबीटी कार्यक्षमतेत रोगकारक जिवाणूंच्या संपर्कात येण्यापूर्वी आणि आल्यानंतर सांख्यिकी दृष्ट्या ($p<0.05$) लाक्षणिक वाढ दर्शविली. म्हणून प्रयोग क्र. २ साठी सुंठ पावडर विविध चार मात्रेमध्ये (५, १०, १५ आणि २० ग्रॅम प्रति १ किलो खाद्य) माशांच्या खाद्यात समाविष्ठ करुन वापरली गेली आणि प्रमाणखाद्य म्हणून सुंठपावडर विरहित खाद्य अशी पाच खाद्ये चार प्रतित सी. आर. डी. प्रायोगिक संरचेत अभ्यासली गेली. प्रयोगाअंती, माशांचे वजन प्रत्यक्ष वाढीचा दर आणि फिड कन्व्हर्जन रेशो यामध्ये १० ग्रॅम प्रति किलो सुंठमिश्रीत खाद्य वापरलेल्या माशांत सांख्यिकी दृष्ट्या ($p<0.05$) परिणाम कारक जास्त वाढ दिसून आली. माशांचे एचबी, आरबीसी, डब्ल्युबीसी, एचसीटी, सिरम अल्ब्यूमीन, ग्लोब्यूलीन, एनबीटी, फॅगोसायटीक आणि लायसोझाइम कार्यक्षमतेवर जिवाणू संपर्कपूर्व आणि जिवाणूसंपर्क पश्चात रोगप्रतिकारक क्षमतेत १० ग्रॅम सुंठ पावडर प्रति किलो खाद्य दिलेल्या पंगस पिल्लांवर सांख्यिकी दृष्ट्या ($p<0.05$) वाढीव फरक निदर्शनास आला. दोन्ही प्रयोगातील जिवाणू संपर्कपूर्व काळातील माशांच्या पिल्लांचे जगणूकीचे प्रमाण १००% नोंदविले गेले. शारीरीक पेशींच्या संरचनेतील अभ्यासात देखील १० ग्रॅम सुंठपावडर मिश्रीत खाद्यातून योग्य पेशी संरचना निदर्शनास आली. दोन्ही प्रयोगाअंती असे सूचित केले गेले की, १० ग्रॅम प्रतिकिलो सुंठमिश्रीत खाद्य पंगस माशांच्या खाद्यात समाविष्ठ केल्यास त्यांच्या वाढीवर, जगणूकीवर आणि सर्वसाधारण रोगप्रतिकारक क्षमतेत लक्षणीय वाढ होते.