EFFECT OF DIETARY PREBIOTIC MANNAN OLIGOSACCHARIDE ON GROWTH AND HEALTH STATUS OF ASIAN CATFISH (*Clarias batrachus*) JUVENILES

A Thesis

By

KHURSHID ZAHAN
Examination Roll No. 1705517
Session: 2017
Semester: July- December, 2018

MASTER OF SCIENCE (MS)

IN

AQUACULTURE

DEPARTMENT OF AQUACULTURE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY DINAJPUR

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Hajee Mohammad Danesh Science and Technology University, Dinajpur
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Approved as to style and contents by

_____	_____
(Prof. Dr. Mst. Nahid Akter)	(Md. Abu Zafar)
Supervisor	Co-supervisor

_____

(Prof. Dr. A.S.M. Kibria)
Chairman
Examination Committee
&
Chairman
Department of Aquaculture

Hajee Mohammad Danesh Science and Technology University, Dinajpur

December 2018

DECLARATION

I declare that this MS thesis the effect of dietary prebiotic mannan oligosaccharide on growth and health status of Asian catfish (*Clarias batrachus)* juvenile, which I submit to Department of Aquaculture, was conducted by me for the degree of Masters in Aquaculture under the guidance and supervision of Prof. Dr. Mst. Nahid Akter, Department of Aquaculture, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

In addition, I took reasonable care to ensure that the work is original and has not been taken from other sources except where such work has been cited and acknowledged within the text.

The Author

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The Author

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ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemist
BAU	Bangladesh Agricultural University
BFRI	Bangladesh Fisheries Research Institute
CM	Centimeter
DEC	Decimal
DMRT	Duncan’s Multiple Range Test
DO	Dissolved Oxygen
DoF	Department of Fisheries
ESR	Erythrocyte sedimentation rate
FAO	Food and agriculture organization
FAO	Food and Agriculture Organization
FCR	Food conversion ratio
FCR	Food Conversion Ratio
FM	Fish meal
FOS	Fructo oligosaccharides
FRSS	Fisheries Research Survey System
g	Gram
GE	Gross energy
GI	Gastrointestinal
H	Hepatocyte
Hb	Haemoglobin
HIS	Hepatosomatic index
Ig	Immunoglobulin
IPF	Intra peritoneal fat
IPF	Intraperitoneal fat
Kg	Kilogram
L	Liter
MCH	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration

Mg	Milligram
Mm	millimeter
MOS	Mannan oligosaccharide
PCV	Packed cell volume
PER	Protein Efficiency Ratio
R_1	Replication-1
R_2	Replication-2
R_3	Replication-3
RBC	Red blood corpuscle
SE	Standard Error
SGR	Specific Growth Rate
SIS	Small indigenous Species
SPSS	Statistical Package for Social Sciences
T_1	Treatment-1
T_2	Treatment-2
T_3	Treatment-3
VSI	Viscera somatic index
WBC	White blood corpuscle
Wg	Weight gain

Abstract

This study was directed to estimate the effects of mannan oligosaccharide (MOS) dietary supplementation on growth, proximate composition and haematological parameters in Asian catfish (*Clarias batrachus*). Triplicate groups of juveniles Asian catfish (initial weight 21.23 ± 1.01 g) were fed twice per day at 3 % of body weight for 12 weeks, with 0 (control), 0.2, 0.4 or 0.6% MOS diets. Water temperature, pH, dissolved oxygen, $NH_4^+$and NH_3 were checked in every two weeks, which were ranged between 17.66-18.42°C, 7.06-7.22, 5.06-6.13 mg L^{-1}, 0.49-0.61 mg L^{-1} and 0.34-0.48 mg L^{-1}, respectively. Compared to control (0.57±0.04), those fed 0.4% (0.82±0.05) or 0.6% (0.77±0.04) MOS had significantly higher (P< 0.05) specific growth rates. Feed conversion ratio and protein efficiency ratio significantly enhanced (P< 0.05) in fish fed 0.4% MOS diet compared to control. The survival rate of the control fed group exposed significantly the lowest (P <0.05) when compared with the treated fish fed with MOS supplemented diets. Significantly lower (P <0.05) HSI was noted when the fish fed 0.2% and 0.4% MOS diets compared to those fish fed control, but it did not differ from those fish fed with 0.6% MOS diet. Similarly, IPF was significantly lowest (P <0.05) in those fish which fed 0.4% diets over the control the group. Whole body proximate composition of Asian catfish exposed that significantly highest *(P <* 0.05) protein content were observed in fish fed with 0.4% MOS supplemented diet, whereas the ash content was significantly highest *(P <* 0.05) in fish group fed with 0.6% MOS. Dietary inclusion of MOS did not show any significant influence on the majority of the haematological parameters of Asian catfish. But it presented a significant positive influence on some of the most important parameters, namely ESR, PCV and WBC. Significantly reduced *($P < 0.05$)* ESR and increased PCV (%) were noted when the fish fed with higher concentration of MOS (0.4% and 0.6% MOS) diets compared to control and lowest concentration of MOS such as 0.2%.

Feeding the fish with MOS supplemented diets showed significant influence *(P < 0.05)* on the WBC compared with the control diet. Thus, 0.4% MOS is sufficient to increase growth, feed utilization and haematological parameters in juvenile Asian catfish.

Key Words: Growth performance, proximate composition, haematological parameters, *Clarias batrachus,* prebiotic.

CHAPTER 1

INTROUCTION

Aquaculture remains one of the fastest-growing food producing sectors in the global fish production and continues to expand faster than world population growth (FAO, 2014). Now, aquaculture contributes 3.69% to Gross Domestic Products (GDP) and 23.81% to the agriculture sectors of Bangladesh while fisheries sector contributes 60% of the total animal protein. Approximately 11% of the population directly and indirectly depends on fisheries for their livelihood (DoF, 2017). Presently, Bangladesh is achieving 5th position in inland culture fisheries (FAO, 2017). We have huge inland fisheries resources, which resource fish and other aquatic animals and plants to millions of people living in the Delta (Hossain *et al*. 2014). To fulfill the demand of over growing population it is essential to intensify aquaculture to contribute more in national GDP.

Aquaculture is the farming of fish and other aquatic organisms, with ‘farming’ implying some form of intervention to enhance productions, and some form of private rights of the stock under intervention (Beveridge and Little, 2002). Aquaculture industry reflects the disease occurrence as the state of restriction to aquaculture production at which seriously affects economic development (Ibrahem *et al.* 2010).

The Asian catfish, *Clarias batrachus* (Linnaeus, 1758) generally known as “*magur*” has a fairly common supply in fresh and brackish

waters of the plains throughout India. It has high commercial importance in India, Bangladesh, Thailand, Philippines, Myanmar and China due to its good taste, high protein (15.0%) and iron content (710 mg/100 gm tissue) with specifically low value of fat (1.0%) as well as therapeutic application. In the natural environment, it spawns once a year. The Spawning period is July–August. The major limitation in the culture of magur is the non–availability of quality seeds from the natural resources due to environmental degradation for rapid industrialization and injudicious use of pesticides, reduction of natural breeding ground due to siltation, over utilization and illegal killing of juveniles and brood fishes (Dhara *et al*. 2013). The genus *Clarias* has been moved to several continents, adapting itself successfully and found throughout Asia and Africa. A comparatively simple culture characteristic with effective food conversion (Ali and Jauncey, 2005) and excellent nutritional profile (Rui *et al*. 2007) makes *Clarias* particularly *C. batrachus* very suitable for commercial intensive culture. This species is revealed as a medicinal fish and traditionally remained a strike among the pregnant and lactating mothers, the elderly and children because of easily digestible high grade protein, high concentration of iron and beneficial lipid content. Intensive *C. batrachus* culture in the rural areas of Bangladesh has much potential towards livelihood development, employment generation and ensuring nutritional enhancement in the regular diet among all of the people.

However, intensive aquaculture has given rise to many difficulties such as degradation of water quality, increase of stress and proliferation of pathogenic microbes which suppress the fish growth (Yousefian and Amiri, 2009). To address these obstacles, antibiotics have been used for fish disease management (Denev *et al*. 2009) but the indiscriminate use of antibiotic has led to increased of antibiotic resistant bacteria and destruction of environmental beneficial microbial flora (Yousefian and Amiri, 2009) as well as reduced immune system (Ringo *et al*. 2010).

Use of dietary supplements that can stimulate immune system function is one of the approaches in which not only provide essential nutrients to support growth and improvement of the cultured organisms but also may be one of the most capable means to influence the culture organisms health and resistance to stress and disease causing agents (Sheikholeslami-Amiri *et al*. 2012).

Probiotic bacteria are now gradually used in aquaculture to overcome antibiotic induced disease resistance, degradation of water quality and growth of farmed fish (Verschuere *et al*. 2000). Probiotics are live microorganisms which when incorporated suitable amounts confer benefits to the health of the host by refining the balance of the intestine microbiota (Azevedo *et al.* 2016). One of the most probable importance's of using probiotic bacteria is the direct effect of probiotic on the growth performance of fish either by direct increase in nutrient uptake, or by providing the nutrients. Probiotics could colonize and implement their positive activity in the intestinal region

of animals which is being considered as a complex harbour of non-pathogenic, pathogenic and commensal microorganisms after feeding probiotic supplemented diets. Among the probiotics commonly used, the genus *Bacillus* is one of the most extensively evaluated as aquaculture feed supplements and has been demonstrated to expand a number of attributes when supplemented in diets of aquatic organisms (Azevedo *et al*. 2016). However, the large scale use of probiotics in commercial aquaculture has been limited due to problems associated with handling, pelleting and storage (Merrifield *et al*. 2010).

Another comparatively new but effective approach to overcome issues associated with antibiotic and probiotic applications in aquaculture is the use of prebiotics. Prebiotics which are defined as non-digestible food ingredients positively affect the host by stimulating growth and/or activity of a limited number of beneficial bacteria in the gastrointestinal tract and have showed to be effective at improving health and growth performance of terrestrial and aquatic animals (Gibson and Roberfroid, 1995). Criteria which permit the classification of a food ingredient as a prebiotic include

1. It must be neither hydrolyzed nor absorbed in the upper portion of the gastrointestinal tract.
2. Selective fermentation by one or a limited number of potentially beneficial bacteria in the colon, and
3. Alteration of the composition of the colonic microbiota toward a healthier composition (Fooks and Gibson 2002).

Among the recognized prebiotics, mannan oligosaccharide (MOS) is most commonly used as the dietary supplementation for fish and crustacean species (Sang and Fotedar, 2010). MOS is a non digestible glucomannan derivative from the cell wall of *Saccharomyces cerevisiae*, being rich source of mannose which is accessible for bacterial adhesion, which adsorbing pathogens stops it's binding to the intestinal wall (Newman, 1994). The use of functional feed additives such as MOS to increase growth and health performance in the aquaculture industry is increasingly significant as which are required the eco-friendly production practices (Dimitroglou *et al.* 2009). Mannan oligosaccharide (MOS) has been shown to develop the overall condition of alimentary canal by restricting the accumulation of agents of disease and strengthening the body immune system (Staykov *et al.* 2007). This prebiotic has been demonstrated to have a variety of beneficial effects on livestock ranging from growth enhancing to immune-stimulation in many species. The growth promotion and immuno-stimulatory benefits of dietary MOS have also been studied in fish but often with different findings. MOS is a natural alternate product for anti-bacterial growth factors, which is known for concerning pathogen microorganisms and toxins to their chemical structure (Newman *et al.* 1994). In this way, pathogenic bacterial growth is prevented and consequently the harmful effect of microflora metabolites is decreased.

In contrast to the progress made in other species, the effects of MOS on Asian catfish have received little attention. Therefore, this study was initiated to evaluate the effectiveness of MOS on magur.

Purposes of the study

Principal objective of the study was to determine the feasibility of using the prebiotic mannan oligosaccharide (MOS) to produce healthy Asian catfish (*C. batrachus*) juveniles.

Objectives of the study

- To determine the optimal inclusion level of MOS in feed ingredients for optimum growth performance, proximate composition and survival of *C. batrachus* juvenile.
- To determine the effect of prebiotic MOS on the hematological parameters of *C. batrachus* juvenile.

CHAPTER 2

REVIEW OF LITERATURE

Aquaculture is one of the fastest growing food production systems in the world, with the majority of its output currently being produced within developing countries, and with expectations for aquaculture to remain its contributions to food security and poverty alleviation. The vast majority of aquaculture practices around the world have been followed with important nutritional and social benefits, and generally with little or no environmental costs. However, it is important for current efforts aiming at the future success of aquaculture in both developing and developed countries, that potential social and environmental problems are appropriately addressed in order to confirm that aquaculture develops sustainably (FAO 2014). Characteristics of the included animal studies.

The term “probiotic” originates from the Greek word meaning “for life”. In 1989, Fuller defined the term probiotic as “a live microbial feed supplement which positively affects the host animal by improving its intestinal balance”.

A prebiotic was first defined as “a non-digestible food ingredient that definitely affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus increases host health.

Subsequently, Roberfroid (1995) stated that “A prebiotic is a selectively fermented ingredient that permits exact changes, both in

the composition and/or activity in the gastrointestinal micro flora that confers aids upon host well-being and health. Many researchers examined three criteria, namely: (a) resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption; (b) fermentation by intestinal micro flora; and (c) selective elevation of the growth and/or activity of intestinal bacteria associated with health and well-being. The following information was studied in favor of the present study which has done around the world and relevant to the study.

2.1 Water quality

Pasala *et al*. (2018) carried out an experiment to assess the effects of probiotic in water quality maintenance and growth of rohu (*Labeo rohita*) fingerlings. The authors resulted that probiotic bacteria are known to improve water quality in many ways.

Ahmad *et al.* (2013) undertook an experiment to determine the effect of mannan oligosaccharide (MOS) on the water quality parameters of a recirculatory aquaculture system growing Indian major carp *L. rohita* was evaluated out for a period of 120 days. The basal diet in all trials contained 35% protein with different dosages of MOS at 0.0, 0.15, 0.30 and 0.45% in triplicates. Uniform sized fish fingerlings averaging 1.3g was used for the experiments. The different water quality parameters viz, temperature, p^H, dissolved oxygen, free carbon dioxide, total alkalinity and ammonia-nitrogen were found to varying amount during the culture period.

2.2 Proximate composition

Akbary *et al.* (2018) carried out an experiment to determine the effect of prebiotic on the proximate composition of grey mullet (*Mugil cephalus*) and observed that the moisture (%), protein (%), lipid (%) and ash (%) content in case of control 72.61, 18.21, 4.59 and 5.63% respectively; for t_1 71.32, 18.14, 24, 5.93% respectively. In case of t_2 76.84, 18.12, 4.67, 5.21 % respectively and for t_3 72.60, 18.19, 4.83 and 5.63 % respectively.

Akrami *et al*. (2018) monitored the effect of dietary A-Max supplementation as a prebiotic on growth performance of great sturgeon (*Huso huso* Linnaeus, 1758) juvenile. The results showed that the total protein were significantly ($p<0.05$) affected by the supplementation of 1.5 g kg^{-1} dietary prebiotic mixture.

Mehrabi *et al.* (2018) carried out an experiment to evaluate the effects of Pre- and Probiotics on Common Carp (*Cyprinus carpio*) to determine proximate composition. The author reported that the percentage of fat, protein, ash and moisture content in case of control as 14.35, 58.81, 13.91 and 23.68 % respectively; T_1 (1g Immunowall®/kg food) 17.71, 60.81, 14.11 and 24.24 % respectively; T_2 (1.5 g Immunowall®/kg food) 21.06, 60.81, 15.89 and 23.33% respectively; T_3 (1g Primalac®/kg food) 20.54, 61.73, 18.15 and 23.07 % respectively; T_4 (1.5 g Primalac®/kg food) 18.39, 62.35, 12.63 and 25.28% respectively and for T_5 (a mix of 1g Immunowall® and 1g Primalac®/kg food) 16.58, 62.88, 13.15 and 25.22% respectively.

Gelibolu *et al.* (2018) evaluated an experiment to assess the effect of prebiotic mannan oligosaccharide (MOS) on proximate composition on sea bream (*Sparus aurata*). The author determined the dry matter (92.43, 91.76, 91.39, 91.92 and 93.23 respectively), protein (45.39, 46.1, 46.5, 46.98 and 45.35 respectively), lipid (20.34, 19.09, 19.29, 19.4 and 20.17 respectively) and ash content (12.51, 12.73, 12.68, 12.8 and 12.37 respectively) in case of control and MOS 1%, MOS 2%, MOS 3% and MOS 4%.

Amirkolaie *et al.* (2015) carried out an experiment to evaluate the effect of immunogen on the proximate composition of common carp (*C. carpio*). The author found the proximate composition of diets such as dry mater 28.66, 29.1, 29.55, 29.15 and 29.63% respectively, protein 14.2, 15.33, 15.35, 15.65 and 15.53% respectively, fat 9.21, 9.6, 9.44, 9.34 and 9.4 respectively %, ash 3.3, 2.9, 3.43, 3.21 and 3.07% respectively for the treatment of control, immunogen 5, immunogen 10, immunogen 20 and immunogen 40.

Denji *et al*. (2015) carried out an experiment on the effect of prebiotic mannan oligoscccharide on Rainbow Trout *(Oncorhynchus mykiss)* juveniles and the result revealed that the crude protein content increased significantly ($p<0.05$) by increasing the supplementation level of MOS.

Ghobadi *et al*. (2015) conducted a research on the effect of dietary prebiotic mannan oligosaccharide (MOS) on growth performance, intestinal microflora, body composition, hematological and blood

serum biochemical parameters of Rainbow Trout (*O. mykiss*) Juveniles. The author reported that the crude protein content increased significantly ($p<0.05$) by the increasing level of MOS.

Aktas *et al*. (2014) carried out an experiment to assess the effect of MOS on the proximate composition of white leg shrimp (*Litopenaeus vannamei)* and reported that the body composition (protein, lipid, ash and dry matter) of the shrimps were not affected by the feeding of MOS supplemented diets. They found the following results moisture, crude protein, lipid and crude ash for control 73.64, 22.68, 1.79 and 1.09 respectively: for MOS 74.66, 22.55, 1.73 and 1.06 respectively: for MOS+ Srt 74.00 22.80, 1.63 and 1.57 respectively: Srt 74.73 22.55, 1.65 and 1.07 respectively.

Ahmad *et al*. (2013) conducted an experiment to evaluate the effect of Bio-Mos® on the whole body proximate composition of Nile tilapia (*Oreochromis niloticus*) and reported that the moisture and ash contents were not significantly affected while the protein content was significant.

Akrami *et al.* (2012) evaluated an experiment to assess the effect of dietary mannan oligosaccharide on growth performance, survival, body composition of carp juvenile (*C. carpio*). The author found that in group treated with 1.0 g kg^{-1} MOS showed higher protein carcass than other group.

Dimitroglo *et al.* (2011) conducted an experiment on the effect of mannan oligosaccharide on Atlantic Salmon Smolts (*Salmo salar* L)

and the results represented that MOS supplementation did not affect growth performance; however, body protein composition was significantly increased. The author reported that the (%) of dry matter 29.15 and 29.20, protein 16.75 and 16.85 and fat 11.35 and 11.48 respectively in case of control and MOS fed fish.

Genc *et al*. (2007) stated an experiment on the effect of prebiotic mannan oligosaccharide on Hybrid Tilapia (*O. niloticus x O. aureus*). The dry matter and protein contents increased significantly ($p<0.05$) with increasing level of dietary MOS. The authors reported the moisture content 10.21, 76.69, 75.51, 74.33 and 72.94; crude protein content 42.83, 21.49, 21.92, 23.12 and 24.49; lipid content 19.64 , 1.35, 1.39, 1.42 and 1.51 and crude ash content were 9.64, 1.36, 1.31, 1.36 and 1.11 respectively in the case of control and (MOS %) 0, 1.5, 3.0, 4.5 diets.

2.3 Growth parameter

Akbary *et al.* (2018) reported an experiment on the effect of prebiotic on the grey mullet (*M. cephalus*) effects of stocking density on growth and survival grey mullet. The author showed a significantly better growth yield in prebiotic fed treatment compared to those fed the control.

Akrami *et al*. (2018) monitored that the effect of dietary A-Max supplementation as a prebiotic on growth performance of great sturgeon (*H. huso* Linnaeus, 1758) juvenile. At the end of the trial, growth factors and haemato-immunological parameters were

assessed. Fish fed 1.5 g kg-1 prebiotic mixture displayed the higher growth performances and feed efficiency compared to the control group.

Gelibolu *et al.* (2018) stated an experiment on the effect of prebiotic mannan oligosaccharide (MOS) on the growth performance of gilthead sea bream (*S. aurata*). The author reported the growth parameter in case of control, 1%, 2%, 3% and 4% MOS were; initial weight (IW) (g) 4.09, 4.08, 4.07, 4.06 and 4.06 respectively; final weight (FW) (g) 89.81, 83.48, 85.84, 86.79 and 84.28 respectively; feed conversion ratio (FCR) 1.28, 1.30, 1.35, 1.35 and 1.33 respectively; protein efficiency ratio (PER) 1.73, 1.68, 1.59, 1.58 and 1.66 respectively.

Bahrekazemi *et al*. (2017) designated an experiment on the effect of diets containing different levels of prebiotic mito on the growth factors, survival, body composition in common carp *C. carpio* fry. The author found the highest final weight in fry treated with 2 g/kg of prebiotic and the lowest value was recorded in the group of fish fed with control diet. The fry of carp fed 2 g/kg of prebiotic mito showed the highest values of SGR, CF and net fish production in comparison with those in fed the control group. Also, the FCR level was lowest in fish fed 2.0 g/kg of prebiotic Mito. The mortality rate of carp fry was zero in all treatments.

Dhanalakshmi *et al*. (2017) carried out an experiment to justify the effect of probiotics and prebiotics supplemented diets on immune

resistance against *Aeromonas hydrophila* in mrigal carp. The mortality of control, probiotic and prebiotic groups where 65%, 40% and 35% respectively.

Djauhari *et al.* (2017) evaluated the effect of prebiotic from sweet potato on common carp (*Cyprinus carpio*) growth. The author reported the best value for daily growth rate and FCR ($p<0.05$) when compared with the control and other treatment groups.

Jaber *et al*. (2017) carried out an experiment on the effect of prebiotic on *C. Carpio* that highest final weight was found in fry treated with 2 g/kg of prebiotic and the lowest value recorded in the control. The fry of carp fed 2 g/kg of prebiotic mito showed the highest values of specific growth rate, condition factor and net fish production in comparison with those in fed the control group.

Forsatkar *et al*. (2017) organized a trial on zebra fish (*Danio rerio*) significant differences were found in the final body weight, body weight intake, percent body weight intake, and specific growth rate among treatments. The regression analyses showed that the optimum MOS level was 4–4.1 g MOS kg^{-1} diet. Fish fed 4 g MOS kg^{-1} feed showed higher survival than the other treatments. Also, using the novel tank test, fish fed with 4 g MOS kg^{-1} diet showed lower anxiety by swimming in the upper portion of the tank.

Azevedo *et al*. (2016) evaluate an experiment on Nile tilapia (*Oreochromis niloticus)* to assess the effect of fish fed diets prebiotic supplemented performed better in average daily gain, feed

conversion rate, specific growth rate, protein efficiency ratio, carcass yield, total and standard length and body height than those maintained on control diets. The results of this study indicated that the MOS and *Bacillus subtilis* supplementation, isolated or combined (symbiotic) could improve growth, body index, intestine morphometric and carcass composition in Nile tilapia. They found FCR for control was 1.87 and use of prebiotic was 1.49.in case of SGR (% day^{-1}) for control 3.55 and use of prebiotic was 3.98.

Gharae *et al*. (2016) reported an experiment on the influence of dietary prebiotic mixture (α - mune on Beluga sturgeon (*H. huso*) to evaluate the growth performance after feeding various levels of prebiotic. The author found the growth parameter in regard to weight gain (WG) (g) 161.0, 173.24, 154.78, 158.17; specific growth rate (SGR) (%/day) 3.27, 3.39, 3.19, 3.23; FCR 1.37, 1.27, 1.41, 1.38 respectively for control, 1.5, 3.0 and 4.5 g kg^{-1}.

Minguez *et al*. (2016) conducted an experiment to evaluate the effect of a prebiotic supplementation of MOS on growth traits and mortality of rainbow trout (*O. mykiss*) and reported that there were significant differences for , FCR in favour of the MOS treatment group ($p<0.05$). The mortality observed was 31% and 17% for the control and the MOS treatment group respectively. The difference between these two groups was statistically significant ($p <0.05$).

Amirkolaie *et al.* (2015) evaluated the effect of immunogen on the growth performance of Common Carp (*Cyprinus carpio*). Five

experimental diets were formulated by incorporating immunogen at 0 (control), 5, 10, 20 and 40 g/kg. The initial weight (g) were as 4.81, 4.82, 4.82, 4.82 and 4.81respectively; the final weight (g) were 10.31, 12.28, 9.7, 9.35 and 8.8 respectively; SGR (%/day) 0.55, 0.67, 0.50, 0.47 and 0.44 respectively; FCR 2.67, 2.5, 2.73, 2.94 and 3.19 respectively and PER 0.98, 1.09, 0.97, 0.90 and 0.85 respectively.

Moghaddam *et al*. (2015) carried out an experiment on the effect of prebiotic mannan oligosaccharide (MOS) to assess the growth performance of common carp (*C. carpio*) fingerlings. The author resulted that the growth performance including final weight, weight gain (WG) and SGR did not differ among the treatments. However FCR was better when the fish were fed 0.05 to 0.20% MOS diets.

Denji *et al*. (2015) carried out an experiment on the effect of MOS on rainbow trout *(O. mykiss)* juveniles. The result showed that in level of 1 g MOS kg^{-1} acquired final weight, body weight increase (BWI), SGR and FCR were significantly higher ($p<0.05$) than the other experimental groups. The highest and the lowest growth performances were observed in 1 g MOS kg^{-1} and control, respectively.

Ghobadi S. (2015) conducted a research on the effect of dietary prebiotic MOS on growth performance, intestinal microflora, body composition, haematological and blood serum biochemical parameters of rainbow trout (*O. mykiss*) juveniles. The author presented that the significantly higher ($p<0.05$) final weight, BWI,

SGR and FCR were observed in fish fed with 1 g kg^{-1}MOS. The highest and the lowest growth performances were observed in 1 g MOS kg^{-1} and control group respectively.

Sado *et al.* (2014) evaluate an experiment on the effect of MOS on pacu (*Piaractus mesopotamicus)* to assess the effect of growth parameters did not differ significantly ($P>0.05$) between fish fed control diet and MOS supplemented diets.

Aktas *et al*. (2014) carried out an analysis on the effect of MOS to assess the growth of white leg shrimp (*L. vannamei*). The results indicate that inclusion of 3 g kg^{-1} MOS into diet enhance the shrimp survival, mounting rate, growth and FCR compared to the control.

Ahmad *et al.* (2013) observed an experiment on Nile tilapia (*O. niloticus*) the diet containing 0.2% Bio-Mos® resulted in the highest ($P<$ 0.05) growth performance compared to other diets. Feed conversion ratio (FCR) improved significantly with the diet contained 0.2 % BioMos®. Looks like FCR, protein efficiency ratio (PER), apparent protein utilization (APU), and energy utilization (EU) were significantly improved by Bio-Mos.

Ramanadevi *et al*. (2013) evaluated an experiment on the effect of prebiotic to estimate the growth *of Amphiprion ocellaris* fingerlings at the end of trial, growth indices and survival rate were determined. Growth indices such as final weight, length and SGR in all treatments showed no statistical significant differences ($p>0.05$) in compared with the control.

Akrami *et al.* (2012) evaluated an experiment to assess the effect of dietary mannan oligosaccharide on growth performance, survival, body composition of carp juvenile (*C. carpio*). The significantly highest and lowest growth performance was observed in the case of fish fed with 1.0 g kg^{-1} MOS and control. However, survival rate and body composition were not affected ($p > 0.05$).

Akrami *et al.* (2012) carried out an experiment on the effect of dietary mannan oligosaccharide on gibel carp juveniles (*Carassius auratus gibelio*) and reported significant differences ($P > 0.05$) in growth and feeding parameters between control and MOS supplementation diets. The highest and lowest growth performances were observed in 4.5 g kg-1 MOS and control group ($P < 0.05$). Subsequently, immune responses (Ig levels, lysozyme activity and ACH50) were significantly higher in 4.5 g kg-1 MOS fed fish ($P < 0.05$). The survival rate of control group was lower than the MOS groups, but not significant ($P > 0.05$).

Zhang *et al*. (2012) evaluated an experiment on the effect of of dietary prebiotic MOS on growth performance, shrimp, *L. vannamei.* After the 8-week feeding trial, growth parameters were assessed. The author found the following result: WG and SGR were significantly higher ($P < 0.05$) in shrimp fed 2.0, 4.0, 6.0 and 8.0 g kg^{-1} MOS-supplemented diets than shrimp fed control diet. WG and SGR of shrimp fed 2.0 g kg^{-1}MOS-supplemented diet was the highest

(*P < 0.05*) in all experimental groups. Survival rate (SR) of shrimp was generally similar (*P > 0.05*) in all experimental groups.

Dimitroglo *et al.* (2011) described an experiment on the effect of prebiotic mannanoligosaccharide (MOS) on growth parameters of Atlantic Salmon Smolts (*S. salar* L). The author found that final fish weight (g) 204.06 and 204.18; FCR 0.86 ± 0.02 0.86 ± 0.04, SGR (%) 1.41 and 1.44 for control and MOS diet respectively.

Yousefian *et al*. (2009) worked an experiment on the effects of prebiotic inulin on the growth performance of Beluga's (*H. huso*). The author reported a negative relationship between some performance indices including WG, SGR, PER, feed efficiency (FE) and supplementation level of inulin (1, 2 and 3%).

Ghosh *et al*. (2008) observed an experiment on dietary probiotic supplementation in growth and health of live bearing ornamental fishes. The length, weight and the survival were significantly higher ($P<0.05$) and the FCR were significantly lower ($P<0.05$) in fishes fed with the probiotic feeds.

Genc *et al*. (2007) stated an experiment on the effect of MOS on growth parameters of Hybrid Tilapia (*Oreochromis niloticus x O. aureus*) and resulted the increasing ($p<0.05$) growth parameters (live weight gain, SGR, FCR) or body indices (hepatosomatic and viscerosomatic) with increasing level of dietary MOS. The author found that incase of diet control, and (MOS%) 1.5, 3, and 4.5 the initial weight (g) were 9.79, 9.76, 9.83 and 9.86, final weight (g)

were 39.31,37.61,39.11 and 37.95; SGR were 1.73, 1.67, 1.73 and 1.68, and FCR were 1.35, 1.42, 1.42 and 1.37 respectively.

2.4 Haematological parameter

Akbary *et al.* (2018) reported an experiment on the effects of adding prebiotic to experimental diets of the grey mullet (*M. cephalus*) led to significant difference ($p<0.05$) in the case of glucose and white blood corpuscle (WBC) while this was not found for haemoglobin (Hb), haematocrit, mean corpuscular hemoglobin concentration (MCHC), red blood corpuscle (RBC), lymphocyte, neutrophil, eosinophil, albumin, globulin and total protein ($p>0.05$). They found the following result in case of Control (0%), Treatment 1 (0.5% prbiotic), Treatment 2(1% prebiotic) and Treatment 3 (2% prebiotic). Haemoglobin (g/dL) found 14.26, 14.76, 16.23 and 13.56 respectively; Haematocrit (%) found 54.50, 54.76, 51.30 and 51.53 respectively. White blood corpuscle WBC ($\times 10^3/mm^3$) found 8.46, 10.14, 11.9 and 12.45 respectively; Red blood corpuscle RBC ($\times 10^4/mm^3$) found 72.45, 79.12, 86.59 and 67.59 respectively; MCHC (g $dL-1$) found 43.82, 44.13, 42.25 and 42.53 respectively.

Akrami *et al*. (2018) monitored the effect of dietary A-Max supplementation as a prebiotic on hemato- immunological parameters of great sturgeon (*H. huso* Linnaeus, 1758) juvenile. The results showed that RBC, WBC, and total protein were significantly affected ($p<0.05$) by 1.5 g kg^{-1} dietary prebiotic mixture. The author found the following result incase of diet 1.5, 1, 0.5 and Control RBC (10^6 mL^{-1})

1.56, 1.040.82 and 0.8 respectively; WBC (10^3 mL^{-1}) 22.76, 20.83 19.2 and 18.6 respectively, hemoglobin (g dL^{-1} 9.91, 9.51, 8.03 and 8.06 respectively; glucose (mg ml^{-1}) 2.3, 2.1, 1.8 and 1.8 respectively.

Akrami *et al.* (2013) studied an experiment on cultured juvenile great sturgeon (*H.huso* Linnaeus, 1754). The author observed no significant differences in the case of serum enzyme activity levels between treatments ($P > 0.05$). However, adding MOS as a supplement to the basal diet resulted in significant differences in lymphocytes and eosin-ophils between the control and the 2 g kg À1 treatment ($P < 0.05$).

Munir *et al.* (2018) worked an experiment to evaluate the effect of supplementation with dietary prebiotics and probiotics and led to significant ($P < 0.05$) improvement in the RBCs, WBCs, packed cell volume (PCV), Hb concentration and serum protein level; these improvements were effective significantly ($P< 0.05$) when the fish were challenged with *A. hydrophila* at the dose of 2×10^6CFU/ml. Haematological parameters of *Channa striata* fingerlings fed a single dose of supplemented diets and a control. They found the following result in case of Control, β-Glucan and MOS. RBC (10^6 mm^{-3}) found 3.75, 3.96 and 3.94 respectively; PCV (%) were 37.04, 42.03 and 41.21respectively; Hb (g dl^{-1}) found 9.26, 12.34 and 11.23

respectively; ESR (mm h^{-1}) were 0.55, 0.40 and 0.40 respectively; WBC (103mm^{-3}) found 22.67, 24.50 and 24.17 respectively.

Nikbakhsh *et al.* (2017) stated an experiment on the effect of diets containing different levels of prebiotic mito on the hematological parameters in common carp *C. carpio* fry. The authors resulted that the number of RBCs showed significant differences ($P < 0.05$) between the control and the other groups. The highest numbers were seen in fish fed 2.0 g kg^{-1} of prebiotic. The number of WBCs showed insignificant differences ($P > 0.05$) between treatments. Hb and haematocrit values significantly differed ($P < 0.05$) between 2 and 3 g kg^{-1} treatments and control.

Dhanalakshmi *et al*. (2017) carried out an experiment to justify the effect of probiotics and prebiotics supplemented diets on immune resistance against *A. hydrophila* in mrigal carp. The authors found the improved serum lysozyme content, serum bactericidal activity, serum protein level, serum globulin level, hematological parameters such as, leukocyte count, red blood cell count, and packed cell volume. The leukocyte count was also high in prebiotic (31.84 ± 104) compared with control group. There was a significant increase in RBC count in both probiotics and prebiotics (1.420 ± 0.04) and (1.390 ± 0.01) treated groups compared with control group. The high level of packed cell volume was observed in the probiotics (26.2 ± 0.83) treated groups compared to control.

Jaber *et al*. (2017) carried out an experiment on the effect of prebiotic Mito on *C. Carpio* and found the number of RBCs showed significant differences between the control and the other groups ($P < 0.05$). The highest numbers were seen in fish fed 2.0 g/kg of prebiotic. The number of WBCs showed insignificant differences between treatments ($P > 0.05$). Hb and haematocrit values significantly differed between 2 and 3 treatments and control ($P < 0.05$). The results show that the addition of 2 g/kg of prebiotic Mito in the diet of carp fry can be used as an appropriate dietary complement for common carp.

Gharae *et al*. (2016) reported an experiment on Beluga sturgeon (*H. huso*) to evaluate the effect of prebiotic on the haematological parameters and displayed significantly higher final weight (FW), special growth rate(SGR), feed conversion ratio (FCR), white blood cell (WBC), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), haemoglobin, haematocrit and lymphocyte levels were also significantly higher in the fish fed 1.5 g kg^{-1} prebiotic diet. Furthermore, the highest hematocrit content and lymphocyte level were found in the fish fed a diet containing 1.5 g kg-1 prebiotic. Alternative complement activity (ACH50), lysozyme activity and Ig concentration were significantly higher in the fed 1.5 g kg^{-1} prebiotic.

Amirkolaie *et al.* (2015) stated an experiment on the effects of dietary supplementation with Immunogen on common carp (*C. carpio*) the

whole body composition of the fish was not significantly influenced by prebiotic inclusion. RBCs counts were increased by prebiotic dietary supplementation at concentrations of 5 and 10 g kg^{-1} prebiotic. The author found the following result in case of control, immunogen 5, immunogen 10, immunogen 20 and immunogen 40. Red blood cells (10^6 µL) found 1.36, 1.71, 1.88, 1.56 and1.43 respectively; Haemoglobin (g dL^{-1}) found 7.03, 7.99, 8.27, 7.39 and 7.09 respectively; Haematocrit (%) 42.33, 48.66, 51.07, 46.53 and 43.53 respectively; White blood cells (10^3 µL) found 14650, 14333, 14500, 13966 and 14100 respectively; Glucose (mg. dL^{-1}) found 78.16, 89.93, 119.34, 107.62 and 107.98 respectively.

Denji *et al*. (2015) carried out an experiment on the effect of MOS on rainbow trout *(O. mykiss)* juveniles. The result showed levels of lymphocyte in the group fed 1 g MOS kg^{-1} was significantly higher ($p<0.05$) than other groups. Also, a non-significant ($p>0.05$) elevation of WBC, haematocrit, Hb and eosinophil levels was found in the fish fed diet 1.0 g MOS kg^{-1}.

Ghobadi S. (2015) conducted a research on the effect of dietary prebiotic MOS on the growth performance, intestinal microflora, body composition, haematological and blood serum biochemical parameters of rainbow trout (*O. mykiss*) juveniles. The author reported that glucose, in the group fed 1 g MOS kg^{-1} was significantly higher ($p<0.05$) than other groups. Also, a non-significant elevation

(p>0.05) of WBC, haematocrit, Hb and eosinophil levels was found in the fish fed diet 1.0 g MOS kg^{-1}.

Ahmad *et al.* (2013) describes the effect of Bio-Mos on Nile tilapia (*O. niloticus*). An improvement in hemoglobin (Hb), RBCs, hematocrite (Ht), total protein, serum albumin concentration and globulin was observed, while glucose level was decreases in fish fed 0.2% Bio-Mos® diet.

Akrami *et al*. (2012) evaluate an experiment to assess the effect of dietary MOS on the growth performance, survival, body composition and some hematological parameters of carp juvenile (*C. carpio*). The author found that a significantly ($p<0.05$) elevation of hematocrit and lymphocyte and non-significantly increased ($p>0.05$) of WBC, RBC, Hb and eosinophil were found in the fish fed diet 1.0 g kg^{-1} MOS.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

This experiment was designed to fulfill the objectives of this current study. For this experiment, study was planned to determine the effect of dietary prebiotic mannan oligosaccharide on growth and health status of Asian catfish (*C. batrachus)* juveniles. This chapter describes the common materials and methods that were applied for this experiment.

3.2 Experimental site

The feeding trial was conducted at the hatchery complex of Caritas, Bochagonj, Dinajpur. Twelve cemented tanks measuring (200 cm × 100 cm × 100 cm) (length × width × height) were used for this study under controlled conditions. Throughout the experiment, water was supplied from an overhead tank. Tanks were cleaned fortnightly to reduce the risk of accumulation of nitrogenous waste.

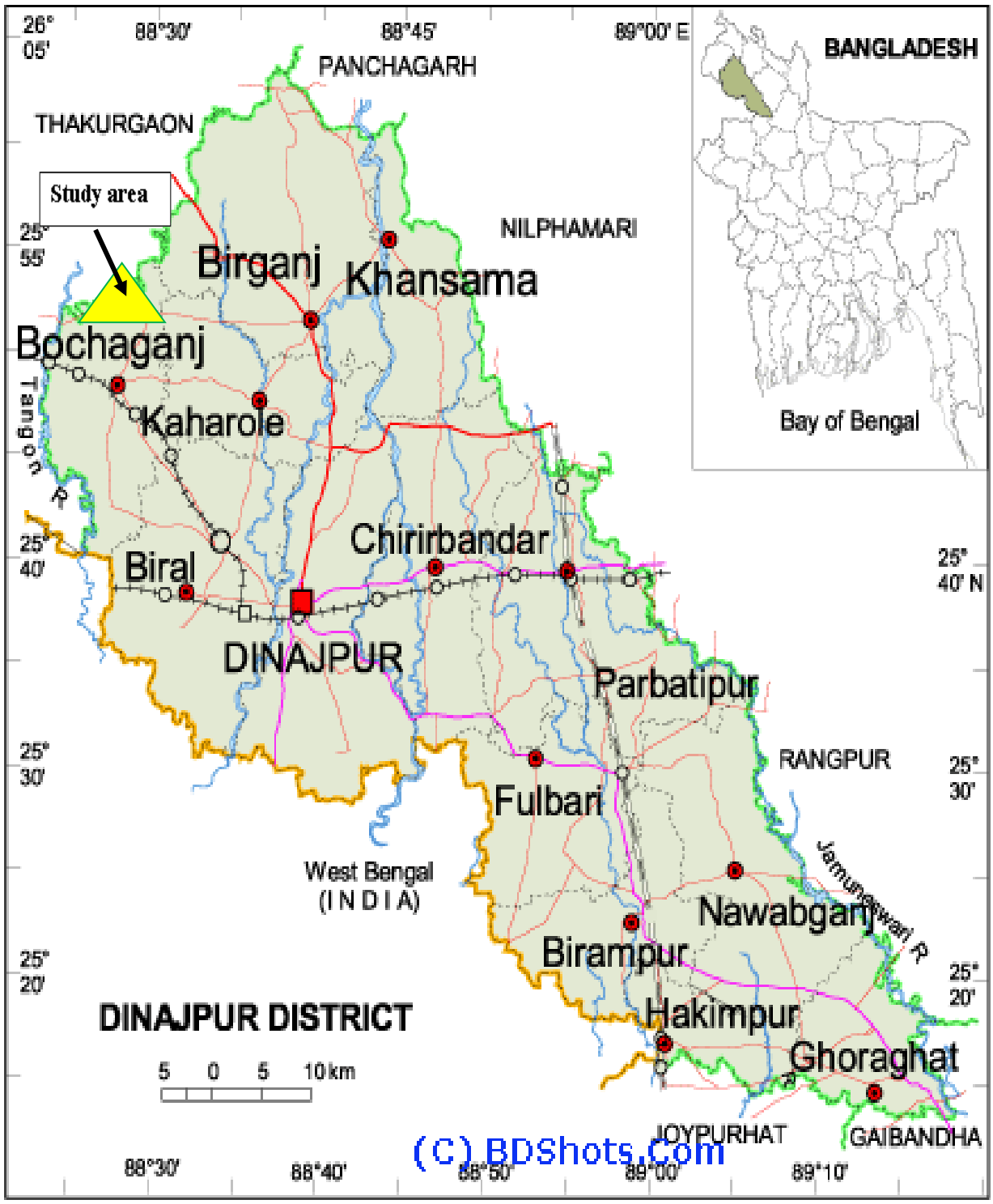

Figure 3.1 Map presenting the study zone of Bochaganj upazila of Dinjpur District.

3.3. Experimental period

The study was conducted from16th Septemberto15th December 2017.

3.4 Experimental design and feeding trial

Asian catfish (average weight 15±1.00 g) were procured from a hatchery in Dinajpur, Bangladesh, and were transported in oxygenated plastic bags filled with freshwater. The fish were acclimatized for two weeks prior to the commencement of the study and were fed to apparent satiation with a commercial feed. Thereafter, the fish (average body weight 21.23 ± 1.01 g) were randomly distributed into twelve cemented tank (200 cm × 100 cm × 100 cm) with a stocking density of 20 fish per tank. Three replicate tanks for each treatment were established. The fish were fed an experimental diet at a rate of 3% body weight. The daily ration was presented in equal portions at 09:00 and 5:00 for a period of 12 weeks. Faeces were regularly siphoned out. Tanks were cleaned fortnightly to reduce the risk of accumulation of nitrogenous waste.

3.5 Diet preparation

Four experimental diets were formulated to contain MOS 0 (control), 0.2%, 0.4%, and 0.6% MOS (International Food Grade, Laboratory of USA, Purity > 90%). The doses of MOS were carefully chosen based on the previous studies (Torrecillas *et al*., 2011; Do Wuuand Jones, 2014). Fish meal and soybean meal were used as a principle source of

protein, while soybean oil and fish oil used as lipid source. Corn starch and wheat flour were used as the carbohydrate source to ensure that the energy levels of all experimental diets were the similar (Phumee, 2011). Diets were prepared by thoroughly mixing feed ingredients (Table 3.1) for 30 minutes in a food mixer at a capacity of 5 kg. After addition of soybean oils and fish oil, the feed ingredients were mixed for an extra 10 minutes (Salaghi *et al*., 2013). Sufficient water was added to make dough which was then extruded through a pelletizing machine (Model MH 237, Miao Hsien Ltd, and Taichung, Taiwan) to make 3 mm diameter pellets. The pellets were air dried for 24 hours, and then manually broken into smaller pieces. The resultant pellets were then packed separately in plastic bags and stored in a freezer at -20^{o}C throughout the feeding trial.

Table 3.1 Ingredients used and proximate composition of the experimental diets containing varying levels of mannan oligosaccharide (MOS) (g kg^{-1})

Ingredients	Treatments			
	Control	0.2% MOS	0.4% MOS	0.6% MOS
Fish meal[1]	247.2	247.2	247.2	247.2
Soybean meal	280.1	280.1	280.1	280.1
Corn Starch	97.5	97.5	97.5	97.5
Wheat floor	270.0	268.0	266.0	264.0
Soybean Oil	32.60	32.60	32.60	32.60
Fish Oil	32.60	32.60	32.60	32.60

Vitamin mix[2]	20.0	20.0	20.0	20.0
Mineral mix[3]	20.0	20.0	20.0	20.0
MOS	0.0	2.0	4.0	6.0

[1]Danish fishmeal: crude protein, 720; crude lipid, 50.

[2] Vitamin mix kg^{-1} (Rovithai Ltd 700/437 Chonburi THAILAND): Vitamin A 50 MIU, Vitamin D_3 10 MIU, Vitamin E 130g, Vitamin K_3 10g, Vitamin B_1 10g, Vitamin B_2 25g, Vitamin B_6 16g, Vitamin B_{12} 100mg, Niacin 200g, Pantothenic Acid 56g, Folic Acid 8g, Biotin 500mg, Antioxidant 0.200g and Anticake 20g.

[3]Mineral mix kg^{-1}: Calcium phosphate (monobasic) 397.5 g; Calcium lactate 327 g; Ferrous sulphate 25 g; Magnesium sulphate 137 g; Potassium chloride, 50 g; Sodium chloride, 60 g; Potassium iodide, 150 mg; Copper sulphate 780 mg; Manganese oxide 800 mg; Cobalt carbonate 100 mg; Zinc oxide 1.5 g and Sodium selenite 20 mg.

Plate 3.1 Diet preparation

3.6 Tank preparation

Cemented tanks measuring (200 cm × 100 cm × 100 cm) (length× width × height) were used for this study under controlled conditions. Throughout the experiment, water was supplied from an overhead

tank. Tank was prepared properly by ensuring continuous circulation of freshwater. All physical and chemical parameters such temperature, light, dissolve oxygen (DO), p^H, NH_3 and NH_4^+ were maintained properly.

3.7 Experimental fish

Asian catfish fry (average weight 15±1.00 g) were procured from a hatchery in Dinajpur, Bangladesh, and were transported in oxygenated plastic bags filled with freshwater. The fish were acclimatized for two weeks prior to the commencement of the study and were fed to apparent satiation with a commercial feed.

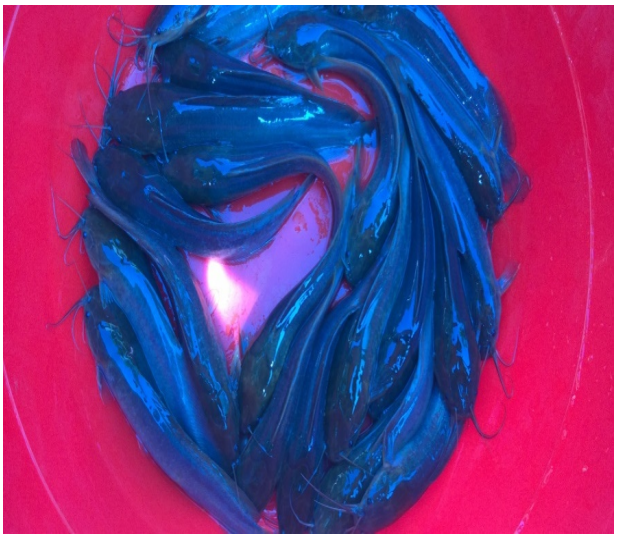

Plate 3.2 Experimental fish

3.8 Feeding

The respective experimental diet was supplied evenly all over the tank's surface two times a day at a rate of 3% body weight throughout the experimental periods. Because in the rate of 3% body weight fish was fulfilled their hunger and there was no wastage of feed. The feed was supplied evenly all over the tanks surface two times a day at 07:00 am, and 5:00 pm.

Plate 3.3 Feeding of the experimental fish

3.9 Water quality assessment

3.9.1 Procedure of the study

In the present study, water sample were collected from each tank. Recording on the spot data and collection of samples were made between 9.00 to 11.30 A.M. Water temperature, pH, DO, NH_4^+, NH_3were recorded every 15 days interval.

3.9.1.1 Temperature

Temperature of water was taken from each tank by using a standard mercury thermometer.

3.9.1.2 Determination pH

pH of water was taken from each pond by using a pH test kit.

3.9.1.3 Dissolved oxygen

The dissolved oxygen was measured by using digital DO5509 meter.

Plate 3.4 Measuring dissolved oxygen during study period

3.9.1.4 Ammonia

The ammonia content of water was measured using an ammonia test kit for freshwater.

3.9.1.5 Harvesting

Fishes were completely harvested after 75 days of nursing, Harvesting of fishes were performed by and dewatering of the tanks. During harvesting, the weights of all fish were also taken.

3.10 Growth performance and production of fish

The following growth performances and survival were evaluated using the following equations:

(i) Growth parameters

a) Weight gain (g)

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

b) % Weight gain

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

c) Specific growth rate (SGR % day^{-1})

The SGR is the momentary change in weight of fish calculated as the percent increase in body weight per day over a given time interval and is written as:

$$\text{SGR } (\% \text{ day}^{-1}) = \frac{\ln w_2 - \ln w_1}{T_2 - T_1} \times 100$$

Where,

W_1 = the initial live body weight (g) at time T_1 (day).

W_2 = the final live body weight (g) at time T_2 (day).

d) Survival rate (%)

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

ii) Feed conversion ratio (FCR)

Feed conversion ratio is defined as the amount of dry feed intake per unit live weight gain. It is calculated as:

$$\text{FCR} = \frac{\text{Total dry feed intake (g)}}{\text{Total wet weight gain (g)}}$$

To calculate FCR, the dry weight of the feed was obtained by using a correction for the analyzed moisture content of the diet. FCR is a measure of the degree of gross utilization of feed for growth.

iii) Protein utilization

Protein efficiency ratio (PER)

Protein efficiency ratio is defined as the gain in weight of fish per gram of crude protein fed. Protein efficiency ratio is calculated as:

$$\text{PER} = \frac{\text{Wet weight gain (g)}}{\text{Total protein intake (g)}} \times 100$$

PER gives an indication of the efficiency of utilization of dietary protein.

3.11 Production of fishes

Net production= No. of fish caught × average final weight (g).

3.12 Proximate composition analysis

Sampling procedure

At the end of the experiment, nine fish were then randomly selected from each replicate (fifteen fish per treatment) and brought to the laboratory for the determination of whole body proximate composition. The selected fish were killed by keeping them in a refrigerator. After being killed, the individual fish were weighed immediately and kept in an oven for drying. Thereafter, the dried fish were then grinned using a blender machine in order to determine the moisture, crude protein, crude lipid and ash content of whole fish body.

Whole fish body composition was determined by using the standard protocols as mentioned by the Association of Official Analytical Chemists (AOAC, 1997).

3.12.1 Moisture

The moisture content of the sample was evaluated by drying the samples in the Hot Air Oven at 105 ℃ for 12 hours until constant weight was obtained as explained in detailed in Appendix B.1. The loss of weight was calculated as percent moisture. The percent moisture content was calculated by applying the following equation (Bhattacharya, 2013):

$$\% \text{ of moisture} = E\backslash C \times 100$$

Where,

E= Weight of moisture. C= Weight of sample.

The moisture free samples were then used in order to determine the crude protein, lipid and ash content.

3.12.2 Crude protein

The crude protein content of the fish samples was calculated indirectly by determining the total nitrogen content of the sampled of the fish by a Kjeldahl method using kjeldhal apparatus. In this case, total nitrogen content was determined by digesting the sample with concentrated sulphuric acid (H_2SO_4), in presence of digestion mixture into boric acid as explained in detail in Appendix B.2. The total nitrogen value was then calculated by using the following formula:

$$\text{Nitrogen\%} = \frac{\text{ml. of titrant used} \times \text{normality of titrant} \times \text{milli equivalent weight of Nitrogen}}{\text{Weight of the sample (g)}} \times 100$$

The amount of crude protein was then calculated by multiplying the % of total nitrogen with the Protein conversion factor 6.25, which is generally used in calculating the animal protein content.

3.12.3 Lipid

The crude lipid content of the samples was determined by removing the lipid from the samples by homogenizing it in 60 ml of chloroform and methanol solution in a ratio of 2:1 (v\v) (Folch *et al.* 1957) and thereafter the solvent was evaporated by heating in the oven at 80 □C as explained in detail in Appendix B.3. The Lipid content of the fish samples was then determined by using following formula:

$$\text{Lipid content \%} = \frac{\text{Weight of beaker with lipid (g)} - \text{Weight of empty beaker (g)}}{\text{Weight of sample (g)}} \times 100$$

3.12.4 Ash

Muffle furnace, desiccator and an electronic balance were used to determine the ash content of the fish sample. The moisture free samples were taken in porceline basin made crucible and weighed. Thereafter the ash content was measured by igniting the samples in a muffle furnace at a temperature of 550 °C for 6 hours. The samples were then cooled in a desiccator. The average weight of each sample of the remaining material was taken as percentage as mentioned in Appendix B.4. Ash content of the whole fish body was then calculated by using the following formula:

$$\text{\% of ash} = \frac{\text{Weight of crucible with ash (g)} - \text{Weight of empty crucible (g)}}{\text{Weight of sample (g)}} \times 100$$

3.12.5 Determination body indices

At the end of the present experiment, body indices of magur such as leptosomatic index (HSI), intraperitoneal fat (IPF) and viscera somatic index (VSI) were determined by using the following formulae:

$$\text{Hematosomatic index (HSI\%)} = \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100$$

$$\text{Intraperitoneal fat (IPF\%)} = \frac{\text{Intraperitoneal fat weight (g)}}{\text{Body weight (g)}} \times 100$$

$$\text{Viscerasomatic index (VSI\%)} = \frac{\text{Viscera weight (g)}}{\text{Body weight gain (g)}} \times 100$$

3.13 Determination of haematological parameter

3.13.1 Blood collection

To determine the haematological parameters, the experimental fish were starved for 24 hours. Then three fish per replicate tank were randomly selected and immediately stabilized to reduce stress during handling.The collection of blood was carried out by inserting a 21 gauge needle attached to a 1 ml syringe into the muscle at the end of the anal fin until it reached the backbone. The collected blood was then transferred to a heparinized tube to prevent the blood from clotting for determining the ESR, PCV, RBC, Hb and WBC.

Plate 3.5 Collection of blood from fish

3.13.2 Determination of erythrocyte sedimentation rate (ESR)

The Erythrocyte Sedimentation Rate (ESR),also called a sedimentation rate or Westergren ESR, is the rate of red cells sediment in a period ofonehour.Erythrocyte sedimentation rate of heparinized blood sample was estimated according to the method of Westergrento perform the test, anti-coagulated blood placed in a microhematocrit tube also known as westergren tube was closed using critoseal and left to stand vertically for 1 hour. After 1 hour of the red blood cells has sedimented than the height of the plasma was measured and the ESR was measured and the ESR was presented as mm h^{-1}.

3.13.3 Determination of packed cell volume (PCV)

Packed cell volume(PCV) was count by using vortex machine. The blood samples were placed into the standard heparinized microhaematocrit capillary tubes until three quarters full and centrifuged immediately for 4 min at 10,000 g using a micro-haematocrit centrifuge. The PCV value was calculated using the following formula:

$$\text{PCV (\%)} = \frac{\text{height of packed red cells (mm)}}{\text{height of packed red cells and plasma (mm)}} \times 100$$

Plate 3.6 Determination of packed cell volume (PCV)

3.13.4 Total red blood cell count (Erythrocyte /RBC X 10^6 MM^3)

The blood sample was diluted 200 times with Nattand Herrick (1952) solution before counting red blood cells, 10 micro liter of the diluted blood sample was placed in a haemocytometer chamber and allowed to settle for 2-3 minutes under a microscope. The total number of red blood cells was calculated in 5 red blood cell square of the haemocytometer. The red blood cells was calculated according to the following formula

RBC (mm^{-3}) = (N ×5 × 10 × 200)

Where, N is the number of cells in 5 squares. A multiplication factor of 5 gives the number of cells in 1 mm^2 and multiplication factor of 10 brings the depth of the chamber from 0.1 to 1 mm. The dilution factor was 200.

Plate 3.7 Red blood and white blood cell counting

3.13.5 Total white blood cells count (Leukocute/WBC ×10^4 MM^3)

The white blood cells were measured using the Natt and Herrick (1952) solution as the diluent and were calculated in a haemocytometer. The blood samples were diluted 200 times with Natt-Herrick solution.WBC or LC was stated as thousands of cells per cubic millimeter, as follows.

$WBC\ (mm^{-3}) = LC \times 500$

Where,

LC is the number of cells in 4 mm^2 squares, and the dilution and volume correction factor is 500.

3.13.6 Glucose determination

Glucose was counted by using glucometer. Strip was added in glucometer chamber and one drop blood dropped into the strip by the syringe. The strip given the exact value of blood glucose.

Plate 3.8 Glucose determination

3.13.7 Haemoglobin (Hb) determination

Hemoglobin was determined by hemoglobinometer. In a generated hemoglobino meter tube taken with 0.1N hydrochloric acid up to the 0 marks.Sucked the thoroughly mixed blood up to 20µm in to the hemoglobinometerpipette. Carefully transferred the blood into the tube counting chloric acid. Mixed it well with the help of glass rod and set stand for 10-20 minutes. Diluted the colored acid by adding distilled water drop by drop with the help of dropper and mixed with the stirrer. Removed the stirrer and match the color of the mixture with the colored standard rod. Read the hemoglobin concentration (%) from the graduations of the tube 13.75% of hemoglobin is known as 100% hemoglobin content

3.14. Data analysis

All data were tested using one-way analysis of variance (ANOVA). Significant results ($P<$ 0.05) were further tested using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) to identify significant difference between means. The data were expressed as average ± SE and statistical analysis was performed using SPSS version 22 and Microsoft Office EXCEL for window.

CHAPTER 4

RESULTS

4.1 Water quality parameters

Water temperature, pH, dissolved oxygen, NH_4^+ and NH_3 were monitored in every two weeksfor a 12 weeks period, which were ranged between 17.66-18.42°C, 7.06-7.22 and 5.06-6.13 mg L^{-1}, 0.49-0.61 mg L^{-1}, 0.34-0.48 mg L^{-1} respectively (Table 4.1).

Table 4.1 Water quality parameters of the experimental tanks

Parameters	Treatments			
	Control	0.2% MOS	0.4% MOS	0.6% MOS
Temperature (°C)	17.92±0.09	18.23±0.10	18.42±1.04	17.66±0.36
pH	7.12±0.11	7.06±0.03	7.11±0.04	7.22±0.08
DO (mgL^{-1})	5.06±0.26	5.87±0.10	5.60±0.57	6.13±0.20
NH_4^+(mgL^{-1})	0.49±0.17	0.61±0.17	0.56±0.29	0.60±0.12
NH_3(mgL^{-1})	0.45±0.16	0.46±0.06	0.34±0.35	0.48±0.24

4.2 Growth performance and body indices

Growth performance, survival and body indices of Asian catfish juveniles fed with varying levels of MOS diets for 12-week are presented in Table 4.2. Generally, growth performance, such as

weight gain and SGR were dose dependent and significantly enhanced ($P < 0.05$) in fish fed 0.4% (21.21±1.16; 0.82±0.05) and 0.6% (18.66±0.96;0.77±0.04) MOS diets over the control (12.94±0.88; 0.57±0.04) fed group. The supplementation of MOS at 0.4% significantly influenced ($P <0.05$) the feed utilization in terms of FCR and PER compared to those fish that were fed with control and 0.2% MOS diets and which was not significant when cared with 0.6% MOS diet fed group.

The survival rate of Asian catfish fed with the control diet presented significantly lowest ($P <0.05$) when compared with the fish fed with MOS supplemented diets.With the exclusion of viscera somatic index (VSI), a significant variation was detected in Hepatosomatic index (HIS) and intra peritoneal fat(IPF) in the fish fed the MOS supplemented diets. Significantly lower ($P <0.05$) HSI was prominent when the fish fed 0.2% and 0.4% MOS diets compared to those fish fed control, but it did not differ from those fish fed 0.6% MOS diet. Similarly, IPF was significantly lowest ($P <0.05$) in those fish which fed 0.4% diets over the control fed group.

Table 4.2 Growth performance, feed utilization and body indices of juvenile Asian catfish, *Clarias batrachus,* fed a diet containing varying levels of mannan oligosaccharide (MOS) for 12 weeks.

Parameters	Treatments			
	Control	0.2% MOS	0.4% MOS	0.6% MOS
Initial Av. Wt.	21.01±0.54	21.60±0.5	21.61±0.82	20.68±0.5

		5		6
Final Av. Wt.	33.95±0.40[a]	38.48±0.75[b]	42.82±1.00[c]	39.34±0.73[b]
Wt. Gain	12.94±0.88[a]	16.88±1.16[b]	21.21±1.16[c]	18.66±0.96[bc]
SGR (%)	0.57±0.04[a]	0.69±0.05[ab]	0.82±0.05[b]	0.77±0.04[b]
FCR	4.71±0.28[d]	2.79±0.35[b]	1.92±0.15[a]	2.16±0.08[ab]
PER	0.59±0.03[a]	1.02±0.14[b]	1.45±0.12[c]	1.27±0.05[bc]
Survival Rate (%)	86.67±1.67[a]	95.00±2.89[b]	100±0.0[b]	98.33±1.67[b]
HSI (%)	0.75±0.08[b]	0.54±0.08[a]	0.42±0.02[a]	0.62±0.07[ab]
IPF (%)	0.55±0.04[b]	0.43±0.08[ab]	0.37±0.02[a]	0.44±0.02[ab]
VSI (%)	3.73±0.39	3.41±0.42	3.56±0.10	3.65±0.22

All values are presented as mean±SDobtained from three replicates tank, (n=3); Data with different superscripts in the same row indicate significant differences *(P < 0.05)*; SGR, Specific growth rate; FCR, Food conversion ratio; PER, Protein efficiency ratio; HSI, Hepatosomatic index; IPF, Intraperitoneal fat; VSI, Viscerosomatic index; MOS, Mannan oligosaccharide.

4.3 Whole body proximate composition

Proximate composition of whole body of fish fed with various concentrations of MOS is shown in Table 4.3. Significantly higher *(P < 0.05)* body protein content was detected in fish treated with 0.4% MOS diet (81.86±0.36) compared to the control group (78.32±0.36). Whereas, significantly lower *(P < 0.05)* lipid content was also detected in fish fed 0.4 % MOS diet when compared to fish that were fed a higher MOS diet at 0.6% and the control. Ash content was significantly highest *(P < 0.05)* in fish fed on 0.6% MOS related to those fish that were fed with control diet.

Table 4.3: Proximate composition of whole body of juvenile Asian catfish, (*Clarias batrachus),* fed varying levels of mannan oligosaccharide (MOS) for 12 weeks

Treatments	Parameters		
	Protein (%)	Lipid (%)	Ash (%)
Control	78.32±0.36^{a}	10.62±1.57^{bc}	5.06±1.08^{a}
0.2% MOS	80.31±1.41^{ab}	8.96±1.0^{ab}	5.77±0.07^{ab}
0.4% MOS	81.86±0.36^{b}	8.62±0.56^{a}	6.32±1.04^{ab}
0.6% MOS	79.99±1.16^{ab}	11.87±0.73^{c}	7.33±1.23^{b}

Data presented as mean±SD, (n=3).

Data with different superscripts in the same column indicate significant differences *(P<0.05).*

4.4 Glucose content of blood

The influence of dietary MOS supplementation on Asian catfish blood glucose content is shown in Figure 4.1. The blood glucose content results exposed that the highest concentration of blood glucose was detect in the blood of Asian catfish fed with highest concentration

(0.6%) of MOS which was not vary significantly fed with 0.4% MOS diet.

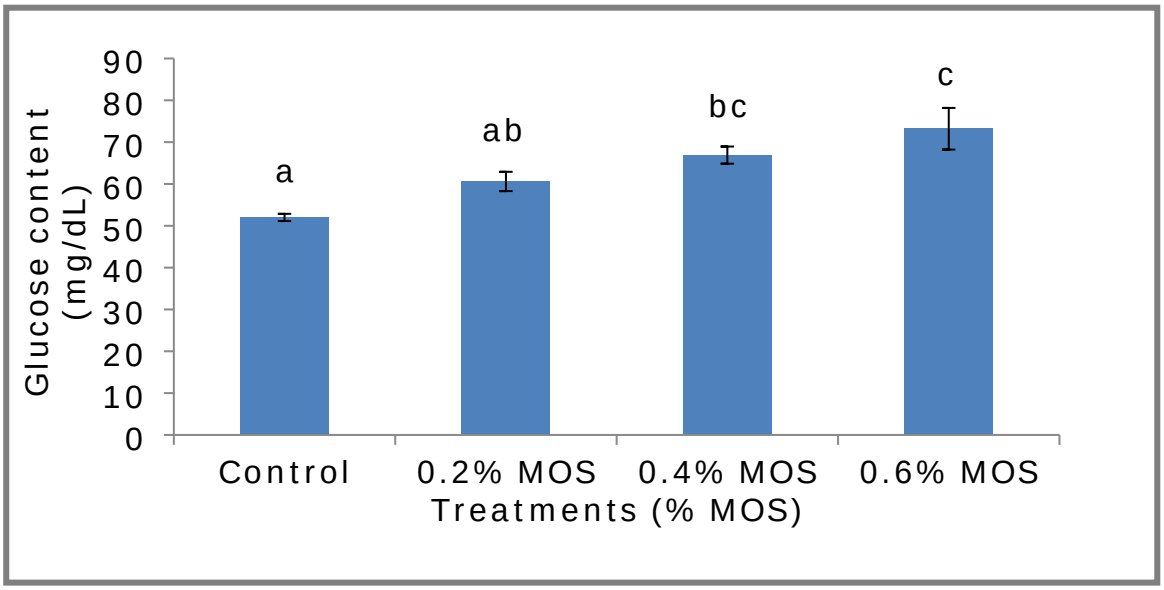

Figure 4.1 The glucose content of juvenile Asian catfish, *Clarias batrachus,* after 12 weeks feeding with experimental diets. Bars with different letter indicate significantly different *(P < 0.05)*. Data presented as mean±SE (n=6; 2 fish per replicate tank).

4.5 Haematological parameters

The influence of dietary MOS supplementation on Asian catfish blood profile is presented in Table 4.4. Among RBC and interrelated haematological parameters only ESR and PCV were significantly influenced by the eating of MOS supplemented diets. Significantly reduced *(P< 0.05)* ESR was prominent when the fish fed with higher concentration of MOS (0.4% and 0.6% MOS) diets compared to those fish that were fed with control and lowest concentration of MOS such as 0.2%. PCV significantly increased *(P < 0.05)* when fish fed with the 0.4% and 0.6% MOS diets compared to the control and 0.2% MOS

diet fed fish. A non-significantincrease (*P*> 0.05) of RBC and Hb content were found when juvenile Asian catfish fed with MOS supplemented diets.Feeding the fish with MOS supplemented diets showed significant influence *(P < 0.05)* on the WBC compared with the control diet.

Table 4.4 Haematological parameters of juvenile Asian catfish, *Clarias batrachus*, fed diets containing varying levels of MOS and control diets for 12 weeks

Haematological parameters	Treatment			
	Control	0.2% MOS	0.4% MOS	0.6% MOS
ESR (mm h^{-1})	1.23±0.08[b]	1.38±0.06[b]	0.65±0.08[a]	0.73±0.09[a]
PCV (%)	34.50±0.56[a]	36.50±0.67[a]	38.83±0.79[b]	39.00±0.77[b]
RBC (x10^6 mm^{-3})	3.97±0.14	4.13±0.26	4.15±0.09	4.34±0.10
Hb (gdL^{-1})	5.63±0.20	5.72±0.18	5.73±0.04	5.81±0.0.27
MCHC (gdL^{-1})	16.33±0.63	15.63±0.61	14.81±0.34	14.90±0.73
MCH (pg $cell^{-1}$)	14.22±0.47	14.24±1.32	13.84±0.31	13.41±0.70
MCV (μm^3)	87.71±4.47	90.70±6.48	93.83±3.71	90.14±2.76
WBC (x10^4 mm^{-3})	4.52±0.23[a]	5.97±0.18[b]	6.25±0.0.32[b]	5.88±0.28[b]

Data presented as mean±SE, (n=6; 2 fish per replicate tank). Data with different superscripts in the same row indicate significant differences *(P < 0.05)*.

CHAPTER 5

DISCUSSION

Prebiotics in aquaculture are used to improve fish growth and disease resistance, improving economic viability and sustainability of fish farming (Ringo *et al*. 2010). Several studies have shown that dietary prebiotics enhances growth and health of aquatic animals (Sakai, 1999; Bricknell and Dalmo, 2005; Mazlum *et al*. 2011). Better growth parameters in fish fed dietary MOS weredetected in rainbow trout (Staykov *et al*. 2007), European sea bass (Torrecillas *et al*. 2007, 2011) and gilthead sea bream *Sparus aurata* (Gultepe *et al*. 2011). However, dietary MOS in fish nutrition are still controversial since some studies did not observe developments on growth parameters.

Growth, feed efficiency and feed consumption of fish are usuallyfocused by few environmental factors (Fry *et al*. 1971). Good water quality is certainly a prerequisite for fish growth and their survival. However, the parameters were within the satisfactory range for fish culture (Jhingran, 1991).

In the present study, the average water temperatures were noted as 17.92±0.09°C, 18.23±0.10°C,18.42±1.04°C and 17.66±0.36°C in control, 0.2%, 0.4% and 0.6% MOS respectively. Previously, different studies reported the water temperatureranges from 25 to 35°C (Aminul, 1996), 26.93 to 27.41°C (Roy *et al.* 2002), 27.60 to 31.00°C (Rahman and Marimuth, 2010), 26.80 to 31.80°C (Kohinoor *et al.* 2016) and 27 to 28°C (Pasala *et al*. 2018). All those values were

within the suitable ranges for fish culture. From this context, it can be said that the experimental tanks were suitable for fish culture.

In addition, pH in the water body absolutely is asignificant factor for successful fish culture. In the current study, the average values of pH in the treatments were found as 7.12±0.11, 7.06±0.03, 7.11±0.04 and 7.22±0.08 in control, 0.2%, 0.4% and 0.6% MOS respectively; Rahman and Marimuth (2010) also noted that the proper range of pH value is 7.40-8.50 for endangered native fish climbing perch; 6.5 to 8.1 for benthic fauna (Shariful *et al.* 2009) and 5 to 9.03 carp SIS polyculture (Roy *et al.* 2002)According to Pasala (2018) pH 7.31 to 7.44 are appropriate for (*Labeo rohita*) fingerling culture. From this context, it can be said that the experimental tanks were appropriate for fish culture.

Usually successful fish culture depends on the careful management of dissolved oxygen at optimum level. DoF (1996) stated that the range of dissolved oxygen content for fish culture should be 5.0-8.0 mgL^{-1}. The average dissolved oxygen levels in the current study were 5.06±0.26, 5.87±0.10, 5.60±0.57 and 6.13±0.20 mgL^{-1} in control, 0.2%, 0.4% and 0.6% MOS respectively; The appropriate range of dissolved oxygen can be very depending on the species being cultured. Kohinoor *et al.* (2012) observed the dissolved oxygen content between 4.23-5.32 mgL^{-1} are acceptable for indigenous stinging catfish (*H. fossilis*) culture, while 4.13 to 4.71 $mgL^{-1}$are adequate for nursing of Thai Koi(*A. testudineus*) (Rahman *et al.* 2013). However, according to Wahab *et al.* (1995) dissolved oxygen

content of a productive pond should not be less than 4 mgL^{-1}. Therefore, the dissolve oxygen content in the present study was acceptable for fish culture.

However, ammonia concentration was foundas 0.45±0.16, 0.46±0.06, 0.34±0.35 and 0.48±0.24mgL^{-1}during the experimental period in control, 0.2%, 0.4% and 0.6% MOS respectively; The ammonia content may vary from 0.20 to 0.57 mgL^{-1} for Nile tilapia and freshwater prawn culture (Rahman, 2005) and 0.01 to 0.82 mgL^{-1} for freshwater prawn post larvae nursing (Asaduzzaman *et al.* 2006).Therefore, it might be concluded that the total ammonia content of this experiment was appropriate for magurnursing.

Body moisture, ash, protein contents of the Asian cat fish magur fingerlings were considerably differed ($P < 0.05$) among all the experimental treatments. This is notalike with the results on the other fish species including Atlantic salmon(Grisdale-Helland *et al*. 2008) rainbow trout and common carp (; Dimitroglou *et al.* 2011), grey mullet (Akbary *et al.* 2016).

However, in the present study protein concentration were 78.32±0.36, 80.31±1.41, 81.86±0.36 and 79.99±1.16 in control, 0.2%, 0.4% and 0.6% MOS respectively; lipid concentration were 10.62±1.57, 8.96±1.0, 8.62±0.56 and in 11.87±0.73 in control, 0.2%, 0.4% and 0.6% MOS respectively; ash concentration were 5.06±1.08, 5.77±0.07, 6.32±1.04 and 7.33±1.23 in control, 0.2%, 0.4% and 0.6% MOS respectively.Similar findings also have been statedfor common

carp (Mehrabi *et al.* 2018) and seabream (Gelibolu *et al.* 2018). A similar effect was described for gilthead sea bream (*S. aurata*) fed MOS supplemented fish meal based diets (Dimitroglou *et al.* 2010). Whole body protein content improved significantly ($P<0.05$) in fish given the 0.4% MOS supplement compared with control. In agreement with the result of this study, 0.4% MOS supplement was shown to increase body protein in Atlantic Salmon Smolts (*Salmo salar*) (Dimitroglou *et al.* 2011). In contrast, MOS supplementation showed to improve growth performance in gilthead sea bream but the body proximate composition continued unaffected (Dimitroglou *et al*. 2010; Gultepe *et al*. 2011).

Mannan oligosaccharide is derivative from the cell walls of the yeast *Saccharomyces cerevisiae* and is used as a dietary supplement in aquaculture (Wu *et al*. 2014) because of its capability to improve survival and immune-modulation functions (Zhou and Li, 2004; Staykov *et al.* 2007; Torrecillas *et al*. 2007, 2011; Salze *et al*. 2008; Sang and Fotedar, 2010). The current research is the first time attempt to demonstrate the effectiveness of MOS on the enhancement of growth and survival of juvenile Asian catfish. In the present study, the juvenile Asian catfishresponded positively to supplementation of the diet with 0.4% based on their significantly improved WG (21.21g), SGR (0.82), FCR (1.92), and PER (1.45) values compared to other treatments.

In the present study supplementation of MOS at 0.4% significantly influenced ($P <0.05$) the feed utilization in terms of FCR and PER

compared to those fish that were fed with control and 0.2% MOS diets and which was not significant when cared with 0.6% MOS diet fed group.

The better FCR found of grey mullet fingerlings in such species as hybrid striped bass Morone chrysops×M. saxatilis (Li and Gatlin 2004), rainbow trout *Oncorhynchus mykiss* (Staykov *et al*. 2007).

Previous studies have exposed variability in the response of fish and crustaceans to MOS supplementation: growth enhancement was observed in rainbow trout (Staykov *et al*. 2007), green tiger prawn (Genc *et al*. 2007), European sea bass (Torrecillas *et al*. 2007), Nile tilapia (Samrongpan *et al*. 2008), tropical juvenile spiny lobster (Sang and Fotedar, 2010), freshwater crayfish (Mazlum *et al*. 2011; Sang *et al*. 2011), and gilthead sea bream (Gültepe *et al*. 2011); however, thesewere not found in the Gulf of Mexico sturgeon (Pryor *et al*. 2003), channel catfish (Welker *et al*. 2007), Nile tilapia (Sado *et al*. 2008), Atlantic salmon (Grisdale-Helland *et al*. 2008), gilthead sea bream (Dimitroglou *et al*. 2010), or giant sturgeon (Mansour *et al*. 2012).

The response of fish to MOS supplementation in diets has not only shown species specific trend but is also dose dependent. In the current study the higher MOS concentration at 0.4% have positive influence on the growth performance of Asian catfish. In contrast, 0.2% MOS was sufficient to improve the growth performance of yellow catfish (*Pelteobagrus fulvidraco*) (Wu *et al*. 2014), whereas,

0.3% MOS showed better growth in the case of crayfish (Mazlum *et al*. 2011). The reasons for the different results are not clearly understood. Previous research reported that prebiotics particularly MOS can utilize varying responses in fish based on the basal diet consumed, inclusion level, source and purity of MOS, processing methods used in their manufacture, acclimation and culture period, animal characteristics (species, age, source), and hygienic conditions of the experiment (Pryor *et al*. 2003; Newman, 2007; Reza *et al*. 2009; Gültepe *et al*. 2011; Taati *et al*. 2011).

Growth in terms of weight gain of fingerlings of *C. Batrachus* was significantly higher ($P < 0.05$) in 0.4% MOS treatment compared to control feed. In this study, fish growth responded positively to increasing MOS levels up to 0.4%, whereas, HSI and IPF responded in an opposite manner.

As the physiological status of animals is greatly influenced by the existence, and proportion of blood circulating cells, currently, an increasing trend is noticeable in evaluating the haematological parameters as an indicator for identifying the stress responses to endogenous or exogenous changes in fish (Cataldi *et al*. 1998).

As the fish is poikilothermic animal, haematological parameters can be easily influenced by the environmental factors. Beside this, a range of other factors, including fish species, age, size, physiological conditions and dietary regime (such as quantity and quality of food, ingredients of diet, and sources of protein) have great influence on

the haematological parameters of fish (Barnhart, 1969; Lim *et al*. 2000; Irianto and Austin, 2002). The study on the effect of MOS on the Asian catfish hematology still rare.

In the current study, the outcome of most of the haematological parameters including RBC, Hb, MCHC, MCH and MCV were not significantly ($P > 0.05$) influenced by the intake of MOS diets in fish groups compared with the control diet. Similarly, many studies also stated that dietary MOS had no effect on haematological parameters of channel catfish (*Ictalurus punctatus*) (Welker *et al*. 2007); Nile tilapia (*Oreochromis niloticus*) (Sado *et al*. 2008); giant sturgeon (*Huso huso*) (Mansour *et al*. 2012); gilthead sea bream (*Sparus auratus*) (Gultepe *et al*. 2011).

Significantly lower ($P < 0.05$) ESR values in MOS fed diets (0.4% and 0.6%) were observed compared to the control and 0.2% MOS fed groups. As the higher ESR level could be associated with damaged RBC because of the poisons released by bacteria during infection (Al-Dohail *et al*. 2011). MOS supplementation at 0.4% and 0.6% exposed significantly ($P <0.05$) improved PCV compared to the control and 0.2% MOS fed groups. The significantly improved PCV indicates that MOS is safe for consumption and their effectiveness in improving health status, as a lower PCV is an indicator of unhealthy fish as a result of not eating properly or are suffering infections (Blaxhall, 1972). Significantly increased ($P < 0.05$) PCV was reported in carp juvenile (*Cyprinus carpio*) fed with a lower (0.1%) MOS level (Akrami *et al*. 2012).

However, the MOS supplementation showed a significant influence on the total WBC count compared with those fish fed with control diet. Similarly, a significantly ($P < 0.05$) elevated level of WBC was reported in *Labeo rohita* fed with 1% MOS diet (Andrews *et al*. 2009). Similar results have been reported in oligofructose-fed beluga juveniles (Hoseinifar 2011).

The increased WBC count results from stress enforced by fish as a result of daily feeding on b-glucan. (Harikrishnan *et al.* 2003) at the same time reported increased WBC counts in *C. carpio* after herbal treatment with *Azadirachta indica*. The various responses obtained on the haematological parameters as a result of feeding MOS diet might be due to different sources and purity of MOS, the concentration used, culture period and different species assessed (Akrami *et al*. 2012).

A non-significantly ($P > 0.05$) increasing trend of RBC and Hb content were noted with the increasing levels of MOS. Increased Hb content in the blood of Asian catfish also indicated improved health status as it directly linked to increase O_2 level in the blood.

Glucose content in blood also influenced by MOS. However, in the present study The blood glucose content results exposed that the highest concentration of blood glucose was observed in the blood of Asian catfish fed with highest concentration (0.6%) of MOS which did not differ significantly with 0.4% MOS diet (Ghobadi *et al*. 2015).

CHAPTER 6

SUMMARY AND CONCLUSION

This study was conducted to observe the effect of prebiotic mannan oligosaccharide on growth and health status of Asian catfish (*C. batrachus*). The water quality parameters and growth performance of Asian catfish (*C. batrachus*) was investigated under the experimental period. Water temperature was recorded as 17.92° to 23.00°C; pH 7.06 to 7.11, dissolved oxygen 5.06 to 6.13mgL^{-1}; ammonia 0.35 to 0.45 mgL^{-1} and ammonium ion 0.49 to 0.62. Observed all the water quality parameters of this current study were suitable for successful culture of Asian catfish.

For estimating the growth performance, sampling was done and the weight (g) of magur were recorded. At the end of the experiment, the average weight gain of Asian catfish were incase of control 12.94±0.88, 0.2% MOS 16.88±1.16, 0.4% MOS 21.21±1.16 and 0.6% MOS 18.66±0.96 respectively.

The survival rates under the four treatments were in control 86.67±1.67, 0.2% MOS 95.00±2.89, 0.4% MOS 100±0.0 and 0.6% MOS 98.33±1.67% respectively.

For estimating the effect of MOS supplemention on the haematological parameters of Asian catfish sampling was done and the results were recorded. The ESR (mm h^{-1}) of Asian catfish magur

were incase of control 1.23±0.08, 0.2% MOS 1.38±0.06, 0.4% MOS 0.65±0.08 and 0.6% MOS 0.73±0.09 respectively.

The PCV (%) were in control 34.50±0.56, 0.2% MOS 36.50±0.67, 0.4% MOS 38.83±0.79 and 0.6% MOS 39.00±0.77 respectively.

The RBC ($x10^6$ mm^{-3}) were in control 3.97±0.14, 0.2% MOS 4.13±0.26, 0.4% MOS 4.15±0.09 and 0.6% MOS 4.34±0.10 respectively.

WBC ($x10^4$ mm^{-3}) were in control 4.52±0.23, 0.2% MOS 5.97±0.18, 0.4% MOS 6.25±0.0.32 and 0.6% MOS 5.88±0.28 respectively.

In conclusion, the results observed in this study clearly indicated that MOS is a beneficial dietary supplement, appears to have a more positive influence on the enhancement of the growth performance and feed utilization of Asian catfish juveniles. It is also able to improve some important haematological parameters, which have direct effect on the non-specific immune response of fish. The results of the present study exposed that MOS supplementation at 0.4% appears to be most effective dose in influencing the growth performance, survival, feed utilization and haematological parameters of Asian catfish. Therefore, the results suggest that the inclusion of MOS at 0.4% concentration can be effectively used as a feed additive for Asian catfish (*C. batrachus)* juvenile's culture.

In order to validate the findings of this research more culture trials will be needed. As a follow up to this current research, future researches proposed are as follows:

- Elucidating the possible pathways and mechanism of action(s) of prebiotic MOS in the digestive and absorptive processes within the intestine of Asian catfish (*C. batrachus*) to stimulate the growth performance and the immune responses.
- The Maximum duration necessary to feed the supplemented diets for sustained effectiveness after reverting to the control diet.
- The changes in immune-relevant gene expression of Asian catfish (*C. batrachus*) exposed to pathogenic bacteria.

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APPENDICES

APPENDIX A: WATER QUALITY ANALYSIS

A.1 Analysis of ammonia

- Removed the cap from the plastic beaker. Rinsed the plastic beaker with water sample before filling it up to the 10 ml mark.
- Added 2 drops of Ammonia Reagent 1 for fresh water replace the cap and mix by carefully swirling the beaker in tight circles.
- Added 8 drops of Nessler Reagent replace the cap and mix by carefully swirling the beaker.
- Removed the cap and transfer the solution into the color comparator cube. Wait for 5 minutes to allow color to develop.
- Determine which color matches, the solution in the cube, and record the results in mgL^{-1}(ppm).
- It is better to match the color with a white sheet of about 10 cm behind the comparator,

APPENDIX B: PROXIMATE COMPOSITION ANALYSIS (AOAC)

B.1 Determination of moisture content

- At first marking the empty petridish according to the sample used, weighed out these petridish by using an electronic balance and was recorded.
- Then the sample ingredients were weighted in the clean weighted petridish by using the balance.
- Then the samples were placed in a hot air oven at 105°C for 12 hours.
- After 12 hours the samples were carefully taken out from the oven by using a specialized forceps and were kept in a desiccator for cooling.
- Finally the weight of each sample with petridish was taken again.
- The differences in weight were represents the moisture content of the samples.
- The percent moisture content of whole body fish sample was calculated by using the following formula:

% of moisture = E\C × 100

Where,

E= Weight of moisture. C= Weight of sample.

B.2 Determination of crude protein (Kjeldhal method)

- The sample as much as 0.1 g is weighted on ash free paper and recorded.
- Each sample is made in triplicate.
- The sample is then taken in Kjeldahl flasks and numbered.
- One teaspoon of catalyst containing K_2SO_4 + $CuSO_4$ and 25 ml sulfuric acid is added to each Kjeldahl flask.
- Then, Kjeldahl flasks are placed on Kjeldahl Digestion unit to digest the sample.
- The flasks are heated for 20 minutes at 250°C and shaken properly.
- The temperature is increased up to 350-380°C for 1-2 hour, or until the color of the solution become light green.
- The samples, then, are digested for another 45 minutes.
- The heater is switched off and left for 10-20 minutes until all the flasks cool down to room temperature.
- The flasks are then connected to the Kjeldahl Distillation unit. 300 ml distilled water is added to each flask automatically in the distillation unit.
- For the distillation process, 100 ml of 40% NaOH is added automatically from the jar contain 40% NaOH in each Kjeldahl flask.
- 25 ml of boric acid and 2-3 drops of indicator (methylene red + bromocresol blue) are prepared in Erlenmeyer flasks, connected to the end duct of the apparatus.

- Make sure the duct is immersed into the solution during the distillation.
- The distillation process is conducted until as much as 75 ml boric acid solution is collected.
- All the distillates are titrated with 0.1 N HCL until the color of the solution turn grayish blue.
- Record the volume of HCL used for titration. Repeat the analysis triplicate for each sample.
- Use the following formula for the calculation of crude protein content

$$\%\text{Nitrogen} = \frac{\text{ml. of titrant used} \times \text{normality of titrant} \times \text{milli equivalent weight of Nitrogen}}{\text{Weight of the sample}} \times 100$$

% Crude protein in sample = % Nitrogen × 6.25

B.3 Determination of crude lipid

- About 1 g of each sample is weighted in triplicate. The sample is homogenized with 60 ml of chloroform: methanol solution at the ratio of 2:1 for 2 minutes.
- Homogenate sample is filtrated through Buchner flask using filter paper (whatman qualitative No.1).
- Fat free residue left on the filter paper. The sample is washed with 40 ml solvent (chloroform: methanol) and transferred to a

separating funnel. 20 ml distilled water is added and the mixture is shaken properly for 1 minute.

- The funnel is vertically left for separation of two phases.
- The upper phase is a mixture of distilled water and methanol, the lower phase is extracted lipid and chloroform.
- After 2-3 hours, the lower phase is collected in a beaker which has been weighed. The beaker together with the sample inside is placed in the oven at 80°C to evaporate the chloroform.
- After 4 hours, the beaker with the dried lipid is taken out and kept in the desiccator until cool. Record the beaker weight. Crude lipid is calculated as:

$$\% \text{ Crude lipid} = \frac{\text{Weight of beaker with lipid (g)} - \text{Weight of empty beaker (g)}}{\text{Weight of sample (g)}} \times 100$$

B.4 Determination of ash content

- The porcelain crucible is dried in the oven at 100 °C for 1 hour.
- After cool it in a desiccator, the weight of each crucible is recorded. About 1 g of sample is weighed and put in the crucible.
- The crucible together with sample is heated up in the muffle furnace at 550°C for 5 hours. After 5 hours, the furnace is switched off.
- After cool down the furnace the crucible is taken out and left in the desiccator until cool.

- The weight of porcelin crucible with the remaining ash is recorded.
- % ash is calculate

$$\% \text{ Ash} = \frac{\text{Weight of crucible with ash (g)} - \text{Weight of empty crucible (g)}}{\text{Weight of sample (g)}} \times 100$$

C. Haematological parameters analysis

C.1 Red blood cells count

Chemicals:Natt-herrick solution

Apparatus: Haematocytometer, microscope,micropippets,tips,heparinizedvaccutainers.

Procedure

- The blood sample was collected from the caudal vein and transferred to a heparinzedmicrotainer.
- The blood was diluted with 1 to 200 of in Natt-Herrick solution (5µl blood in 995 µl diluent).
- The suspension was well mixed gently by pipetting it up and down for a few minutes.
- The cover side was then placed on the surface of the haemacytometer chamber.
- The chamber was filled with 10 µldiluted blood by holding the pipette at an angle of 45^{o} and by lightly touching the tip against the edge of the cover slip.(Ensure that the chamber must not overflow into the channels.Too much fluid in the chamber could raise the cover glass, thereby causing a variation in the depth and resulting in errors).
- The chamber was fixed under the microscope and the cells were allowed to settle for several minutes by using 40x magnification (4mm objective and × 10

eye piece) and the objective was focused on the square millimeter of the counting chamber and the cells counted in five squares (1/25 mm^2 each),the four corner squires and the central one of the ruled area.

- The final results were expressed as the number of cells per cubic millimeter, as follows:

Number of cells per mm^3 = N × 5 × 10 × 200

Number of cells as millions per mm^3 =(N × 5 × 10 × 200)/10^6

RBC (10^6 /mm^3) = N/100

Where,

N = Number of cells counting in the 5 squares.

5 = multiplication factor to give the number of cells in mm^2

10 = multiplication factor to bring the depth of the chamber from 0.1 to 1mm.

200 = the dilution factor.

For more explanation

Chamber depth = 0.1 mm

Chamber volume = 0.1 microliter = 0.1 mm^3

The counting chamber (central box)

Area = 1mm^2

Volume = 0.1 mm^3

Contains 25 squares (1/25 each)

C.2 White blood cell count

Chemicals: Natt-Herrick solution

Apparatus: Haemactometer, microscope, micropippets, tips, heparinized microtainers.

Procedure

- The blood sample was collected from the caudal vein and transferred to a heparinized microtainer.
- The blood was diluted with 1 to 200 of Natt-Herrick solution (5 µl blood in 995 µl diluent).
- The suspension was well mixed gently by pippeting it up and down for a few minutes.
- The cover slide was then placed on the surface haemacytometerchamber.
- The chamber was filled with 10 µl diluted blood by holding the pippet at an angle of 45^{o} and by lightly touching the tip against the edge of the cover slip. (Ensure that the chamber must not overflow into the channels.too much fluid in the chamber could raise the cover glass, thereby causing a variation in the depth and resulting in errors).
- The chamber was fixed under the microscope and the cells were allowed to settle for several minutes.by using 40x magnification (4mm objective and × 10 eye piece) and the objective was focused on the square millimeter of the counting chamber and the cells counted in

four(1 mm^2 each),the four corner squires and the central one of the ruled area.

- The final results were expressed as the number of cells per cubic millimeter, as follows:

Number of cells per mm^3= LC × 500

Where,

LC = The number of cells in 4 mm^2 squares

500 = dilution and volume correction factor.

L/C mm^3= Total number of cells counted in the four squares of $1mm^2/4/10 \times 1/200$

LC/ mm^3 = LC in 4 $mm^2 \times$ 2000 / 4

LC/ mm^3 = LC in $4mm^2 \times$ 500.