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THE DIETARY CHITOSAN POSITIVELY MODULATES THE GROWTH AND SURVIVAL OF *BARBONYMUS GONIONOTUS* FRY

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Abstract

The dietary chitosan has been largely used in the supplemental diets of terrestrial animals to improve growth and development. However, there is little information on the roles of dietary chitosan in growth and development of aquatic animals like fishes. Thus, this study aimed at determining the effects of dietary chitosan on growth performances and survival of *Barbonymus gonionotus* fry. A total of 600 *B. gonionotus* fry (2.12±0.02 g) were considered for this study. This experiment consisted of four treatments (0, 1, 2 and 3 g Kg^{-1} formulated feeds) and each treatment had three replications with a stocking density of 50 fry/tank. The dietary chitosan was supplied as 8% of body weight of each fry twice daily for 60 days. Fish fry treated with the dietary chitosan exhibited significant ($P < 0.05$) improvement in growth (body weight gain, % body weight gain, specific growth rate, and feed conversion ratio) and survival of *B. gonionotus* in comparison with untreated control. The water quality parameters, such as temperature, pH and dissolved oxygen showed no significant variations, and maintained suitable range throughout the study period for fish growth. Among the treatments, application of 1 g chitosan kg^{-1} feed showed the highest positive effects on growth and survival of *B. gonionotus* fry, indicating their potentials for practical application in promoting sustainable aquaculture.

Keywords: Diets, aquatic animal, formulated feeds, FCR, fish growth.

Introduction

Aquaculture is a promising source of animal nutrition which plays a significant role in fulfilling the global fish food demand over the decades. It contributes about 51.4 million tonnes of fish food which is 47% of the world's total fish production (FAO, 2018). Due to high consumption rate, the demand for fish food is increasing while the open water fisheries resources are diminishing day by day (Abdel-Ghany and Salem, 2020). In these circumstances, inland aquaculture has the abilities to ensure the sustainable supply of animal nutrition throughout the year. In Bangladesh, carp species contributes about 1.2 million tonnes of fish food throughout the year that comprises almost 33% of country's total fish production (BBS, 2016). *Barbonymus gonionotus* (Bleeker, 1850) commonly known as silver barb belonging to the family Cyprinidae is the most popular and economically important exotic food

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fish in Bangladesh for its good taste, fast growth, highest protein content, bright silvery appearance and high yielding potential (Mollah *et al*., 2011; Zaman *et al*., 2014). In traditional culture practices of *B. gonionotus*, lack of effective formulated fish feeds are the main constraint to the sustainable as well as energetic fry management, which increases the fry mortality rate under poor culture conditions. Use of immunostimulants as a feed supplements increase the survival rate and non-specific immunity in aquaculture (Anderson, 1992). Therefore it is important to explore the potential immune stimulator as a promising source of feed supplement in fish culture practices which may improve immunity against unfavorable culture conditions to sustain the production of healthy and quality fry.

Chitosan is a linear homopolymer of β-(1,4)-2-amino-2-deoxy-D-glucose attained from N-deacetylation of chitin, a naturally occurring immunostimulants and the second abundant polysaccharide after cellulose, which act as a potential growth promoter and also known as a vital ingredient for the improvement of growth during aquaculture practices (Jolles and Muzzarelli, 1999; Niu *et al.,* 2011). Chitosan enhances the absorption of essential nutrients and promote growth performance by developing the morphological structure of the small intestine (Shi *et al*., 2005; Zaki *et al*., 2015). Multiple researches on different fish species confirmed that optimal level of chitosan improved growth performances of fish (Najafabad *et al*. 2016; Chen *et al*., 2014; Geng *et al.*, 2011; Wang and Li, 2011; Abd El-Naby *et al*., 2019; Akbary and Younesi, 2017). Moreover, the dietary chitosan has already been proved as a potential immunostimulant in a number of fish species all over the world (Meshkini *et al*., 2012; Ranjan *et al*., 2014; Akbary and Younesi, 2017; Sajid *et al*., 2010; Alishahi *et al*., 2014). The survival rate of fish fry in the hatcheries are often not at the desirable level because of unexpected seed mortality. Some hatcheries use imported probiotic bacteria for reducing seed mortality which is sometimes resistance in the gut of fish. In this aspect, prebiotic dietary chitosan could be used as viable and eco-friendly agents in enhancing growth performance and survival of fish fry. Therefore, the objectives of the present study were to assess the effect of the prebiotic dietary chitosan on growth and survival of *B. gonionotus* fry.

Materials and Methods

Collection of fish fry

A total of 600 (initial mean weight 2.12±0.02 g) of *B. gonionotus* fry were collected from a mini hatchery of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur. The fry were reared in the Wet Laboratory, Faculty of Fisheries, BSMRAU. The fry were fed with commercial diet before starting of feeding trial.

Chitosan collection

Chitosan was collected from a well-recognized pharmaceutical company named research-lab fine Chem Industries, Mumbai, India (Cat. No. 2095A).

Experimental design

This experiment was designed according to completely randomized design (CRD) and this experiment was divided into four treatments, including control, T1, T2 and T3. Each treatment had three replicates. A

total of 12 plastic tanks (300 L) were used for fry rearing and each tank was considered as a replication. Fish fry were stocked at 50 fry tank^{-1} by random selection. Fish fry fed feed the dietary chitosan control (0g chitosan kg^{-1} diet), T1 (1g chitosan kg^{-1} diet), T2 (2g chitosan kg^{-1} diet) and T3 (3g chitosan kg^{-1} diet). The experimental system had the facility of good inlet, outlet, aerators and shed.

Feed formulation

The indigenous ingredients (maize flour, soybean meal, mustard oil cake, and wheat flour), fish meal and vitamin premix were used to formulate the feeds. All ingredients were ground using blender, mixed with one another properly. The formulated feed contained about 37% protein as determined using Pearson's square method (Table 1). Then mixture of ingredients was divided into 4 parts to use for 4 treatments. Required amount of chitosan was added in each treatment. Dough were prepared with required amount of water. Pellets were prepared and dried under sunlight. After drying, the pellets were ground using blender according to the mouth size of fry. The pellet feeds were kept in air tight container. The proximate composition of formulated feed was analyzed according to AOAC (1980) (Table 2).

Rearing and feeding trial with chitosan incorporated diets

Fish fry were reared for 60 days and fed with chitosan mixed at feed 8% of body weight twice daily (9:00 AM and 17.00 PM). Uneaten feeds were collected in the morning from each tank. Water of the tank was renewed every two days.

Table 1. Composition of experimental feed with the graded level of chitosan for rearing of fry *B. gonionotus*

Ingredients	Inclusion level (%)			
	Feed 1 (Control)	Feed 2 (T1)	Feed 3 (T2)	Feed 4 (T3)
[1]Fish meal (%)	28.90	28.90	28.90	28.90
[2]Soybean meal (%)	28.90	28.90	28.90	28.90
[3]Mustard oil cake (%)	15.00	15.00	15.00	15.00
Maize flour (%)	12.18	12.18	12.18	12.18
Wheat flour (%)	10.00	10.00	10.00	10.00
Molasses (%)	5.00	5.00	5.00	5.00
[4]Vitamin premix (%)	1.00	1.00	1.00	1.00
[5]Chitosan (g)	0.00	1.00	2.00	3.00

[1]Locally purchased, crude protein 70%, crude lipid 9%; [2] Mega Feed Limited, Bangladesh, crude protein 49%, crude lipid 20%; [3] Locally purchased, crude protein 40%, crude lipid 20%; [4] Vitamin premix (mg/kg diet): thiamin, 25; riboflavin 20; pyridoxine 21; cyanocobalamine, 0.03; folic acid 5; calcium pentothenate, 45; inositol, 100; niacin 100; biotin 0.1; starch, 3400; ascorbic acid, 100; Vitamin A, 100; Vitamin D, 20; Vitamin E, 50; Vitamin K, 12.

[5] Collected from Chem Industries, Mumbai, India, (Cat. No. 2095A)

Table 2. Proximate composition of different feeds (dry matter basis) for rearing fry of *B. gonionotus*

Treatment	(%) Dry matter	(%) Lipid	(%) Protein	(%) Ash	(%) Crude Fiber	(%) Carbohydrate
Control	88.87	10.05	37.05	14.15	6.3	32.40
T1	88.92	9.84	37.23	14.21	6.18	32.38
T2	88.85	10.08	37.11	14.30	6.09	32.45
T3	88.82	10.17	37.15	14.5	5.87	32.26

Sampling of fish fry were done every 15 days intervals. Uneaten diets were collected to correct feed intake and feed conversion ratio (FCR). Feed adjustments were done for each tank every 15 days after sampling

Water quality parameter

Everyday the water quality parameter such as water temperature, water pH, dissolved oxygen (DO) of water were recorded. A digital DO and thermometer (LUTRON PDO-519, TAIWAN) were used to check the temperature and DO of water. A digital pH meter (EZODO, Taiwan pH 5011) was used for the measurement of pH of water.

Growth parameters

For the determination of the effects of chitosan incorporated diets in the growth of fry of fish, weight gain (WG), % BWG (percent body weight gain), SGR (specific growth rate) were measured. Feed utilization were determined through measuring FCR (feed conversion ratio).

Weight gain was calculated from the following equation:

$$WG\ (g) = Wf - Wi$$

% Body weight gain was calculated from the following equation:

$$\%\ BWG = \frac{Wf - Wi}{Wi} \times 100$$

Specific growth rate was calculated from the following equation:

$$SGR\%\ /day = (Ln\ Wf - Ln\ Wi) \times 100/t$$

Where,

Wi = The initial body weight (g) of fish fry

Wf = The final body weight (g) of fish fry

Feed conversion ratio calculated from the following equation,

FCR= Feed taken by fish fry (g)/ Weight gain of fish fry (g)

Survival rate

After 60 days of experiment, the survival rate of fish fry was calculated from the following equation:

$$\text{Survival rate } (\%) = \frac{(\text{Total no. of fish fry at the end of experiment})}{(\text{Total no. of fish fry at the start of experiment})} \times 100$$

Statistical analysis

Collected data such as water quality parameters, WG, %BWG, SGR, FCR and survival rate of fish fry were statistically analyzed using ANOVA (one-way analysis of variance) test between means. Standard deviation (±SD) was calculated to identify the range of means. All statistical analyses were performed with the aid of computer software Statistix 10.0 version.

Results and Discussion

Water quality parameter

The water quality parameters such as water temperature, dissolved oxygen and pH of fish fry rearing tanks were measured in the morning and evening as shown in Table 3. The water temperature ranged from 28.03 ^{0}C to 29.8 ^{0}C while the water pH ranged from 6.25 to 6.60 and DO from 4.1 to 4.44 mg/L (Table 3). Interestingly, there were no significant variation in water quality parameter

Table 3. The observation of water quality parameter including water temperature, pH and DO in the morning at 9:00 AM and evening at 5:00 PM during the experimental period

Treatments	Parameters	No. of sampling				
		Initial	1st	2nd	3rd	4th
Control	Temperature (°C)	M-29.13^a± 0.05 E-28.43^a± 0.05	M-29.4^a± 0.11 E-28.3^a± 0.04	M-29.05^a± 0.07 E-28.33^a± 0.02	M-29.13^a± 0.02 E-28.03^a± 0.01	M-29.1^a± 0.02 E-28.27^a± 0.03
	pH	M-6.47^a± 0.2 E-6.34^a± 0.23	M-6.6^a± 0.27 E-6.27^a± 0.03	M-6.42^a± 0.3 E-6.34^a± 0.22	M-6.29 ^{a} ± 0.17 E-6.25^a± 0.20	M-6.34^a± 0.18 E-6.32^a± 0.15
	DO (mg/l)	M-4.3^a± 0.15 E-4.33^a± 0.3	M-4.3^a± 0.1 E-4.34^a± 0.09	M-4.37^a± 0.2 E-4.38^a± 0.16	M-4.38^a± 0.22 E-4.39^a± 0.13	M-4.42^a± 0.3 E-4.43^a± 0.26
T1	Temperature (°C)	M-29.17^a± 0.01 E-28.4^a± 0.03	M-29.09^a± 0.01 E-28.32^a± 0.03	M-29^a± 0.1 E-28.3^a± 0.08	M-29.11^a± 0.01 E-28.03^a± 0.09	M-29.5^a± 0.013 E-28.15^a± 0.03
	pH	M-6.34^a± 0.16 E-6.26^a± 0.18	M-6.5^a± 0.22 E-6.4^a± 0.28	M-6.47^a± 0.16 E-6.38^a± 0.18	M-6.31^a± 0.17 E-6.27^a± 0.09	M-6.33^a± 0.10 E-6.31^a± 0.3
	DO (mg/l)	M-4.4^a± 0.23 E-4.3^a± 0.3	M-4.35^a± 0.24 E-4.4^a± 0.32	M-4.4^a± 0.25 E-4.3^a± 0.19	M-4.4^a± 0.3 E-4.2^a± 0.23	M-4.44^a± 0.16 E-4.43^a± 0.5
T2	Temperature (°C)	M-29.13^a± 0.11 E-28.35^a± 0.03	M-29.21^a± 0.15 E-28.33^a± 0.09	M-29.45^a± 0.12 E-28.2^a± 0.03	M-29.05^a± 0.04 E-28.7^a± 0.06	M-29.8^a± 0.09 E-28.13^a± 0.05
	pH	M-6.32^a± 0.26 E-6.3^a± 0.4	M-6.27^a± 0.17 E-6.25^a± 0.2	M-6.42^a± 0.19 E-6.34^a± 0.25	M-6.29^a± 0.3 E-6.26^a± 0.24	M-6.31^a± 0.27 E-6.3^a± 0.22
	DO (mg/l)	M-4.3^a± 0.09 E-4.4^a± 0.11	M-4.37^a± 0.16 E-4.39^a± 0.1	M-4.3^a± 0.2 E-4.1^a± 0.15	M-4.41^a± 0.13 E-4.42^a± 0.09	M-4.42^a± 0.17 E-4.45^a± 0.11
T3	Temperature (°C)	M-29.2^a± 0.07 E-28.08^a± 0.03	M-29.5^a± 0.05 E-28.33^a± 0.03	M-29.17^a± 0.04 E-28.29^a± 0.06	M-29.6^a± 0.14 E-28.6^a± 0.13	M-29.34^a± 0.15 E-28.56^a± 0.07
	pH	M-6.32^a± 0.15 E-6.25^a± 0.21	M-6.47^a± 0.16 E-6.33^a± 0.24	M-6.43^a± 0.3 E-6.34^a± 0.2	M-6.32^a± 0.18 E-6.27^a± 0.13	M-6.33^a± 0.16 E-6.31^a± 0.12
	DO (mg/l)	M-4.35^a± 0.17 E-4.36^a± 0.09	M-4.38^a± 0.25 E-4.4^a± 0.22	M-4.3^a± 0.21 E-4.38^a± 0.11	M-4.41^a± 0.1 E-4.43^a± 0.21	M-4.43^a± 0.16 E-4.42^a± 0.18

Here, Morning, M; Evening, E; Values are expressed as mean. Different letters on the rows indicate significant difference by LSD test ($p < 0.05$); ($n = 3$)

among the treatments during experiment. These results indicate that the water quality parameters were in suitable range throughout the study period which is supported by Khan *et al*. (2018) and Jahan *et al*. (2020).

Effects of chitosan on growth performances and survival of fry of *B. gonionotus*

In order to assess the effect of dietary chitosan on growth parameters and survival, the fish fry were fed chitosan treated formulated feeds for 60 days. After 15, 30, 45 and 60 days, chitosan treated formulated feeds significantly ($p<0.05$) enhanced the weight gain in T1, T2 and T3 compared to control without the dietary chitosan in diet (Fig. 1A). Significantly ($p<0.05$) highest weight gain (10.55g) was found in T1 where fish fry fed feed with 1g chitosan kg^{-1} (Fig. 1A) and the lowest weight gain (8.84 g) was found in the control group. Similarly, significantly the highest %BWG (404.68%) and SGR (%/day) (2.69%) were recorded in T1 (fish was fed feed 1g chitosan kg^{-1}) compared to other chitosan treated treatments T2 (fish was fed feed 2g chitosan kg^{-1}), T3 (fish was fed feed 3g chitosan/kg) or control (fish was fed feed 0g chitosan/kg) (Fig. 1B and Fig. 1C). A decreasing trend of body weight gain and %BWG were found in treatments of fish with higher doses of the dietary chitosan than at 1g chitosan/kg (Fig. 1A and Fig.1B). Similar trend of effects of the dietary chitosan was also found in specific growth rate (SGR% /day) of fish fry (Fig. 1C). On the other hand, a reverse trend of results were obtained in the case of feed conversion ratio (FCR) (Fig. 1D). The highest FCR was recorded in the fish treated with no dietary chitosan. FCR was significantly ($p<0.05$) increased with the increasing doses of the dietary chitosan (Fig. 1D). A decreasing trend of FCR was found in the treatment of fish fry with lower doses of the dietary chitosan (Fig. 1D). Moreover, significantly ($p<0.05$) the highest number of survival rate (76%) was found in fish fry fed feed supplemented with the dietary chitosan at the lowest dose of 1 g chitosan kg^{-1} feed compared to the fish fed feed supplemented with T2 and T3 (Fig. 2).

In the present study, we found that dietary chitosan supplementation diet had positive significant effects on the growth attributes, such as weight gain, %BWG, SGR (%/day) and FCR as well as survival rate of fry of *B. gonionotus*. Some reports showed that dietary chitosan supplemented feeds significantly improved immunity and growth performances of fishes (Wang and Chen, 2005; Niu *et al*., 2013; Najafabad *et al*., 2016; Fadl *et al*., 2020; Oushani *et al*., 2020). On the contrary, the effects of chitosan treated supplemented diets on growth performances are controversial in the published literature (Kono *et al*., 1987; Shiau and Yu, 1999; Chen *et al*., 2014).

The low-dose dietary chitosan (0, 1800, 4000 and 7500 mgk^{-1}) did not have effect on the growth of gibel carp (*Carassius auratus gibelio*). Kono et al. (1987) reported that the dietary chitosan treated feed had no effect of the growth of red sea bream, the Japanese eel and yellow tail. On the other hand, Shiau and Yu (1999) observed that the dietary chitosan and chitin at the rate of 2, 5 and 10% level depressed the growth of tilapia. They also predicted that the growth suppression of tilapia may be due to the interposition of the chitosan and chitin in the absorption of nutrients. These results indicate that the optimum level of chitosan in diet may be beneficial

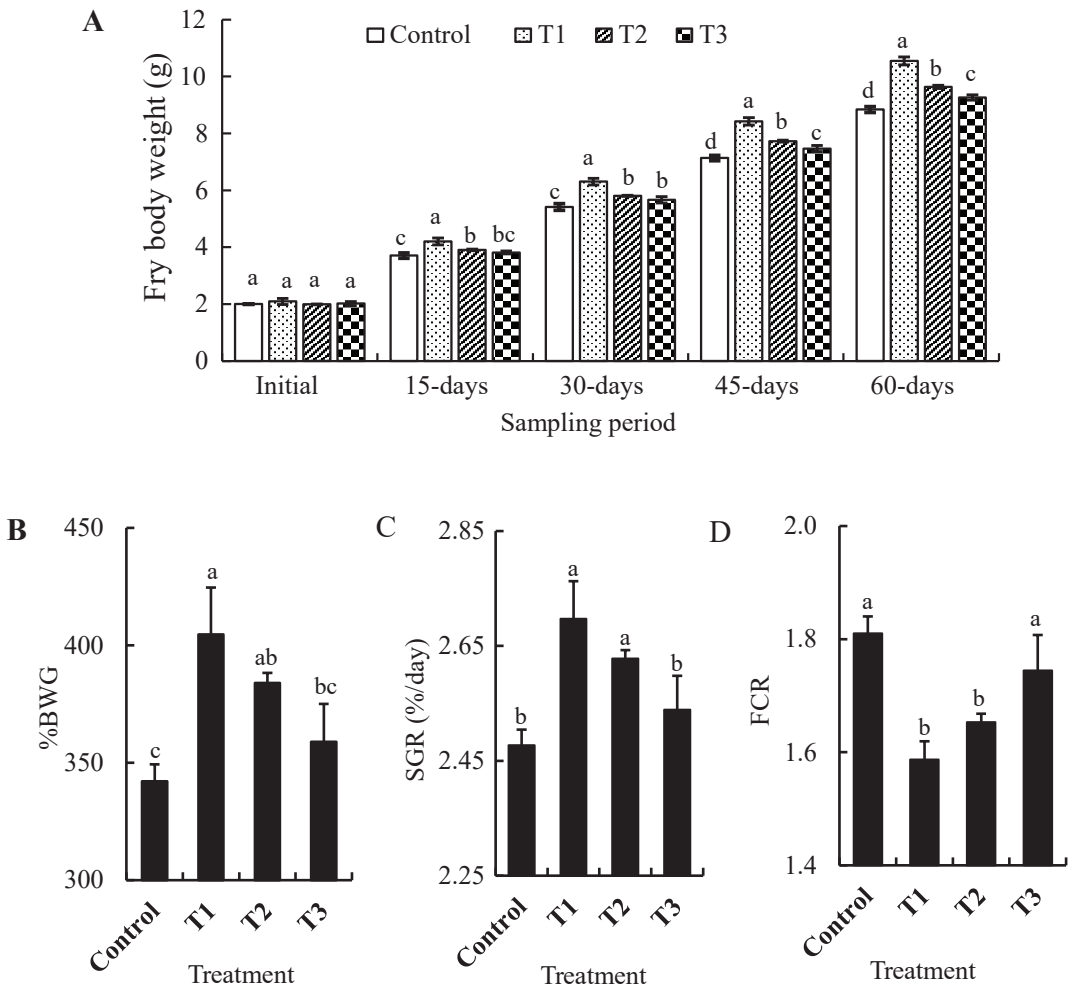

Fig. 1. Assessment of effects of the dietary chitosan on growth parameters (A, B, C and D) of fry of *B. gonionotus*. One way ANOVA was performed for analyzing the data of three replicated experiment and data in column varies significantly in LSD at $p < 0.05$ (Statistix 10). Different letter bars indicates significant variations in (A) Fry body weight (B) % body weight gain (% BWG) (C) specific growth rate (SGR%/day) and (D) food conversion ratio (FCR) of fry of *B. gonionotus* $p < 0.05$ (Statistix 10.0). Error bar = ±SD.

for growth performances and survival of *B. gonionotus* fry. However, the high quantities of chitosan may weaken the effect of chitosan on the growth performance and survival of *B. gonionotus* fry. The chitosan has positive effect on the physiology of fishes because it may enhance the digestive enzyme activity in the gut at lower dose (Gopalakannan and Arul, 2006; Niu *et al*., 2013). Moreover, this biopolymer also may keep the gut microflora including probiotic lactobacillus bacteria probiotic yeasts which accelerate the digestion and absorption of fish fry. Chitosan materials extracted from the shell of shellfish such as shrimp and crab and extracted from the scales of finfishes play many significant

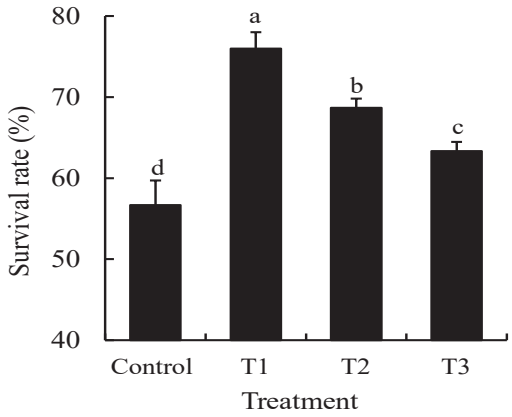

Fig. 2. Evaluation of effects of the dietary chitosan on survival rate of *B. gonionotus* fry. One way ANOVA was performed for analyzing the data of three replicated experiment and data in column varies significantly in LSD at $p < 0.05$ (Statistix 10). Different letter bars indicates significant variations in survival rate of fry of *B. gonionotus* at $p < 0.05$ (Statistix 10.0). Error bar = ±SD.

roles in physiological activities of fish. Therefore, when it was incorporated in the diet at optimum level, it could activate in the biosynthesis of the organism rapidly, which could positively affect the fry of *B. gonionotus* by enhancing the digestion and absorption of nutrition of the optimum doses, consequently, this benefic might create the proper growth performances and survival (Niu *et al*., 2013; Oushani *et al*., 2020).

Conclusions

The present study demonstrated that the optimum level of dietary chitosan improves the growth performances and survival of fry of *B. gonionotus.* Supplementation of dietary chitosan at 1 g kg^{-1} feed could be recommended for fish fry. Further investigation is required to identify the mechanisms of chitosan action in the health improvement of fish.

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References

Abd El-Naby, F. S., M. A. Naiel, A. A. Al-Sagheer and S. S. Negm. 2019. Dietary chitosan nanoparticles enhance the growth, production performance, and immunity in *Oreochromis niloticus*. *Aquaculture.* 501: 82–89.

Abdel-Ghany, H. M. and M. E. S. Salem. 2020. Effects of dietary chitosan supplementation on farmed fish; a review. *Rev. Aquacult.* 12(1): 438–452.

Akbary, P. and A. Younesi. 2017. Effect of dietary supplementation of Chitosan on growth, hematology and innate immunity of grey Mullet (*Mugil cephalus*). *Veteri. Res. Biol. Product.* 30(3): 194–203.

Alishahi, M., A. E. Rad., M. Zarei and M. Ghorbanpour. 2014. Effect of dietary chitosan on immune response and disease resistance in *Cyprinus carpio*. *Iran. J. Vet. Med.* 8(2): 125–133.

Anderson, D. P. 1992. Immunostimulants, adjuvants and vaccine carriers in fish: applications to aquaculture. *Annu. Rev. Fish Dis.2*. Pp. 281–307.

AOAC (Association of Official Analytical Chemists).1980. Official Methods of Analysis of the Association of Official Analytical Chemists. Ed. W. Hoewitz. (13th ed). Washington D. C. 78 P.

BBS (Bangladesh Bureau of Statistics). 2016. Yearbook of Agricultural Statistics -2015. Statistics and Informatics Division (SID), Ministry of Planning Government of the People's Republic of Bangladesh. 27th Series. Pp. 466.

Chen, Y., X. Zhu, Y. Yang, D. Han, J. Jin and S. Xie. 2014. Effect of dietary chitosan on growth performance, haematology, immune response, intestine morphology, intestine microbiota and disease resistance in gibel carp (*Carassius auratus gibelio*). *Aquac. Nutr*. 20(5): 532–546.

Fadl, S. E., G. A. El-Gammal, W. S. Abdo, M. Barakat, O. A. Sakr, E. Nassef and H. S. El-Sheshtawy. 2020. Evaluation of dietary chitosan effects on growth performance, immunity, body composition and histopathology of Nile tilapia (*Oreochromis niloticus*) as well as the resistance to *Streptococcus agalactiae* infection. *Aquac. Res.* 51(13): 1120–1132.

FAO (Food and Agriculture Organization). 2018. The state of world fisheries and aquaculture. The meeting the sustainable development goals. Rome.

Geng, X., X. H. Dong, B. P. Tan, Q. H. Yang, S. Y. Chi, H. Y. Liu and X. Q. Liu. 2011. Effects of dietary chitosan and *Bacillus subtilis* on the growth performance, non-specific immunity and disease resistance of cobia, *Rachycentron canadum*. *Fish Shellfish Immunol.* 31(13): 400–406.

Gopalakannan, A. and V. Arul. 2006. Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. *Aquaculture*. 255: 179–187.

Jahan, N., M. A. Salam, A. Ahsan, M L. Rahman, S. I. Paul, S. K. Das and S. K. Mazumder. 2020. Domestication Technique of Commercially Important Freshwater Mud Eel, *Monopterus cuchia* (Hamilton, 1822). *Malays. Appl. Biol.* 49(33): 95–104.

Jolles, P. and R. A. Muzzarelli. 1999. Chitin and chitinases. Basel; Boston: Birkhäuser Verlag, ©1999.EXS (Basel).

Khan, W., A. Vahab, A. Masood and N. Hasan. 2018. Water quality requirements and management strategies for fish culture (a case study of ponds around Gurgaon canal Nuh Palwal). *Inter. J. Trend Sci. Res. Develop.* 2(1): 388-393.

Kono, M., T. Matsui and C. Shimizu. 1987. Effects of chitin, chitosan and cellulose as diet supplements on the growth of cultured fish. *Nipp. Suisan. Gakka.* 53: 125–129.

Meshkini, S., A. A. Tafy, A. Tukmechi and F. Farhang-Pajuh. 2012. Effects of chitosan on hematological parameters and stress resistance in rainbow trout (*Oncorhynchus mykiss*). *Vet. Res. Forum*. 3(1): 49.

Mollah, M. F. A., M. Moniruzzaman and M. M. Rahman. 2011. Effects of stocking densities on growth and survival of Thai Sharpunti (*Barbonymus gonionotus*) in earthen ponds. *J. Bangladesh Agric. Univ.* 9(2): 327-338.

Najafabad, M. K., M. R. Imanpoor, V. Taghizadeh and A. Alishahi. 2016. Effect of dietary chitosan on growth performance, hematological parameters, intestinal histology and stress resistance of Caspian kutum (*Rutilus frisii kutum*, Kamenskii, 1901) fingerlings. *Fish Physiol Biochem*. 42(4): 1063-1071.

Niu, J., C. H. Li, L. X. Tian, Y. J. Liu, X. Chen, K. C. Wu, W. Jun, Z. Huang and H. Z. Lin. 2013. Suitable dietary chitosan improves the growth performance, survival and immune function of tiger shrimp, *Penaeus monodon*. *Aquac. Res*. 46(7): 1668-1678.

Niu, J., Y. J Liu, H. Z. Lin, K. S. Mai, H. J. Yang, G. Y. Liang and L. X. Tian. 2011. Effect of dietary chitosan on growth and stress tolerance of postlarval *Litopenaeus vannamei*. *Aquac Nutr.* 17: e406–e412.

Oushani, A. K., M. Soltani, N. Sheikhzadeh, M. H. Mehrgan and H. R. Islami. 2020. Effects of dietary chitosan and nano-chitosan loaded clinoptilolite on growth and immune responses of rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol*. 98: 210–217.

Ranjan, R., K. P. Prasad, T. Vani and R. Kumar. 2014. Effect of dietary chitosan on haematology, innate immunity and disease resistance of Asian seabass, *Lates calcarifer* (Bloch). *Aquac. Res*. 45(6): 983–993.

Sajid, M., S. Prabjeet, M. H. Samoon and A. K. Balange. 2010. Effect of dietary chitosan on non-specific immune response and growth of *Cyprinus carpio* challenged with *Aeromonas hydrophila*. *Int. Aquat. Res*. *2*(2): 77–85.

Shi, B. L., D. F. Li, X. S. Piao and S. M. Yan. 2005. Effects of chitosan on growth performance and energy and protein utilisation in broiler chickens. *Br. Poult. Sci.* 46(4): 516–519.

Shiau, S. Y. and Y. P. Yu. 1999. Dietary supplementation of chitin and chitosan depresses growth in Tilapia, *Oreochromis niloticus×O. auratus*. *Aquaculture* 179: 439–446.

Wang, S. H. and Chen, J. C. 2005. The protective effect of chitin and chitosan against *Vibrio alginolyticus* in white shrimp *Litopenaeus vannamei*. *Fish Shellfish Immunol*. 19: 191–204.

Wang, Y. and J. Li. 2011. Effects of chitosan nanoparticles on survival, growth and meat quality of tilapia, *Oreochromis nilotica*. *Nanotoxicology*. 5(3): 425–431.

Zaki, M. A., E. Shatby and E. Shatby. 2015. Effect of chitosan supplemented diet on survival, growth, feed utilization, body composition and histology of sea bass (*Dicentrarchus labrax*). *World J. Engineer. Technol*. 3(04): 38.

Zaman, M., M. N. Naser, A. T. M. Abdullah and N. Khan. 2014. Nutrient contents of some popular freshwater and marine fish species of Bangladesh. *Bangladesh J. Zool.* 42(2): 251–259.