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Growth, Survival and Haemato-Biochemical Profiles of the Freshwater Catfish, *Pangasius sutchi* (Fowler, 1937) Fingerlings Fed on *Tinospora cordifolia* Leaf Extract Supplemented Diet.

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ABSTRACT

The present study documents the possible effects of *Tinospora cordifolia* leaf extract supplemented diets on growth, survival and haemato-biochemical profiles of the catfish, *Pangasius sutchi* fingerlings. *P. sutchi* fingerlings were fed with formulated diets, supplemented with four different concentrations of *T. cordifolia* leaf extract (viz. 100, 200, 400 and 800 mg kg^{-1} of feed) for 45 days. Fingerlings fed with basal diet served as control. Various parameters of serum biochemical and haematology such as serum total protein content, albumin content, globulin content, albumin globulin ratio, glucose, erythrocytes count, leucocytes count were evaluated along with growth parameters. The results indicated that Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), survival and Haemato-biochemical profiles such as total serum protein, albumin, globulin, albumin globulin ratio and serum glucose were high in the fingerlings fed with *T. cordifolia* leaf extract supplemented diets, irrespective of dosage, compared to control. Among the four concentrations of *T. cordifolia* leaf extract used, 200 mg/kg of feed group showed increased growth, survival and enhanced the health status of *P. sutchi* fingerlings.

INTRODUCTION

The health status of the early developmental stages of any cultivable organisms is essential for an economic viability of any aquaculture practices (**Misra *et al.*, 2009; Stoskopf, 1993**) and it can be improved through feeding of formulated diet to increase growth of reared fish (**Abdel-Tawwab *et al.*, 2006**). Even though, fish health is affected and lead to production loss due to unmanaged fish culture practices and adverse environmental conditions. Hence, the fish aquaculturists have to carry out careful husbandry practices (**Sakai, 1999**) by undertaking prophylactic, diagnostic and therapeutic measures to achieve optimal fish production during aquaculture of fish.

Nowadays, antibiotics and different chemicals have been extensively used to prevent or treat fish diseases. In the Ayurvedic and Unani systems of the medicine, *Tinospora cordifolia*, commonly known as Guduchi or Amruthavalli, is used as a medicine for centuries. The antidiabetic, antioxidant, antihepatotoxic and immunomodulatory properties, due to the presence of various constituents like alkaloids, steroids, glycosides and polysaccharides, of *T. cordifolia* extract are well documented (**Singh *et al*., 2003; Panchabhai *et al*., 2008; Sharma *et al*., 2012; Thatte, 1992**).

The freshwater cat fish, *Pangasius sutchi* is a promising candidate for aquaculture practices due to its omnivorous nature of feeding, rapid growth, high fecundity and high stocking density tolerance (**Hung *et al.*, 2001; Rahman *et al.*, 2000**). In the present study, an attempt was made to evaluate the potential use of methanolic extract of *T. cordifolia* leaf, supplemented through diet, on growth, survival and serum biochemical profiles of the freshwater fish, *P. sutchi* fingerlings.

MATERIALS AND METHODS

Fingerlings collection and maintenance

The healthy, disease-free and active fingerlings of the freshwater cat fish, *P. sutchi*, average body weight of 22.5±1.5g, were brought from the Bharath Fish Seed Farm located in Poondi, Tamilnadu, India. The fingerlings were brought to the laboratory and acclimatized them to the laboratory condition in 1000L circular FRP tank and were fed with the basal diet (without any dietary supplementation) for 15 days. After acclimatization, fingerlings were randomly selected and subjected to feeding trial experiment for 45 days. Ground water was used for rearing the fingerlings and the water quality parameters, such as temperature (26.5±1.5°C), pH (7.2 to 8.3), dissolved oxygen (6.3-7.8 mg L^{-1}), ammonia (0.15-0.23 mg L^{-1}), nitrite (0.002-0.005 mg L^{-1}) and nitrate (0.04-0.09 mg L^{-1}) were maintained throughout the experimental period. All the above estimations were carried out using the standard methods of **Strickland and Parsons (1972**).

Collection and preparation of *T. cordifolia* extract

The fresh *T. cordifolia* leaves were hand-picked from the natural gardens from the University campus. The leaves were washed in distilled water and shade dried, powdered and stored at 4°C until use. The leaf powder of *T. cordifolia* were subjected to methanolic extract by following the method of **Harikrishnan and Balasundaram (2005**). Briefly, 50g leaf powder was taken into a sterilized 1000 ml conical flask. To this, 500ml of methanol was added at a ratio of 1:10 w/v and shaked continuously for 72 hr. After that, the mixture was filtered using Whatman No.1 filter paper. The filtered extract was concentrated using a rotary vacuum evaporator (Buchi SMP, Switzerland).

Preparation of formulated feed

Formulated diet was prepared according to **Misra *et al* (2009)**. Formulated diet was supplemented with graded levels of methanolic extract of *T. cordifolia* leaf. In total, 5 experimental groups were used for the present study and were designated as D_c, D_1, D_2, D_3 and D_4. Group D_c was fed with a formulated diet without any supplementation and was considered as control group. Group D_1, D_2, D_3 and D_4 were incorporated with 100, 200, 400 and 800 mg/kg diet concentrations of methanol extract of *T. cordifolia* leaves respectively and were denoted as experimental groups. After cooling, other ingredients

like fish oil, vitamins and minerals along with the prerequisite leaf extract were added (**Garg *et al*., 2002**). Finally, uniform-sized pellets of the feed were obtained using a hand pelletizer, spreaded on a paper and dried under shade and also by keeping in a hot air oven at 55±1°C for overnight to ensure complete dryness. Then, the pellets were packed separately in polythene bag and stored at 4°C. All the control and experimental *P. sutchi* fingerlings were fed at the rate of 3% of their body weight with respective diet for 45 days. The feeding rate was adjusted accordingly with respect to increase in the bio-mass.

Growth performance

To assess the growth performance, both control and experimental fingerlings were counted and weighed every two weeks. The growth parameters such as specific growth rate (SGR) (**Ricker, 1979**), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (FCR) (**Cho, 1992**) and percent survival (S%) of both control and experimental fingerlings were calculated at the end of the feeding experimental period.

$$\text{SGR} = 100 \times \frac{\ln \text{final weight} - \ln \text{initial weight}}{\text{total duration of the experiment}}$$

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

$$\text{PER} = \frac{\text{Protein fed (g)}}{\text{wet weight gain (g)}}$$

Haematological parameters

For hematological parameters and serum studies, the blood samples of the fingerlings of both control and experimental groups were collected either by puncturing the heart and/or caudal vein using syringe (insulin syringe- 1ml), which was previously rinsed with 2.7% EDTA solution, once in 15 days interval. For serum studies, the blood samples were collected as above, without anticoagulant.

Hematological parameters and serum biochemical profiles such as red blood cell and white blood cell counts (by haemocytometry according to **Rao and Chakrabarti, 2004**), haemoglobin concentration (spectrophotometrically at 540 nm with cyanmethemoglobin method of **Citarasu *et al*., (2006**), haematocrit (microcentrifuge method), mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration (**Rao and Chakrabarti, 2004**), protein (Biuret and BCG dye binding method of **Reinhold (1980**), albumin (by the bromocresol green binding method of **Doumas *et al*., 1997**), globulin (calculated by subtracting albumin values from total serum protein), albumin/globulin ratio (calculated by dividing albumin values by globulin values) and glucose (**Sasaki, 1972**) were estimated by following standard methods.

Statistical analysis

All results are presented as the mean and standard deviation of five independent observations. The nonparametric ANOVA (SPSS) was used to determine whether there was a significant difference ($P < 0.05$) in the growth, survival and hemato-biochemical parameters compared to control and tested groups.

RESULTS

All the water-quality parameters, during the experimental period, were within the optimal range for rearing freshwater catfish.

Growth parameters and survival percentage

The results on the growth performance revealed that the experimental fingerlings showed significantly increased the SGR, PER and S% in compared to control on the 45th day (Table 1). Among the various experimental groups, D_3 fingerlings registered high SGR, PER and S% (1.49±0.10; 2.49±0.12 and 94.67±2.08% respectively) and D_1 fingerlings registered low values for the above (1.14±0.07; 1.91±0.11 and 83.33±2.33% respectively), whereas FCR was found significantly decreased in D_3 (1.21±0.10) fingerlings (Table 1). Statistical analysis revealed that the variations in SGR, FCR, PER and S% of fingerlings were significantly differed in the experimental groups compared to the control ($P < 0.05$).

Table 1. Growth performance and survival of *P. sutchi* fingerlings fed with herbal extract supplemented diet

Experimental group	SGR (%)	FCR (g/g)	PER (g/g)	Survival (%)
D_c	1.00±0.05^a	1.67±0.12^a	1.80±0.07^c	75.34±2.06^a
D_1	1.14±0.07^b	1.57±0.08^a	1.91±0.11^c	83.33±2.33^b
D_2	1.29±0.12^c	1.42±0.11^b	2.11±0.06^d	88.00±2.00^c
D_3	1.49±0.10^d	1.21±0.10^c	2.49±0.12^e	94.67±2.08^d
D_4	1.20±0.04^e	1.53±0.07^a	1.95±0.09^c	89.34±2.51^e

$\overline{X}$ ±S.D of 5 observations; the values in the row with different letters are significantly different ($P<0.05$).

Haematological Parameters

In the present study a variety of haematological parameters were used to augment the possible physiological and metabolic alterations of *P. sutchi* fingerlings fed with methanolic extract of *T. cordifolia* leaf supplemented fingerlings and were compared with the control group fingerlings.

All the hematological parameters studied, such as erythrocytes and leucocytes count, haematocrit percentage, haemoglobin concentration, mean corpuscular haemoglobin concentration, level of mean corpuscular hemoglobin, mean corpuscular volume were found to be increased in experimental fingerlings compared to control. Among the experimental groups, D_3 fingerlings registered maximum values for all the above parameters (2.02±0.06×10^6 cells mm^{-3} and 30.65±0.28×10^3 cells mm^{-3}, 30.06±0.25%, 7.81±0.14g dl^{-1}, 25.97±0.45%, 39.01±2.42pg cell$^{-1,}$ 150.18±10.74 respectively), on the 45th day. Whereas these values in control fingerlings showed variations (1.60±0.06 to 1.78±0.05×10^6 cells mm^{-3}, 23.61±0.34 to 24.56±0.37×10^3 cells mm$^{-3,}$ 26.36±0.21 to 27.56±0.23%, 6.22±0.08 to 6.45±0.16g dl^{-1} respectively (Figure 1,2,3,4,5&6). Statistical analysis revealed that all the above parameters are differed significantly when compared to control fingerlings ($P < 0.05$).

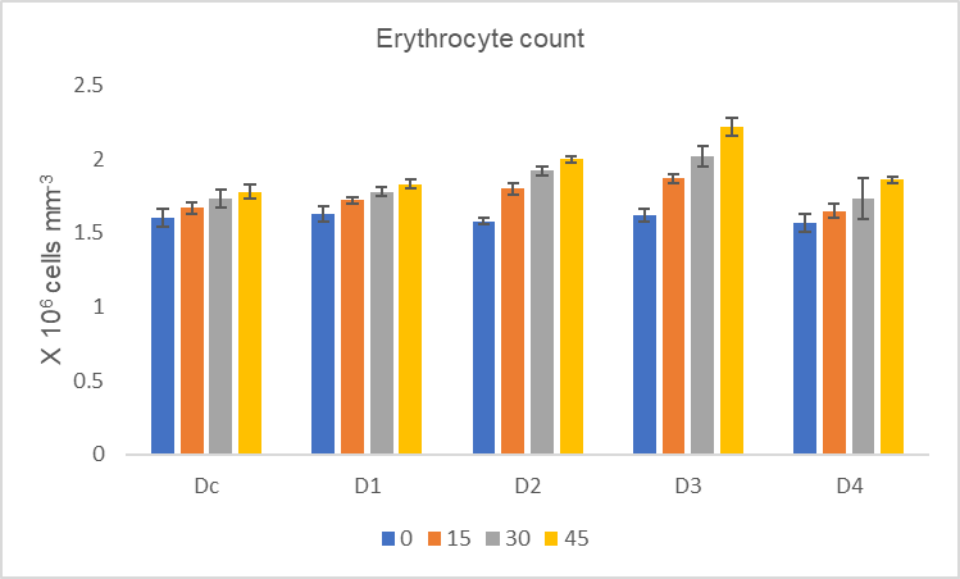

Fig. 1: Total erythrocytes count of *P. sutchi* fingerlings fed with herbal extract supplemented diets

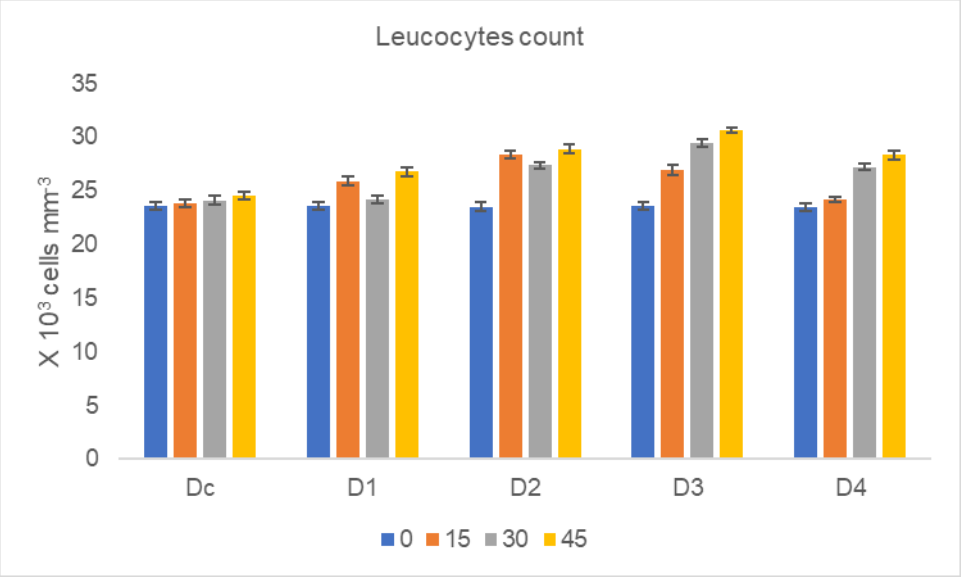

Fig. 2: Total leucocytes count of *P. sutchi* fingerlings fed with herbal extract supplemented diets

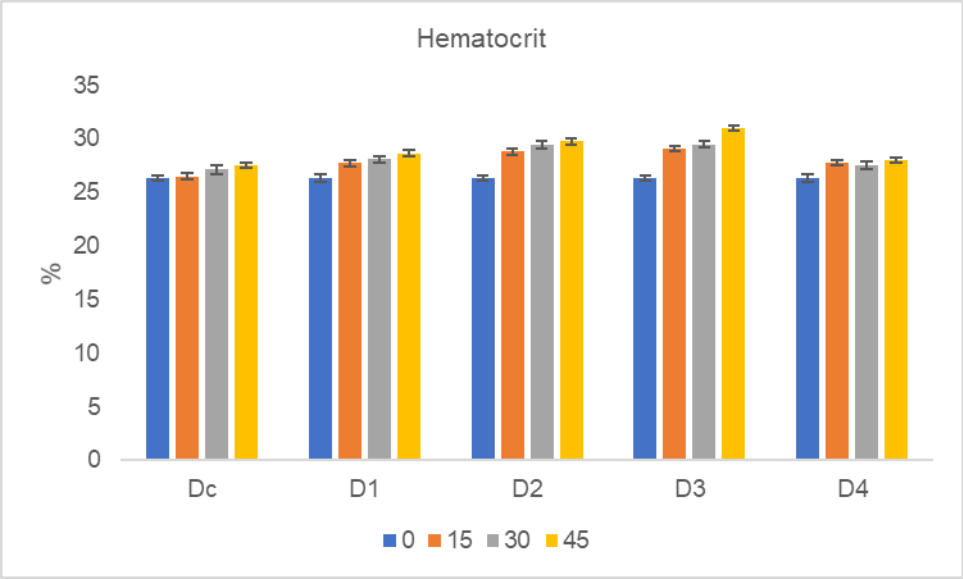

Fig. 3 : Haematocrit percentage of *P. sutchi* fingerlings fed with herbal extract supplemented diets

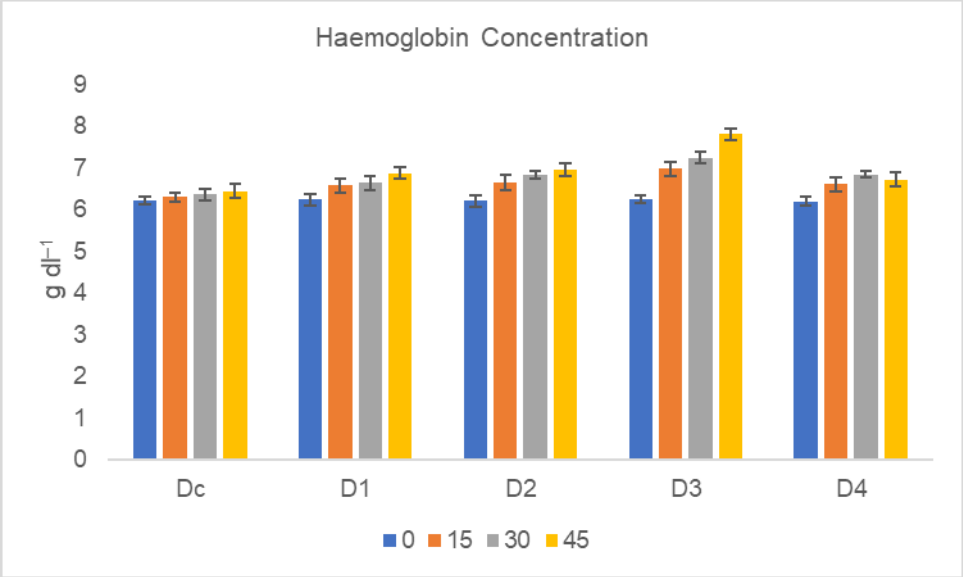

Fig. 4: Haemoglobin concentration of *P. sutchi* fingerlings fed with herbal extract supplemented diets

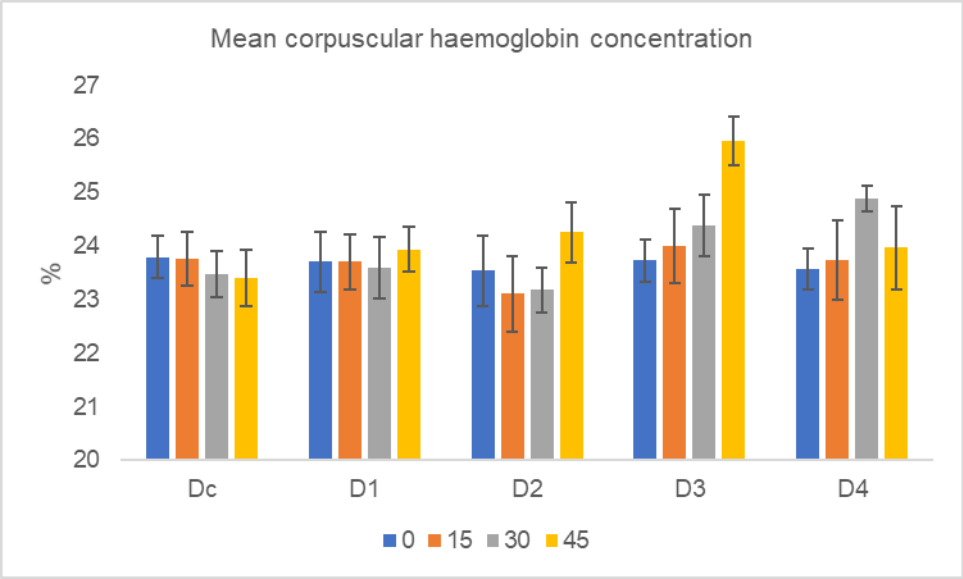

Fig. 5: Mean corpuscular haemoglobin concentration of *P. sutchi* fingerlings fed with herbal extract supplemented diets

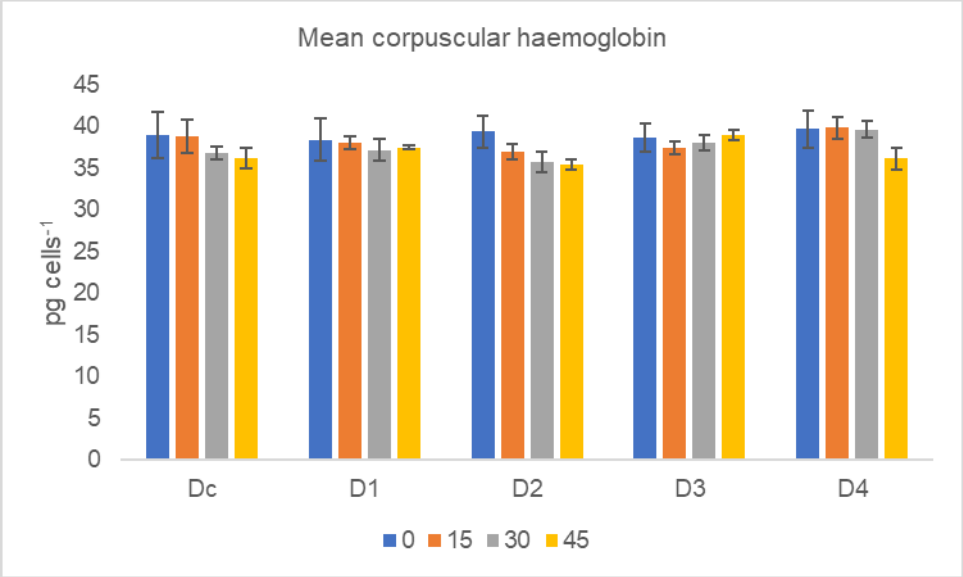

Fig. 6: Mean corpuscular haemoglobin of *P. sutchi* fingerlings fed with herbal extract supplemented diets

Serum Biochemical Profiles

Biochemical analysis of serum was carried out to understand the suitability of methanolic extract of *T. cordifolia* leaf supplementation in preserving the health status of *P. sutchi* fingerlings. Total serum protein, albumin, globulin and albumin/globulin ratio of fingerlings were analyzed in fingerlings before and after the feeding experiment. The results revealed that all the studied biochemical properties of the serum, except serum glucose, such as protein, albumin, globulin serum and albumin/globulin ratio and reached maximum experimental fingerlings compared to the control fingerlings. Among the experimental fingerlings, D_3 fingerlings registered maximum value for protein, albumin,

globulin and serum albumin/globulin ratio and serum glucose (3.92±0.05g dl^{-1}, 1.64±0.05g dl^{-1}, 2.39±0.08g dl^{-1}, 0.72±0.07g dl^{-1} respectively) on 45th day, whereas the serum glucose level was found to be decreased (74.35±0.62mg dl^{-1}) in D_3 fingerlings compared to control (Figures 7, 8, 9, 10 & 11). Statistical analysis revealed that all the studied hematobiochemical profiles varied significantly in the experimental groups compared to the control ($P < 0.05$).

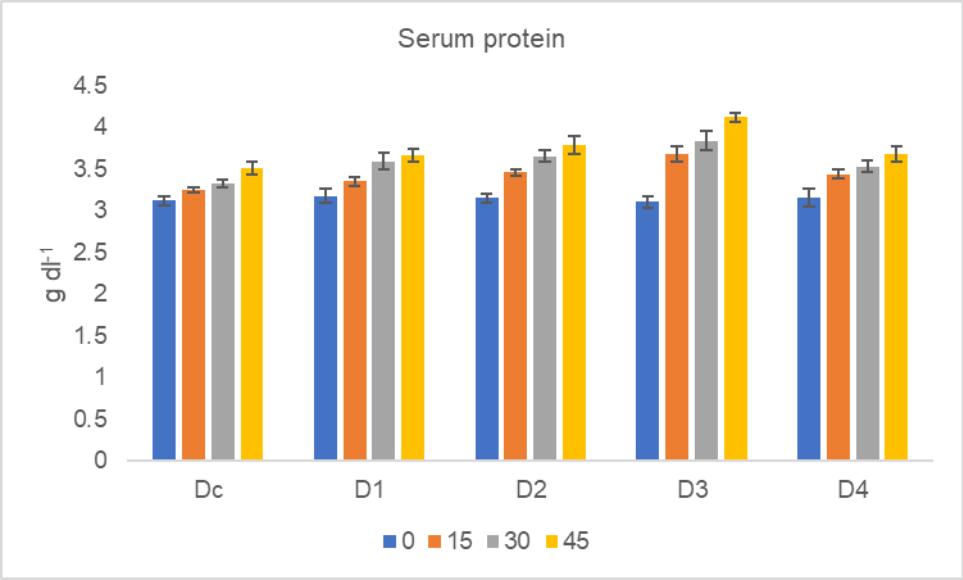

Fig. 7: Serum protein content of *P. sutchi* fingerlings fed with herbal extract supplemented diets

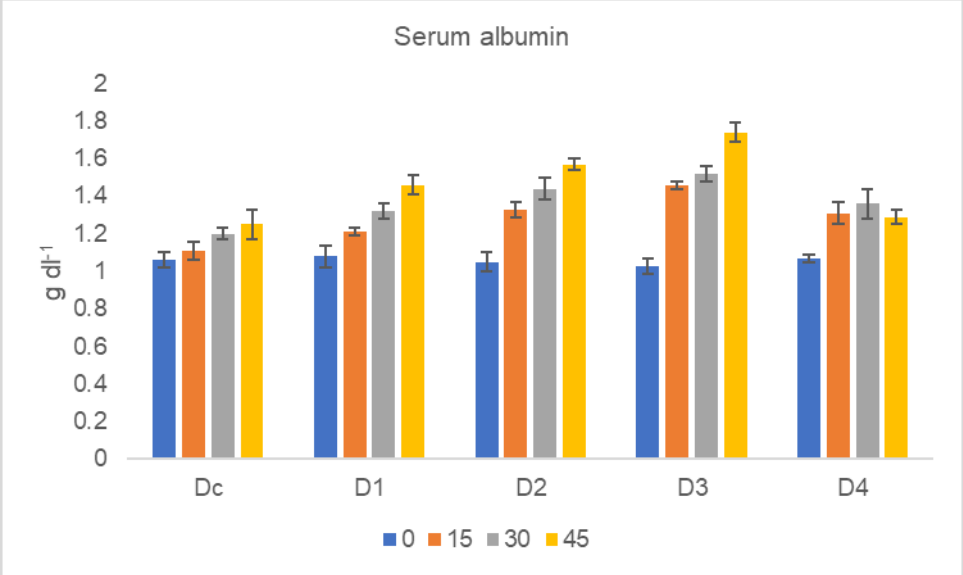

Fig. 8: Serum albumin content of *P. sutchi* fingerlings fed with herbal extract supplemented diets

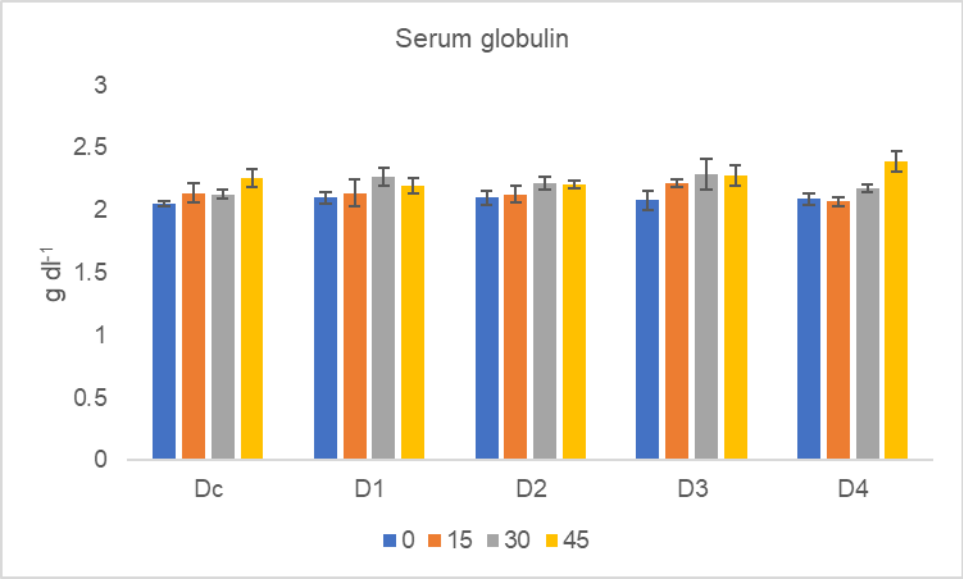

Fig. 9: Serum globulin content of *P. sutchi* fingerlings fed with herbal extract supplemented diets

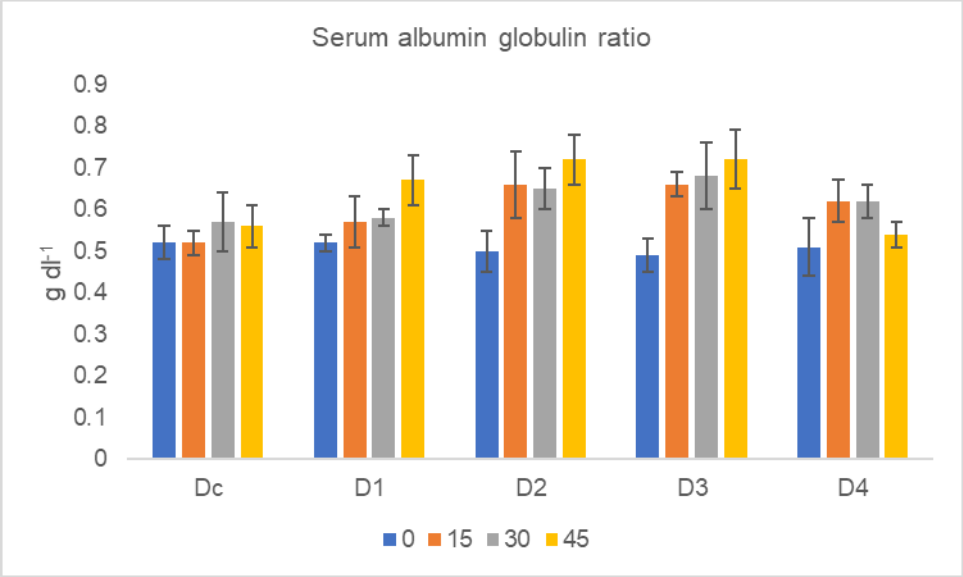

Fig. 10: Serum albumin/globulin ratio of *P. sutchi* fingerlings fed with herbal extract supplemented diets

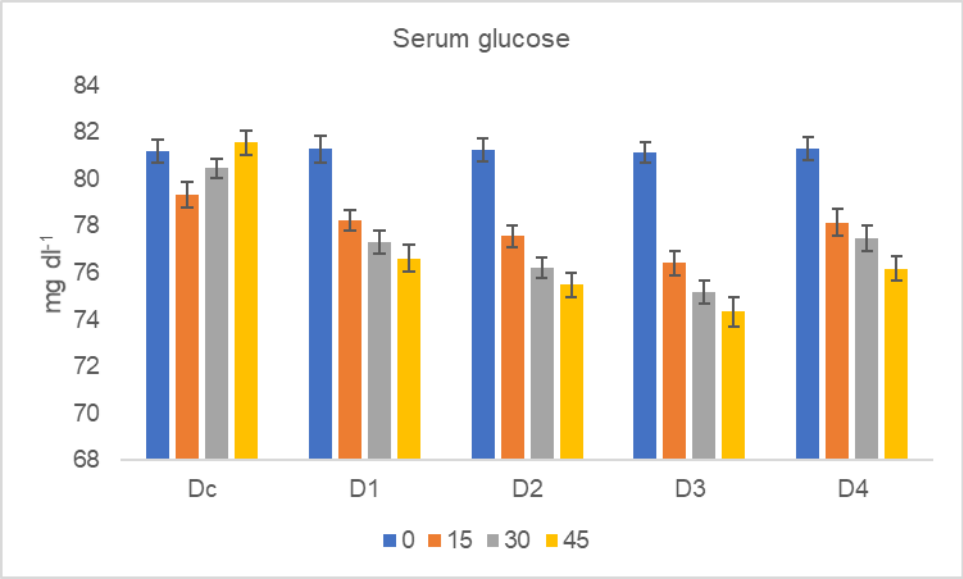

Fig. 11: Serum glucose content of *P. sutchi* fingerlings fed with herbal extract supplemented diets

DISCUSSION

Inclusion of herbal protein has become a common alternative for replacement of fish meal in the diet of many fishes. Therefore, dietary manipulation for growth enhancement and health management has been a prioritized topic in aquaculture research (**Li *et al.*, 2006**). The present study evaluated the effect of *Tinospora cordifolia* leaf (herbal) extract to enhance the growth performance in the fingerlings of the catfish *Pangasius sutchi*. The results revealed that the dietary administration of *T. cordifolia* leaf extract significantly increased the growth parameters in *P. sutchi* fingerlings compared to the control fingerlings. Growth factors were more favorable in *T. cordifolia* leaf extract supplemented diets, fed group D_3 (400mg kg^{-1}). This may possibly be due to the ability of the herb to improve nutrient digestibility, the secretory stimulation of extracellular enzymes by gut micro flora that reduce the bacterial load. The above results can be correlated with observations of **Rao and Chakrabarti (2004**). Similarly, herbal extracts supplemented diet increase growth parameters in *P. monodon* post larvae (**Citarasu *et al.*, 2006**), *Radix astragalin*, *R. angelicae sinensis (***Jian and Wu, 2004**), *Myristica fragrans* (**Sivaram *et al.*, 2004**), *Labeo rohita (***Rao *et al.*, 2006; Sahu *et al.*, 2007**), *Paralichthys olivaceus (***Ji *et al.*, 2007**). In the present study, the efficiency of FCR reduced in fish fingerlings fed with herbal supplemented diets. Of interest, **Glencross *et al.* (2004**) declared that incorporation of yellow lupin at 12.5% into fish diets of rainbow trout enhanced FCR.

Due to the great importance of blood in pathological studies, hematological parameters of fish are commonly used to assess the health status and in diagnosing of disease. It can also be used to study immunopotentiators. In the present study, fingerlings fed with herbal, *T. cordifolia* leaf extract supplemented diet showed increased erythrocyte count probably due to an immunostimulatory activity of herbal. These findings are similar to that of **Sahu (2007**), who reported an increased in RBC counts in *L. rohita* fingerlings fed with the kernel of *Magnifera indica*.

Hematological parameters such as haemoglobin concentration and haematocrit values provide a key to understand the fish health for fishery biologists (**Blaxhall, 1972**) and in monitoring stress responses (**Soivio and Oikari, 1976**). Haematocrit is the proportion of blood volume that is occupied by RBC's. In the present study haemoglobin and haematocrit concentrations were, increased in the fingerlings fed with *T. cordifolia* supplemented diets, indicating an improvement on the health status. Blood indices such as MCV, MCH and MCHC are particularly important for the diagnosis of anemia in animals (**Coles, 1986**). In the present study, a significant increase of MCHC in fingerlings fed with group D_3 fingerlings of herbal supplemented diets and MCH in herbal extract supplemented group D_3.

Assessing the serum biochemical profiles often provide vital information about the health status of the cultured fish species as they are related to their nutritional status (**Cnaani, 2003; Řehulka *et al*., 2004; Abdel-Tawwab *et al*., 2008**). Generally, increases in the levels of serum protein, albumin and globulin in fish are thought to be associated with a stronger innate response (**Eskander and Won Jun, 1995**). Present study showed a significant increase in serum biochemical profiles such as protein, albumin and globulin in the cat fish fingerlings fed with *T. cordifolia* leaf supplemented diets compared to the control. Several investigators have recorded increases in total protein, albumin and globulin in fish serum after administration of herbal compounds or immunostimulants (**Jian and Wu, 2004; Ji *et al*., 2007**). Generally glucose levels increase in infected or stressed animals (**Jian and Wu, 2004**). In the experimental catfish fingerlings, the glucose level found decreased; which clearly indicates the herbal supplementation does not pose any stress to the fish. An inverse relationship between glucose level and concentration of herbal extract had been reported in common carp and rohu fed with the extracts of garlic and mango kernel (**Ji et al., 2007**). This observation may be attributed to the hypoglycemic activity of some plant extracts to increase the level of serum insulin[37] and for the enhancement of peripheral metabolism of glucose (**Skim et al., 1999**). In contrast, the glucose level increased in common carp treated with aqueous neem, *A. indica,* leaf extract after challenge with *Aeromonous hydrophila (***Harikrishnan et al., 2003**). Conversely to the present study, feeding rainbow trout with 1 % and 2 % lupin, mango and stinging nettle for 2 months led to an increase in glucose level in all the treated groups, compared to controls.

CONCLUSION

The methanolic extract of *Tinospora cordifolia* leaf can be supplemented in order to increase the growth and hematobiochemical profile of the freshwater fish, *Pangasius sutchi* fingerlings.

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