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Effect of 0.5% and 1.5% dietary supplementation of cinnamon (*Cinnamomum verum*) on growth performance and blood parameters of grass carp (*Ctenopharyngodon idella*)

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Abstract

Feed additives are essential for the enhancement of growth and nutritional value of fish. There is a need to use different feed additives to meet the economic requirements and formulate cheaper fish feed with all essential nutrients. In this view, the present study was designed to assess the effect of 0.5% and 1.5% dietary supplementation of cinnamon (*Cinnamonum verum*) on growth performance and blood parameters of grass carp (*Ctenopharyngodon idella*) for an experimental duration of 60 days. The uniform-sized fingerlings were collected from Fisheries Research Farms, University of Agriculture, Faislabad, and stocked at the rate of 10 fish per tank. Fish were stocked in three aquaria with two replicates; one served as control T₀ and the other two were the experimental units i.e. T₁ with 0.5% and T₂ with 1.5% cinnamon supplemented feed. Fish were fed two times a day at the rate of 7 % of fish body weight. Weight gain in T₀ was 17.1 g, for T₁ 20.1 g, and T₂ 14.55 g. SGR and survival rates were higher for T₁.Observed RBCs values were 1.15, 1.26, and1.18 (10⁶/µL) for T₀, T₁, and T₂. WBCs values determined were 11.35, 15.65, and 13.60 (10³/µL) respectively for T₀, T₁ and T₂ groups were as 29.1%, 34.35%, and 32.60%, correspondingly. Predominantly, it was observed that 0.5% cinnamon level was acquiring the most significant values in terms of weight gain, length gain, SGR, and blood parameters. So, it was concluded to be most effective than others as a feed supplement for improvement in growth, performance, and blood parameters of grass carp *Ctenopharyngodon Idella*.

Keywords: Aquaculture, Grass carp, Fish feed, Feeding innovations, Cinnamon feed, Trends in aqua feeds.

Introduction

Feed is the basic requirement for growth and survival of all the living organisms. The proper feed should contain all the active compounds an organism needs for its nourishment. Feed should be cheaper and accessible to all who depend upon that feed. In aquaculture, feed comprises 40%-50% of all the production costs (Rakhmawati and Ubaidillah, 2017). There is a need to find feed substitutes as the present feed ingredients are expensive and less available. Feed additives can be regarded as the substances that are required in fish feed in minute amounts for enhancement of growth performance, survival rate and reproduction of the fish stock. Feed additives can be categorized as living and non-living feed additives. The living feed additives include the use of probiotics, plants and algae in fish feed. The plant additives can be in the form of leaves, extracts, oils etc. (Ogunkalu, 2019).

Ctenopharyngodon idella, one of the widely cultured Chinese carp, is being reared for more than thousand years and is being cultured in more than 40 countries in the present scenario. Grass carp is an herbivorous fish which is able to utilize plant proteins and produce high quality meat (Kristan*et al.*, 2018).

Grass carp is widely found in Pakistan in slow running and standing waters. Grass carp is one of the exotic fishes introduced in Pakistan in last four decades. Exotic Carps are present abundantly in three provinces in Pakistan viz Sindh, K.P.K and Punjab (Khan *et al.*, 2008).

Carp species are eminent all over the world for their fast growth, good performance, high feed conversion ratio, easy cultivation, convenient harvesting and high nutritional value(Song *et al.*, 2012).

World health organization is on its way of promoting the usage of remedial herbs and plants to replace or lessen the chemical use(Levic*et al.*, 2008).

The use of chemicals is strongly condemned at national and international levels if the same results can be achieved by herbs or medicinal plants (Cabuk *et al.*, 2003). Because, these agents increase microbial resistance for infection they impart less-dangerous impacts on health of fish and alternatively the human (Baruah *et al.*, 2008).

Cinnamomum verum isa notable species of plant family Lauracea. This species is known for centuries for its remedial and herbal property, and also forimplicit defense effects such as fungicidal and antimicrobial activity (Becerril et al.. 2007). Approximately 49.9% of nutritional content is cinnamaldehyde in the bark of the plant (Wong et al., 2014). Active concentration of this ingredient may vary depending on various environmental factors and as well as to the form used powder or essential oil (Afifi et al., 2009).Cinnamon also contains large amounts of bioactive molecules including essential oil, tannins, polyphenol, carbohydrates and flavonoids (Gruenwald et al., 2010).

These are cost-effective too than the commercially available fish feed additives and growth stimulators and are imperative to be used in fisheries for the purpose of sustainable and ecologically friendly aquaculture(NRC, 2011; Nugroho, 2016).

Cinnamon contains many essential molecules in large amounts like essential oils which include cinnamic aldehyde and cinnamyl aldehyde, flavonoids, saponins, phenols, and carbohydrates as well (Anderson, 2008). These molecules are responsible for many vital and lifesaving reactions; enzyme modulation, anti-microbial, antioxidant and antiinflammatory activity, redox chelation reactions and contribution of reactive O-oxygen and N-nitrogen species in the fish biological systems (Lopez *et al.*, 2007).

Flavor and storage ability of feed is also improved by supplementation with spices. Cinnamon leaf extract, cinnamon bark oil and cinnamon powder is known for significant increase in energy utilization, PER, SGR, APU, FCR, PER, FER and protein retention in fish. The resultant enhanced antimicrobial activity is associated with cinnamaldehyde, a compound present in cinnamon bark. Cinnamon exhibits various biological activities i.e. antioxidant, antiallergic, antidiabetic and antimicrobial (Begum et al., 2018). Cinnamon improves growth performance, health and immunity (Tawwabet al., 2018); essential for improvement in blood glucose metabolism and breakdown of fatty acids (Setiawatiet al., 2016); improves immunity (Kanghearet al., 2005) and enhances growth performance (Rolinet al., 2015).

It is required to study the effect of different level supplementation of cinnamon on grass carp growth performance and blood parameters. Keeping in view the earlier mentioned reading the study was established to assess the production performance and blood parameters of Grass carp as influenced by dietary administrative 0.5% and 1.5% *Cinnamonum verum*.

Materials and Methods

The present study entitled "Effect of 0.5% and 1.5% dietary supplementation of cinnamon (*Cinnamomum verum*) on growth performance and blood parameters of grass carp (*Ctenopharyngodon idella*)" was conducted at Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. As set before, the trial period was 60 days (2 months), that is, from 5th July to 5th September.

The fingerlings of *Ctenopharygodon idella* with average weight of 10 ± 1.5 g were acquired from Fisheries Research Farms, University of Agriculture, Faisalabad. Before initiation of the experimental period, collected grass carp were kept in glass aquaria and allowed to become habituated to lab conditions for the duration of one week. Basal diet was provided to the fish during this period. Fish were stocked at the rate of 10 fish per tank. Tanks were equipped with capillary system aeration system to meet the optimum growth conditions. The aeration was controlled round the clock with the help of a digital timer attached to electric vacuum aeration pump. Sampling of growth parameter (length(cm) and weight (mg)) was done

weekly on regular basis and feed was adjusted accordingly. During the study period, physiological and water quality parameters were controlled to optimum level. temperature was maintained with the help of exhausts and fanning. Water was changed twice a day to avoid toxic by-products of fish metabolism. Water replacing strategy also minimized temperature, DO and pH fluctuations. pH meter, DO meter and thermometer, were respectively used for pH, DO and temperature records. pH was maintained between 7.3-8.5; temperature between 26-30°C; and DO between 5.5-7.9 mg/L.

Experimental diet preparation

Feed ingredients were gathered from market in raw form and were later processed to make the control and experimental feed. The ingredients included fish meal, rice polish, wheat flour, sunflower oil, minerals and vitamins, and additionally cinnamon only for experimental diets. All elements were grounded to fine powder in morter and pestle. After the preparation of basal feed, powdered cinnamon (also processed in pestle-morter) was added in 0.5% (0.5g/100g feed) and 1.5% (1.5g/100g feed), respectively, to form experimental diet. The prepared feed was stored in cool dry place and in strictly airtight containers. The jars were also labeled in accordance with the treatments viz, A with no added cinnamon (jar 1), B with 0.5% cinnamon (jar 2) and C with 1.5% cinnamon (jar 3).

Feed was given to fish in morning and evening. Also, the feeding rate adjusted in accordance with fish weight after weekly sampling.

Figure1: Preparation of experimental diet of *Ctenopharyngodon idella*



Length gain

gain in length(cm) = final length(cm) - initial length(cm)

Weight gain

weight gain(g) = final weight(g) - initial weight(g)

Composition of experimental diets T₀, T₁ and T₂

Ingredients	Cinnamon levels % in the diet			
	0.0% (control)	0.5%	1%	
		Cinnamon	Cinnamon	
Fish meal	48	48	48	
Soybean meal	38	38	38	
Rice polish	5	5	5	
Wheat flour	5	5	5	
Vitamins and minerals	2	2	2	
Sunflower oil	2	1.5	1	
Cinnamon	0	0.5	1	
Total	100	100	100	

Specific growth rate (SGR)

$$SGR = \frac{\log(final\ body\ wt.) - \log(Initial\ body\ wt.)}{Total\ duration\ of\ experiment} \times 100$$

Feed conversion ratio (FCR)

 $FCR = \frac{Feed \ fed \ (dry \ wt.)}{Weight \ attained \ (net \ wt.)}$

% Survival

 $Survival rate (\%) = \frac{Initial no. of fish stocked - No. of fish died}{Initial no. of fish} \times 100$

Condition factor (K)

Condition factor (K) was calculated as proposed by Hatun Han (1978).

$$K = \frac{Weight (gm)}{Length (cm)} \times 100$$

Determination of Hematological Parameters

Results and Discussion

All the hematological parameters such as red blood cells (TEC), white blood cells (TLC), blood platelet count (BPC), hemoglobin content (Hb), hematocrit(Hct), MCV, MCH and MCHC were determined using different methods.

Growth parameters were measured weekly in terms of total body weight (g) and total body length (cm), and parameters like SGR, FCR, feed conversion ratio etc. Blood parameters including TLC, TEC, hemoglobin level (Hb), Hct, MCV and MCH were analyzed at the termination of study period. Water quality parameters were monitored weekly and values were recorded in notebook. The results were analyzed statistically. Setiawati *et al.* (2015) reported the role of cinnamon in enhanced growth performance of striped catfish at different feeding periods. It was concluded that fish grew best at 0.5% cinnamon feed supplementation for higher growth rate, better meat quality, good texture, high protein content of meat and improved feed efficiency.

Rahmawati and Ubaidillah (2017) evaluated the growth rate and survival of *Oreochromis nilotica* by supplementation of diet with cinnamon leaves. It was seen that survival rate was 100% in all treatments, but the 0.25% level showed the most significant growth rate and feed conversion rate enhancement.

Growth Parameters

- At first week average weight for T₀, T₁ and T₂ was 10, 9 and 10.6 g respectively. At the end, average weight 27.1, 29.1 and 25.15 g observed was in the three aquariums T0, T1 and T2 respectively. The observed average weight was highest for 0.5% cinnamon being 19.54 g; others being 18.54 and 17.75, for T₀ and T₂, respectively.
- Weight gain in T₀ was 17.1 g, for T₁ 20.1 g and for T₂ 14.5 g. Weight gain was highest for 0.5% cinnamon treatment.
- Initial body length recorded in T₀, T₁ and T₂ was 5.4, 5.15 and 5.8 cm, respectively. The final body length was 11.2, 12.7 and 10.95 cm for T₀, T₁ and T₂,respectively.
- The total length recorded was maximum in 0.5% cinnamon treatment being 7.55 cm over a period of 8 weeks. Control group showed 5.8 cm increase

in length and cinnamon 1.5% showed 5.15 cm length gain.

- Like other parameters, the SGR was highest in 0.5% cinnamon and also the growth was consistent throughout the study period. SGR of T₁ was 1.66% per day. T₀ and T₂ showed 1.10% and 1.61% SGR, respectively.
- Feed conversion ratio was lowest for 0.5% cinnamon being 0.42, but it showed highest standard deviation. FCR for T₂ was highest being 0.64 and for T₀ was 0.50.
- CF was highest for T_2 i.e., 3.32 and lowest for T_0 i.e., 3.31. for T_2 it was 3.00. Statistical analysis showed significant difference between three treatments.
- 0.5% cinnamon group showed highest survival rate throughout the study period. Control showed highest mortality. Mortality rate of T₀, T₁ and T₂ was 20%, 0% and 10%, respectively.
- Higher count was observed in T₁ group.
- WBCs values determined were as 11.35, 15.65 and 13.60 $(10^3/\mu L)$ respectively for T₀, T₁ and T₂ (Table 41). It represented 0.5% cinnamon to be effective in boosting WBCs count.
- Hb values obtained after blood analysis were 4.9, 6.03 and 5.36 (g/dl) T_0 , T_1 and T_2 . Analysis of variance indicated significant variations among three treatments
- Haematocrit % determined for T_0 , T_1 and T_2 groups were as 29.1, 34.35 and 32.60 correspondingly
- MCV value was highest in T₁. Statistically highly significant variations were observed
- Observed RBCs values were 1.15, 1.26 and 1.18 $(10^6\!/\mu L)$ for $T_0,\,T_1$ and T_2

Table showing values of different growth parameters during the study period

Growth parameters	T ₀ (Cinnamon=0)	T_1 (Cinnamon=0.5%)	T ₂ (Cinnamon=1%)	P-value	
Initial weight (g)	10±0.14	9±0.50	10.6±0.14	0.0004**	
Final weight (g)	$18.54{\pm}1.41$	19.54±1.61	17.75±1.18		
Weight gain	17.1±0.28	20.1±0.75	14.55±0.69	0.0000**	
Initial length (cm)	5.4±0.07	5.15±0.04	5.8±0.07	0.0005**	
Final length (cm)	8.63±0.50	9.13±0.63	$8.40{\pm}0.44$	0.0005***	
Length gain (cm)	5.8±0.09	7.55 ± 0.08	5.15±0.04	0.0026**	
SGR (%)	2.03±0.15	2.40±0.21	1.76±0.17	0.0000**	
FCR	0.50 ± 0.04	0.42 ± 0.03	0.64 ± 0.07	0.0000**	
Survival rate (%)	80%	100%	90%		
CF	3.31±0.39	3.00±0.46	3.32±0.32	0.0104*	

NS= non-significant (P>0.05); *= significant (P<0.05); **= highly significant (P<0.01)

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Hematlogical parameters	T ₀ (Cinnamon=0)	T_1 (Cinnamon=0.5%)	T_2 (Cinnamon=1%)	P-value
RBCs (10 ⁶ /µL)	1.15±0.005	1.26±0.01	1.18±0.01	0.0035**
WBCs (10 ³ /µL)	11.35±0.350	15.65±0.650	13.60±0.600	0.0266*
Hb (g/dl)	4.90±0.100	6.03±0.08	5.36±0.04	0.0043**
Hct (%)	29.10±0.60	34.35±0.65	32.60±0.70	0.0233*
MCV (fI)	190.64±2.105	235.40±1.700	223.17±2.13	0.0011**
MCH (pg)	34.48±0.38	36.08 ± 0.62	38.30±0.40	0.0250*
MCHC (g/dl)	16.85±0.25	19.00 ± 0.10	18.05±0.25	0.0129*

Hematological values \pm SE of Ctenopharyngodon idella in T₀, T₁ and T₂

NS= non-significant (P>0.05); *= significant (P<0.05); **= highly significant (P<0.01)

Graph showing weight gain (g) and length gain (cm) of Ctenopharyngodon idella in various treatments



Conclusion

It was observed that 0.5% cinnamon level was acquiring the most significant values in terms of weight gain, length gain, SGR and blood parameters. Also, the survival rate was highest for T_1 as compared to T_0 and T_2 . So, supplementation of grass carp's feed with 0.5% *Cinnamomum verum* was concluded to be most effective than others as a feed supplementation for improvement in growth, performance and blood parameters. Growth rate and survival rate as well as immunity can be improved by addition of medicinal plants to fish feed. If farmers start using herbs and natural plants as their feed enhancers, they can get more profit out of little effort.

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