ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2016; 4(5): 328-333

www.fisheriesjournal.com
Received: 15-07-2016
Accepted: 16-08-2016

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Potential use of *Ganoderma lucidum* polysaccharides as a feed supplement in diets on survival and growth performance of the grass carp, *Ctenopharyngodon idella*

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Abstract
The aim of the present study was to assess the effects of dietary *Ganoderma lucidum* polysaccharides (GLPs) on the survival and growth performance of the freshwater fish, *Ctenopharyngodon idella*. *C. idella* juveniles were fed diet containing 0 (control), 0.1, 0.5 and 1.0 g kg^{-1} of GLPs supplemented diets for a period of 60 days. The sampling was made at 15 days interval (after 15th, 30th, 45th, and 60 days of feeding trial) to analyze the growth parameters and the results showed that the survival, weight gain, length gain, feed intake and specific growth rate were found to be significantly (P<0.05) higher in 0.1-1.0 g kg^{-1} of GLPs supplemented diets when compared to control in all samplings days. The feed conversion ratio was found to be significantly decreased in fish fed with 0.1-1.0 g GLPs kg^{-1}supplemented diets when compared to control feed fed fish group. Hence, present study suggests that 1.0 g kg^{-1} GLPs can be taken as a dietary supplement for regulating better production of *C. idella*.

Keywords: *Ctenopharyngodon idella*, *Ganoderma lucidum* polysaccharides, survival, growth performance

1. Introduction
Grass carp, *Ctenopharyngodon idella* is one of the very popular freshwater fish species for aquaculture because of its fast growth, rich in nutrients, highly reproductive, good taste and flesh quality [1]. It is one of the commercial herbivorous species, and is widely distributed in many countries [2].

Sustainable and successful freshwater fish culture on scientific basis principally depends upon the use of adequate, economically viable and environment friendly artificial feeds. Since the feed cost very 40% to 60% of the total managerial expenditure in fresh water fish culture system, provision of artificial feed increase the survival and growth of fish. Carbohydrates are generally the major source of energy in most domestic animal diets, and carbohydrate-containing feedstuffs are available in great quantities at low prices [3]. As the cheapest dietary energy sources and good binding agents [4], carbohydrates utilization by different species of cultured fish is of interest to fish nutritionists and food producers (major carp, *Labeo rohita*: [5]; grass carp, *Ctenopharyngodon idella*: [6]).

In the aquaculture research, dietary administration of polysaccharides can stimulates the immune response and reduced pathogens load which leads to better survival and growth performance of fish and crustaceans [7-12].

The medicinal mushroom, *Ganoderma lucidum* is extensively used as a traditional Chinese herb to treat various human diseases like bronchitis, allergies, hepatitis, hypertension, immunological disorders, and cancer [13-15]. This mushroom composed with huge amount of polysaccharides (β-D-glucans, heteropolysaccharides and glycoproteins), flavonoids, alkaloids, antioxidants, proteins, vitamins, minerals, etc. [16]. Among these substances, *G. lucidum* polysaccharides (GLPs) has been identified as major bioactive components which showing multiple pharmacological effects such as immunomodulation, anti-oxidation, hepatoprotection, anti-proliferation, and anti-angiogenesis [17]. Hence, current study was performed to assess the effects of dietary GLPs on survival and growth parameters such as weight gain, length gain, feed intake, specific growth rate and feed conversion ratio of *Ctenopharyngodon idella* juveniles.

2. Materials and Methods

2.1 Collection of *Ganoderma lucidum* fruit bodies

Matured *Ganoderma lucidum* fruit bodies were collected from December 2014 to February 2015 on Ooty hill region (located in Nilgiri District of Tamil Nadu), and authenticated the Mycology Division of Indian Forest Genetics and Tree Breeding Institute, Coimbatore, Tamilnadu, India (Voucher No: RT-25406/9-1-2015). The name of the host tree (*Azadirachta indica* and *Cassia fistula*) was also recorded at the time of the sample collection. Mostly undisturbed areas are selected for collection of samples.

2.2 Preparation of *Ganoderma lucidum* polysaccharides

Ganoderma lucidum (200 g) were mixed with 1000 ml of 80 % (v/v) ethanol and shaken at 30 ºC for 24 hours to remove of the polyphenols and monosaccharides. After the mixture was filtered, the residues were dried in air and then extracted three times with hot water (100 ºC) (1:20, w/v). The extract was concentrated in a rotary evaporator under reduced pressure, precipitated by 95 % (v/v) ethanol at 4 ºC for 24 h and then centrifuged (5000 g, 10 min). The precipitate was vacuum freeze-dried, and 15 g of polysaccharides were obtained and further used.

2.3 Experimental Fish

Hundred juvenile *C. idella* with an average weight of 10 g were obtained from sirago fish form Nerunjipettai in Erode District. All fish were healthy and acclimatized at 22 ºC for three weeks in 400 L aerated tanks prior to use. The fish were fed with a commercial diet at 3% body weight per day.

2.4 Preparation of feed

The ingredients and proximate composition of control and experimental diets were formulated in Table 1. The feed ingredients, such as ground nut oil cake, fish meal rice bran, tapioca flour and eggs were purchased from local markets. Cod liver oil and vitamin tablets were purchased from local pharmacy. The 3.0 ± 0.64 mm diets were prepared according to the method of Muralisankar *et al* [18]. Briefly, fish meal, ground nut oil cake, rice bran and tapioca flour were mixed and steam cooked for 20 min at 105 ºC. After steam cooking, the heat sensitive ingredients, such as vitamin mineral mixture, egg albumin, cod liver oil and different concentration (0, 0.1 0.5, and 1.0 g kg^{-1}) of GLPs were added and thoroughly mixed until stiff dough was formed. The dough was pelletized by an indigenous hand pelletizer. The pellets were dried at room temperature (28 ± 1.30 °C) until constant weight was reached. The proximate composition of the experimental diets was determined by standard method of AOAC [19].

Table 1: Ingredients and proximate composition of control and experimental diets (g kg^{-1})

Ingredients	Experimental diets (g kg^{-1})			
	Control	**0.1**	**0.5**	**1.0**
Fish meal[1]	315	315	315	315
Groundnut oil cake[1]	345	345	345	345
Rice bran[1]	180	180	180	180
Tapioca flour[1]	120	120	120	120
Egg albumin[2]	20	20	20	20
Cod liver oil[2]	10	10	10	10
Vitamin and mineral premix[2*]	10	10	10	10
GLPs [3]	0	0.1	0.5	1.0
Proximate composition (g / kg)				
Dry matter	900.51	900.55	900.56	900.61
Crude protein	350.12	350.34	350.45	349.98
Crude fat	60.13	60.23	60.13	60.64
Crude fiber	80.23	80.23	80.23	80.45
Carbohydrate	280.44	281.66	282.34	284.15
Ash (%)	14.67	14.56	14.67	14.49
Moisture (%)	8.00	8.60	8.50	8.10
Gross energy (kJ g^{-1})	15.24	15.36	15.35	15.47

[1]Kannan Departmental stories, Erode, Tamilnadu, India
[2] Deepa medicals, Erode, Tamilnadu, India
[*] Vit/min premix (kg/premix): Vitamins: A=1.200.000 UI; D3=200.000 UI; E=12.000 mg; K3=2.400 mg; B1=4.800 mg; B2=4.800 mg; B6=4.000 mg; B12=4.800 mg; folic acid=1.200 mg; pantotenate Ca=12.000 mg; C=48.000 mg; biotin=48mg; choline=65.000mg; niacin=24.000mg; Minerals: Fe=10.000 mg; Cu=600 mg; Mn=4.000 mg; Zn=0 mg; I=20 mg; Co=2 mg e Se=20 mg.
[3] *Ganoderma lucidum* polysaccharides

2.5 Experimental setup

Four groups of *C. idella* fingerlings (7.5 ± 0.02 cm length and 10.03 ± 0.01g weight) were assigned for this experiment in triplicate for 60 days. Three groups were fed with 0.1, 0.5 and 1.0 g kg^{-1} *G. lucidum* polysaccharides supplemented diets respectively. The remaining one group was served as control (fed with '0' concentration of GLPs supplemented diet). Each group consisted of 15 fish in an aquarium maintained with 30 L of tap water. The water medium was renewed every 24 h by siphoning method with minimum disturbance to the fish and aerated adequately. The experimental fish were fed with these feeds at 3 % of body weight twice per day. The unfed feed, excreta, and molts were removed during the feeding experiment while renewing the aquarium water. At end of feeding experiment, three fish from each treatment were sampled for analyzing of each parameter.

2.6 Analysis of growth parameters

Survival and growth parameters such as weight gain (WG), length gain (LG), feed intake (FI), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated according to following formulas:
Survival (%) = no. of live prawns/ no. of prawns introduced × 100;

Length gain (cm) = final length (cm) - initial length (cm);
Weight gain (g) = final weight (g) - initial weight (g);
Feed intake (g day^{-1}) = feed intake (g)/ total number of days
Specific growth rate (%) = $_{log}W_2$- $_{log}W_1$ /t × 100 (where, w_1 and w_2= initial and final weight (g), and t = duration of experiment in days);
Feed conversion ratio = feed intake (g)/ weight gain (g);

2.7 Statistical analysis
The Data were analyzed using one-way analysis of variation (ANOVA) using the statistical package for the social sciences (SPSS) computer software (version 22.0). Duncan's multiple range tests at a significant level of 0.05 % was used to determine significant difference between treatments.

3. Results and Discussion
3.1 Analysis of survival and growth parameters
The survival, growth performance such as weight gain, length gain, feed intake and specific growth rate significantly increased (P <0.05) in fish fed with 0.1-1.0 g GLPs kg^{-1} supplemented diets when compared with the control diet fed fish. Among these supplementations, 1.0 g GLPs kg^{-1} showed better performance. The feed conversion ratio was found to be significantly decreased in fish fed with 0.1-1.0 g GLPs kg^{-1} supplemented diets when compared to control feed fed fish group.

3.1.1 Survival
The survival was significantly (P <0.05) increased in GLPs supplemented diets fed fishes when compared with control (Figure 1)

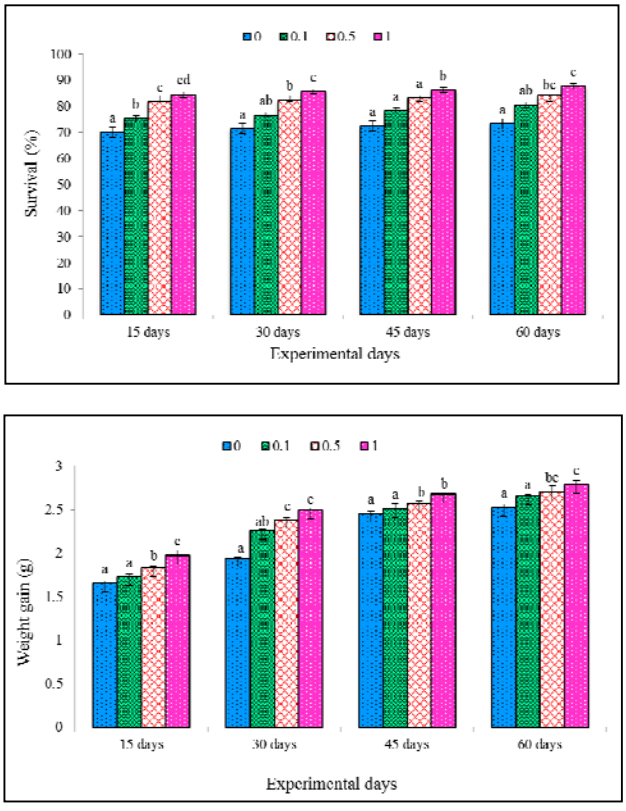
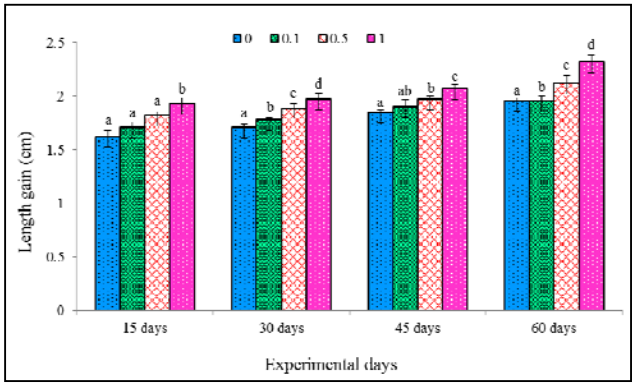


Fig 1: Survival (%) and weight gain (g) of *C. idella* juveniles fed with GLPs supplemented diets on different treatment days. Data are expressed as mean ± SE at the same sampling day with different superscript letters as significantly difference (p<0.05)

3.1.2 Weight and length gain
The initial body weight and length of the fish recorded to 10.03 g and 7.5 cm respectively. After 15th, 30th, 45th and 60th days feeding the final weight and length of fish was found to elevate in experimental groups when compared with control. However, the weight gain and length gain was found to maximum in fish fed with GLPs supplemented diets (Figure 1 and 2). These differences were found to statistically significant (P <0.05).



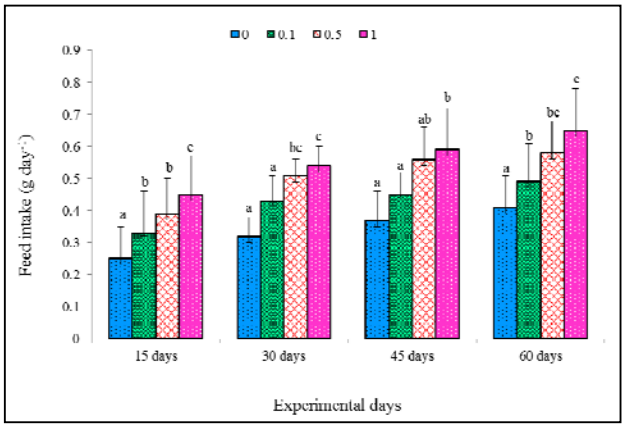
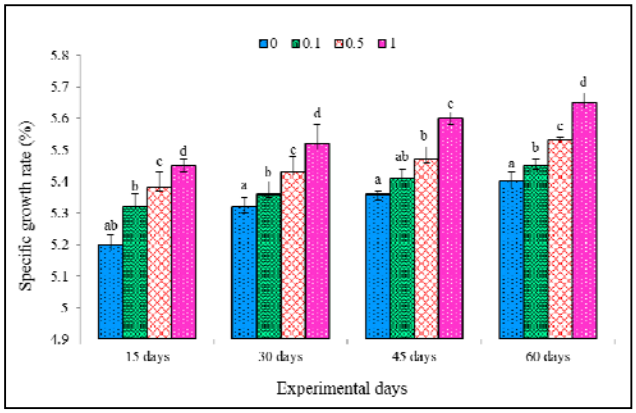


Fig 2: length gain (cm) and feed intake (g day^{-1}) of *C. idella* juveniles fed with GLPs supplemented diets on different treatment days. Data are expressed as mean ± SE at the same sampling day with different superscript letters as significantly difference (p<0.05)

3.1.3 Feed intake and specific growth rate
The feed intake and specific growth rate were found to be significantly increased (P <0.05) in GLPs supplemented feed fed fish group when compared to the control (Figure 2 and 3).



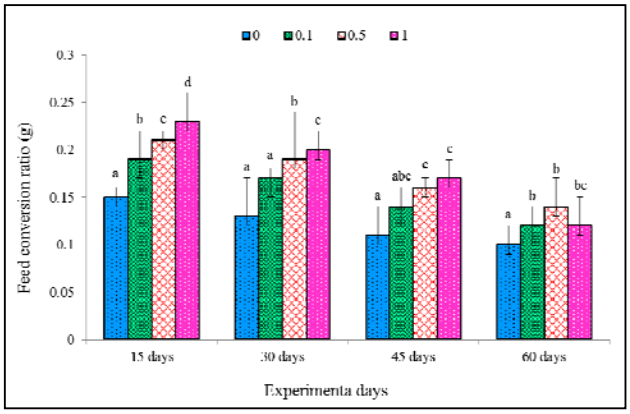


Fig 3: Specific growth rate (%) and feed conversion ratio (g) of *C. idella* juveniles fed with GLPs supplemented diets on different treatment days. Data are expressed as mean ± SE at the same sampling day with different superscript letters as significantly difference ($p<0.05$)

3.1.4 Feed conversion ratio

The feed conversion ratio was observed to be lower in experimental fish group when compared with control (Figure 3). The lower feed conversion ratio recorded in experimental fish group reflects the better quality of diets formulated with incorporation of prebiotics. These differences were found to be statistically significant ($P<0.05$).

Nutritional status is considered as one of the important factors that determine the ability of animals to withstand infections. The growth promoting effects of polysaccharides was partly due to their immunomodulating effect which helps to balance immune function, combat auto immune disease and encourages healthy immune function. Prebiotics are non-digestible feed ingredients that beneficially affect the host by stimulating growth or activity of one or a limited number of bacterial species already resident in the gut and thus improving host health [20]. Dietary polysaccharides are digested in the body and used as potential energy sources which can decrease the utilization of nutrients as an energy source and it ultimately increases the storage of nutrients in organisms.

In the present study, significant improvements in survival, weight gain, length gain, feed intake and specific growth rate indicates that the administration of 1.0 g kg^{-1} GLPs have ability to promote the feeding, followed by better survival and growth performance of fish. Polysaccharides such as β glucan, chitin, fucoidan, fructooligosaccharide and *Ganoderma lucidum* polysaccharides supplemented feed fed prawns and fishes showed better survival, growth performance, feed intake and specific growth rate have been reported [21-29].

One well studied aspect regarding prebiotics effect in fish is their effect on growth performance and feed utilization [30, 31, 7, 32]. The enhanced feed efficiency (FE), nutrient digestibility and growth improvements associated with dietary prebiotics may be due to changes in digestive enzymes or in gut morphology. Anguiano *et al* [33] studied the digestive enzymes and gut histomorphology of red drum (*Sciaenops ocellatus*) fed FOS, MOS, TOS, and GroBiotic®-A, and of hybrid striped bass fed GroBiotic®-A. The authors concluded that the observed improvement of nutrient digestibility in response to dietary prebiotic supplementation was more likely related with changes in gut structure than with improvements on digestive enzyme activities. On the other hand, the higher growth of allogynogenetic crucian carp (*Carassius auratus gibelio*), Caspian roach (*Rutilus rutilus*), and blunt snout bream (*Megalobrama amblycephala*) when fed prebiotics was well related with increased activities of digestive enzymes [9, 34-35]. In any case, the observed results may change among studies since prebiotic effects may change due to several factors, such as prebiotic source, supplementation level, fish species and age, rearing conditions and diet composition. In fact, results with different species and prebiotics are still confusing, since some studies report growth beneficial effects [36, 37, 9, 34, 35, 38]. Better food conversion rate (FCR) may be due to increased digestive enzyme activity [39]. The high rate of food intake does not always bring out an effective growth rate, there by leading to lower FCR [40]. In present study lower FCR was recorded in 1.0 g kg^{-1} GLPs supplemented diets on *C. idella*. It indicates that better quality of feed.

4. Conclusion

In conclusion, the present study indicates that supplementation of 1.0 g kg^{-1} *Ganoderma lucidum* polysaccharides in *Ctenopharyngodon idella* diets enhanced the survival and growth performance.

5. Acknowledgments

Authors thank the authorities of Sri Vasavi College, Erode for providing the essential infrastructure facilities needed for the study. I extend my sincere thanks to the in-charge of Sirago Fish Farm, for their timely help with the supply of fish samples throughout my study.

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