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Safety dose of three commercially used growth promoters: nuricell-aqua, hepaprotect-aqua and rapid-grow on growth and survival of Thai pangas (*Pangasianodon hypophthalmus*)

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PEER REVIEW

Peer reviewer

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Comments

Although it is the duty of the feed additive manufacturers to first optimize the dose for specific fish species based on geographic location, various physico-chemical factors, and then market the product, the researchers did a good job where they demonstrated that Nutricell-Aqua, Hepaprotect-Aqua and Rapid Grow can be successfully used as growth promoters when applied at a dose about one and a half times higher than the recommended dose for satisfactory growth and survival.
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ABSTRACT

Objective: To optimize the dose of 3 commonly used growth promoters, *viz*., Nuricell-Aqua (composition: glucomannan complex and mannose polymer), Hepaprotect-Aqua (composition: β-glucan, mannose polymer and essential oil) and Rapid-Grow (composition: organic acid and their salt, β-glucan, mannose oligosaccharide and essential oil), using Thai pangas (*Pangasiandon hypophthalmus*) as cultured species.

Methods: Thai pangas fingerlings with an average length and weight of 11 cm and 10 g were reared under laboratory condition and growth promoters were fed after incorporating them with a test diet at a ratio of 10% of their body weight for a period of 28 d. Estimation of data on growth such as weight gain (g), specific growth rate, survivability (%) test in each aquarium were conducted and data were analyzed using statistical software.

Results: After 28 d of feeding with Nutricell-Aqua, 10 mg/(20 g feed·day), which was the dose recommended by the manufacturer, was found better. When Hepaprotect-Aqua and Rapid-Grow were employed, performance was found to be better with the dose of 60 mg/(20 g feed·day) which was 1.5 times higher than the dose recommended by the corresponding manufacturer.

Conclusions: These results suggest that chemicals and feed additives marketed in Bangladesh Fish Feed Market need further testing under Bangladesh climatic condition before being marketed.

KEYWORDS

Growth promoters, Thai pangas, Safety dose, Mannose polymer, Immunostimulants

1. Introduction

Growth promoters are a kind of feed additives that are principally chemical and/or biological substances added to fish feed with the aim to improving fish growth[1,2]. In intensive and semi-intensive aquaculture systems, growth promoters have become an essential part of fish diet in improving food conversion ratio (FCR), significant reduction in mortality, enhancing digestion of fish, *etc*. The use of such feed additive has been proven to be successful in aquaculture[3]. It is necessary for fattening fishes and improving the utilization of food to obtain better production and financial results.

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Although their mechanism of action varies, positive effect can be expressed through better appetite, improved FCR, stimulation of the immune system, improved self-defense system against foreign particles and increased vitality, regulation of the intestinal micro-flora, *etc*. In any cases, expected results of improved production and increased financial returns can be obtained through controlled use of these growth promoters. Because of the fact that growth promoters have different mechanisms of action, it is vitally important to test each group individually and present the expected results to the aquaculturists. Also it is equally important to test their effectiveness for concerns about food safety[4].

In aquaculture, the most utilized growth-promoting agents include probiotics, prebiotics, hormones, antibiotics, ionospheres and some salts[5,6]. Probiotics may serve as dietary supplements to keep the host intestinal microbial balance and improve growth performance of the respective culture species[7]. The use of probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species and enhancement of nutrition of host species through the production of supplemental digestive enzymes[8,9]. On the other hand, prebiotics are non-digestible food components/ingredients which have positive effect on host in their selective growth and/or activation of certain number of bacterial strains present in intestines[10]. These include a large group of compounds such as oligosaccharides: fructo-oligosaccharides, gluco-oligosaccharides and mannan-oligosaccharides (MOS). Their advantage compared to probiotics is that they promote growth of useful bacteria which are already present in the host and are adapted to all conditions of the environment[9]. As for the use of hormones and antibiotics, their use has become restricted in aquaculture over the last decade due to putative negative effects of using these agents in fish farms including cancers in human[11,12]. The use of different nutritional factors like vitamins, immunostimulants (also called immunomodulators), adjuvants and other chemicals has, therefore, increased in recent years to enhance aquaculture production[13,14]. Glucans and mannans possess non-specific immunostimulatory effect[15]. β-Glucans are major components of yeasts, mushrooms, and fungal mycelia and mannan, a plant polysaccharide, is a polymer of the sugar mannose. Mannan leads to lysis in the mannan-binding lectin pathway. As feed additives, immunostimulants provide significant protection against pathogens by upregulating phagocytosis, bacterial killing, and oxidative burst[16].

Aquaculture has boomed in Bangladesh over the past decades, and the country now ranks as the 5th largest aquaculture producer in the world[17]. With the expansion of aquaculture, a wide range of chemicals and drugs have been introduced by different manufacturers and pharmaceutical companies. It was reported that 33 companies were found either producing or marketing aquaculture targeting products[18]. Both traditional and formulated chemicals, antibiotics in health management are used and it was found that some feed additives and growth promoters were widely used such as Nuricell-Aqua (Ab Marui Co. Ltd.), Rapid-Grow (Anova Joint Venture Company Ltd.) and Hepaprotect-Aqua (Biomin Pty Ltd.). These growth promoters should be used in appropriate doses for the aquaculture species concerned. However, due to lack of technical knowledge of fish farmers on fish health feeding and health management, farmers face problems during applying these chemicals in their ponds. It was reported that most of the farmers in Bangladesh do not know the appropriate dosage and method of application of aqua-medicines. It has been recognized that farmers were using those aqua-medicines without knowing their efficacy, due to lack of information regarding the consequence of aqua-medicines using in aqua-health-development.

In recent years Thai pangas (*Pangasianodon hypophthalmus*) culture has been increasing in Bangladesh and at present production reached to a volume of about 300000 t[19]. For better production, farmers use various growth promoters in Thai pangas culture particularly Nutricell-Aqua, Rapid-Grow and Hepaprotect-Aqua. Chandra *et al*.[20] recently have reported the effect of Rapid-Grow on the growth performance and feed utilization of monosex tilapia, but there are no reports on Thai pangas under Bangladeshi climatic condition. The present study was, therefore, conducted to determine the optimal doses of 3 commonly used growth promoters, *viz*., Nuricell-Aqua, Rapid-Grow and Hepaprotect-Aqua using Thai pangas (*Pangasiandon hypophthalmus*) as cultured species.

2. Materials and methods

2.1. Experimental design

Twelve aquaria (2.5 foot×1.5 foot×1.5 foot) with average water holding capacity of 55 L were set at the Laboratory of Fishing Technology, Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh, where treatments with the 3 growth promoting agent, *viz*., Nuricell-Aqua, Rapid-Grow and Hepaprotect-Aqua were used for experimentation (each with 3 replications and 3 for control). Thirty healthy Thai pangas fingerlings with average body length and weight of 11 cm and 10 g were stocked in each aquarium. Aerators were set in each aquarium and water was changed everyday. All the aquaria were covered by a synthetic net to prevent escaping of fish. Water quality parameters were regularly monitored using commercially available water quality kits from Hanna Instruments Ltd., Germany.

2.2. Preparation of test diets and chemical analysis

Pelleted feeds were prepared in the laboratory using small pellet machine where the following ingredients and amounts of raw materials for 5 kg were used: wheat bran 600 g, rice bran 1000 g, maize flour 700 g, soybean meal 750 g, fishmeal 1700 g, molasses 200 g, vitamin premix 50 g, salt 50 g. Proximate composition of the diet was determined at the Laboratory of Fish Nutrition, Department of Aquaculture, Bangladesh Agricultural University, and it was found that moisture content was 11.75% ±0.30%, ash 10.34%±0.10%, protein 30.71%±0.52%, lipid 8.64%± 0.12% and fiber 5.35%±0.10%. For preparation of 3 treatments of test diet, dose of growth promoters recommended by the manufacturer was manipulated and 3 doses, *viz*., T1 (less than recommended dose), T2 (recommended dose) and T3 (more than

recommended dose) for each growth promoter mentioned in Table 1 was used.

Table 1
Amount of growth promoters used for preparation of test feed.

Growth promoters	Treatments (mg/20 g feed)		
	T1	T2*	T3
Nutricell-Aqua	5	10	15
Rapid-Grow	20	40	60
Hepaprotect-Aqua	20	40	60

*T2 is the dose recommended by the manufacturer.

For its preparation, first a little bit of flour was boiled with small amount of water and required amount of growth promoter was taken into the boiled water and mixed well. Then this semi-liquid mixture was mixed with pelleted feed and dried using fan. The feed was supplied to the fish when the feed was completely dried.

2.3. Measurement of fish growth and other parameters

Fish were fed with test diet at a ratio of 10% of their body weight for a period of 28 d. Estimation of experimental data on growth such as weight gain (g), specific growth rate (SGR) was conducted. Survivability (%) test of fishes in each aquarium was also conducted.

2.4. Data analysis

The data obtained in the experiment were recorded and preserved in computer. The data obtained in the experiment were analyzed by using SPSS version 11.5 (Chicago, USA). Significant differences were determined among treatments at the 5% level ($P<0.05$).

3. Results

For the determination of appropriate doses of three widely used growth promoters in Thai pangas cultured, feeding experiment was conducted under laboratory condition by incorporating different doses of Nuricell-Aqua, Hepaprotect-Aqua and Rapid-Grow in the test diet.

3.1. Water quality parameters

Water quality parameters were monitored throughout the experimental period for both control and treatment aquarium and it was found that water temperatures varied from 28.50 to 30.2 °C. During the study period, the values of pH ranged from 7.9 to 8.2 in aquarium condition while dissolved oxygen values ranged from (3.38±0.14) to (6.50±0.01) mg/L. There was hardly presence of nitrate, ammonia and phosphate in aquarium water while total alkalinity values varied from (110.0±11.2) to (160.0±3.6) mg/L indicating optimum condition for growth of Thai pangas.

3.2. Growth performance for growth promoters

Nutricell-Aqua was manufactured by Ab Marui Co. Ltd. and marketed in Bangladesh by EON Animal Health Products Ltd. In Nutricell-Aqua treated aquaria, it was found that the initial weight of Thai pangas for aquarium treated with T2 dose was 10 g. The weight of fish after 7, 14, 21 and 28 d were found to be 12.0 g, 13.5 g, 15.0 g and 16.5 g respectively. For aquarium treated with T1 dose, average initial weight of fish was 10 g, which after 7, 14, 21 and 28 d reached 10.5 g, 11.75 g, 12.25 g and 13.50 g respectively. As for aquarium treated with T3 dose, average initial weight of each fish was 10 g. Then average weight of fish after 7, 14, 21 and 28 d were found to be 12.50 g, 14.00 g, 16.50 g and 17.50 g respectively. After 28 d of feeding trial, performance with T3 was found to be significantly better than the other two doses (T1 and T2). Rapid-Grow was manufactured by Anova Joint Venture Company Ltd. and marketed in Bangladesh by Fish Tech. Initial weight for aquarium treated with T2 dose of Rapid-Grow was found to be 10 g. Then average weight of fish after 7, 14, 21 and 28 d reached to 12.0 g, 13.0 g, 14.25 g and 15.0 g respectively. For aquarium treated with T1 dose, average initial weight of fish was 10 g. Then average weight of fish after 7, 14, 21 and 28 d were found to be 11.0 g, 11.5 g, 11.75 g and 12.25 g respectively. As for aquarium treated with T3 dose, average initial weight of fish was 10 g which reached to values of 12.50 g, 13.75 g, 15.00 g and 16.00 g after 7, 14, 21 and 28 d respectively. This indicates that after the end of feeding trial, growth performance with T3 dose was better than the other two doses. In case of Hepaprotect-Aqua, the initial average weight of fish in aquarium treated with T2 dose was 10 g. The weight of fish after 7, 14, 21 and 28 d were found to be 12.0 g, 13.0 g, 15 g and 16.50 g respectively. For aquarium treated with T1 dose, average initial weight of fish was 10 g. Their average weight of after 7, 14, 21 and 28 d were found as 11.0 g, 11.5 g, 12.0 g and 12.5 g respectively. As for fish in aquarium treated with T3 dose, the weight reached to 12.5 g, 14.0 g, 16.5 g and 17.5 g after 7, 14, 21 and 28 d respectively from an average initial weight of 10 g. After 28 d feeding trial, performances of T3 dose was found to be better than the other two doses.

3.3. SGR

A term SGR is used in aquaculture to estimate the production of fish after a certain period. In the present study, SGR of 3 widely used growth promoters are presented in Figure 1. It was found that highest average weight gain with Nutricell-Aqua was obtained from T3 dose [(17.5±0.5) g] after 28 d with a corresponding highest average SGR of 0.01. Similarly for Rapid-Grow and Hepaprotect-Aqua, it was

also observed that highest average SGR was obtained from T3 doses which were 0.007 for both chemicals (Figure 1).

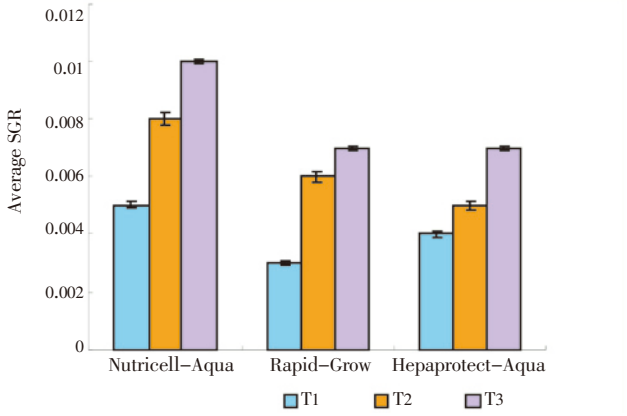

Figure 1. Average SGR of Thai pangas for Nutricell-Aqua, Rapid-Grow and Hepaprotect-Aqua under aquarium condition after 28 d.

Also, while comparing SGR, it was clear that for Nutricell-Aqua, T3 showed better performance which was significantly different from rest of the treatments (P<0.01). On the contrary, for Rapid-Grow and Hepaprotect-Aqua, it was observed that their performance with T3 doses was significantly higher than the other two doses.

3.4. Survivability of fishes after using growth promoters

Survivability of fishes after using three growth promoters was monitored and data are given in Table 2. It was found that survivability was significantly higher in T3 doses for all three growth promoters *viz*., Nutricell-Aqua, Rapid-Grow and Hepaprotect-Aqua.

Table 2
Survivability of Thai pangas for three growth promoters under laboratory condition.

Selected dose		Survivability of Thai pangas (%)			
		Day 7	Day 14	Day 21	Day 28
Nutricell-Aqua	T1 (5 mg/20 g feed)	80	75	75	75
	T2 (10 mg/20 g feed)	85	80	80	80
	T3 (15 mg/20 g feed)	90	85	85	85
Rapid Grow	T1 (20 mg/20 g feed)	70	65	65	65
	T2 (40 mg/20 g feed)	80	78	78	78
	T3 (60 mg/20 g feed)	85	80	80	80
Hepaprotect-	T1 (20 mg/20 g feed)	70	68	68	68
Aqua	T2 (40 mg/20 g feed)	75	70	70	70
	T3 (60 mg/20 g feed)	80	78	78	78

4. Discussion

This is the first report of such study on Thai pangas, although Chandra *et al*.[20] conducted a study about Rapid-Grow in which they observed growth in tilapia in pond system. The nutrient values of raw materials used in feeds for Thai pangas culture are still poorly documented[21]. Their nutrient requirements are mostly adapted from other species of catfish especially that of *Ictalurus* species or other fresh water species. Aquafeed cost accounts for approximately 60%-80% of operational cost due to the ever increasing cost. So, it is essential to ensure efficient utilization of feed ingredients of the targeted culture species to assure profitability. Studies were, therefore, undertaken to determine the appropriate doses of three commonly available growth promoters, *viz*., Nuricell-Aqua, Rapid Grow and Hepaprotect-Aqua which are suggested to improve feed utilization in Thai pangas and other aquaculture fish species.

Water quality parameters of aquarium, growth performance, SGR and survivability of Thai pangas were determined under laboratory condition as it was suggested that these work of screening additives was based on synergistic blends of digestive phytobiotics, natural emulsifying agents and co-factors of digestion for improving the feed utilization which is need to be conducted under controlled environment[21]. It was observed that water temperatures varied from 28.50 to 30.20 °C with average pH ranging from 7.9 to 8.2 and dissolved oxygen values from (3.38±0.140) to (6.50±0.01) mg/L. These values are typical for culture condition in summer in Bangladesh[22]. Some of our previous studies have also shown more or less similar water quality parameter values for experiment under laboratory condition[23-25].

Nuricell-Aqua is a growth promoter having a composition of glucomannan complex and mannose polymer. It is manufactured by Ab Marui Co. Ltd. and marketed in Bangladesh by EON Animal Health Products Ltd. According to manufacturer's instruction, benefits of Nutricell-Aqua in fish are improved, including self defense against foreign substances, improved specific and non-specific immunity, reduced poisonous reaction, increased activity of monocyte, increased FCR. Feeding trial of this feed additive in Thai pangas using three different treatments showed that after 28 d, growth performance with T3 was found to be significantly higher than the other two doses (T1 and T2). This MOS as nutritional supplements has been found to be able to improve gastrointestinal health, overall health as well as wellbeing, energy levels and performance. Dimitroglou *et al*.[26] showed that MOS incorporation into live feeds retarded *Vibrio* species levels and can improve fish health and production. They also reported that MOS supplementation can improve growth performance and feed utilization in gilthead sea bream[27]. In our present study, SGR and survivability was found to be significantly higher when Nuricell-Aqua was incorporated in Thai pangas diet at a rate of 15 mg/(20 g feed·day). It is expected that incorporation of Nutricell-Aqua at this dose in Thai pangas will not only ensure proper growth, but also help gain expected economic benefit.

In present aquaculture system, fish production largely

depends on suitable growth promoters, when growth promoters doses are used accurately. If the growth promoters doses are not in proper concentration, it may hamper the production. The other two growth promoters under investigation, *viz*., Rapid-Grow and Hepaprotect-Aqua are similar in their composition. Rapid-Grow composes of organic acid and their salt, β-glucan, mannose oligosaccharide and essential oil. It is manufactured by Anova Joint Venture Company Ltd. and marketed in Bangladesh by Fish Tech. Company Ltd. According to the manufacturer, it can enhance metabolism and energy of fish body cells, raise the efficiency of feed utilization by supplying oxygen to whole body, improve the immune responses, excrete heavy metals and maintain the normal endocrine system. Vetvicka *et al*.[15] reported that administration of β-glucan through immersion, dietary inclusion or injection has been found to enhance many types of immune responses, resistance to bacterial and viral infections to environmental stress in many fish species. From the studies that were conducted to determine optimum dose of Rapid-Grow, we found that after end of feeding trial, growth performance with T3 [60 mg/(20 g feed·day)] dose was better than the other two doses. Interestingly this dose was 1.5 times higher than that recommended by the manufacturer. The third experimental feed additive Hepaprotect-Aqua which is manufactured by Biomin Pty Ltd. and marketed in Bangladesh by Renata Ltd composes of β-glucan, mannose polymer and essential oil. Hepaprotect-Aqua can enhance digestion of fish, prevent and treat liver disease. It was found that dose of 60 mg/(20 g feed·day) (T3) showed significantly higher rate of growth than the other two doses (T1 and T2). This dose was also 1.5 times higher than that recommended by the manufacturer. Chandra *et al*.[20] reported that Rapid-Grow could significantly enhance growth in tilapia and bring best economic efficiency. Similarly, our study of these two group of feed additives, *viz*., Rapid-Grow and Hepaprotect-Aqua, if used appropriately, they can give significantly higher production and thereby promote Thai pangas culture in this time of Pangas culture crisis in Bangladesh[28].

Since the present study was conducted in laboratory condition, further studies are necessary to test their efficacy in pond condition. However, there are reasons to believe that chemicals and feed additives marketed in Bangladesh feed market may have not been tested properly before they get approval. Government and research institutes should come forward so that chemicals and drugs are properly tested for safety dose determination and farmers can get maximum benefit so that their investment return is ensured. Our study showed that growth promoters used in Thai pangas culture may be useful for enhancing production if appropriate dose is used.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Growth promoters have gained considerable attention in recent years that heaps production in both intensive and semi-intensive culture systems. With increased intensification in Bangladeshi aquaculture system, it is imperative to check suitability of different growth promoters and suggest the most appropriate one with recommended doses to benefit fish farmers.

Research frontiers

The researchers worked to optimize doses of 3 growth promoters using Thai pangas as culture species for a period of 28 d where they estimated weight gain (g), SGR, survivability (%) in aquarium condition.

Related reports

The growth promoters which include probiotics, prebiotics, hormones, antibiotics, ionospheres and even salts serve as dietary supplements to improve the host intestinal microbial balance and growth performance of the respective culture species. The materials and methods used in the study where growth promoters were incorporated into fish feed are sufficient to conclude their findings.

Innovations & breakthroughs

Nutricell-Aqua, Hepaprotect-Aqua and Rapid-Grow are growth promoters used for increasing digestibility and feed utilization in fish. In the present study, authors demonstrated that the dose recommended by the manufacturer were not optimum, rather one and a half times the recommended dose of the promoters gave satisfactory growth and survival.

Applications

From the literature survey it has been found that Nutricell-Aqua, Hepaprotect-Aqua and Rapid Grow are safe for application and the investigators reported the optimum dose. It is expected that the described dose will benefit the Thai pangas fish farmers.

Peer review

Although it is the duty of the feed additive manufacturers to first optimize the dose for specific fish species based on geographic location, various physico-chemical factors, and then market the product, the researchers did a good job where they demonstrated that Nutricell-Aqua, Hepaprotect-Aqua and Rapid-Grow can be successfully used as growth promoters when applied at a dose about one and a half times

higher than the recommended dose for satisfactory growth and survival.

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