

Effect of feeding of pointed gourd (*Trichosanthes dioica*) fruit and leaves on mortality and serum biochemical profile of common carp (*Cyprinus carpio*)

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ABSTRACT : In present study paste of *Trichosanthes dioica* fruit (TDF) and leaf (TDL) was fed to common carp at 3 different inclusion levels of 5%, 10% and 15% respectively of total feed. The experimental design incorporated total 6 groups, 3 each of TDF (G1, G2 and G3) and TDL (G4, G5 and G6) fed @5%, 10% & 15% of total feed given daily for 15 days. Groups fed with TDF paste showed high mortality rate whereas no mortality was seen in case of groups fed with TDL paste. No serum biochemical studies could be done in TDL fed groups because of high mortality. Fish fed with 15% TDF paste showed greater reduction in the levels of total blood glucose, aspartate amino transferase, alanine amino transferase, creatinine, whereas highest increase in total protein, albumin and globulin level was observed. The present study suggests that TDF can be used as a feed additive in fish feed upto 15% of inclusion level without any deleterious effect on fish health.

Key words: Biochemical, common carp, *Trichosanthes dioica*

The population of the world is growing at an exponential rate and the need of the hour is to increase the food production with the same rate to ameliorate inadequate food supplies and the consequential malnutrition. One of the promising solutions to the shortage of animal protein intake in developing countries is the proper development of aquaculture (Osineye *et al.*, 2009). Fish feed is the most expensive input in aquaculture operations. Most of the high cost of feed arises from extensive reliance on protein sources such as fish meal and shrimp meal. The shortage of feed and its high cost severely constrained the development of low cost aquaculture systems suitable for small-scale farmers in developing countries. Hence, there is a need to assess the potential of non-conventional raw ingredients like orange peels (Ashade *et al.*, 2011), corn cob (Ansah *et al.*, 2012), kola pod husk (Oshineye *et al.*, 2008), bread waste (Omole *et al.*, 2011), shrimp waste (Osineye *et al.*, 2009), yam peels (Lawal *et al.*, 2012), potato peels (Faramarzi *et al.*, 2012) etc. It would therefore be more economical to utilize plant protein in fish feeding than animal protein materials to enhance fish production.

Trichosanthes dioica is also known as the pointed gourd, parwal/parval (from Hindi) and often called green potato. Fruits of *Trichosanthes dioica* are used as vegetable for human consumption. Being very rich in

protein and vitamin A, it has certain medicinal properties, and many reports are available regarding its role in lowering of blood sugar and serum triglycerides (Sheshadri, 1990). The fruits are easily digestible and diuretic in nature. It is very beneficial in overcoming problems like constipation, fever, skin infection, wounds and also improves appetite and digestion and lowers cholesterol (Tanwar *et al.*, 2011 and Banu *et al.*, 2007). The juice of leaves of *T. dioica* are used as tonic, febrifuge, in oedema, alopecia and in subacute cases of enlargement of liver and spleen (Shankar *et al.*, 2005).

Although, various leaf, seed and root meals have been tested as potential fish feed ingredient to decrease diet cost, the use of pointed gourd leaf, root or plant meal has not been tested in fish. The present study deals with changes in serum biochemical parameters of *Cyprinus carpio* fed with leaf and fruit paste of pointed gourd.

MATERIALS AND METHODS

Collection and acclimatization of experimental fish:

The experiment was carried out at College of Fisheries, G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand). Total duration of experiment was 15 days and it was conducted during the

month of March, 2015. Experimental fishes were obtained from the Instructional Fish Farm, College of Fisheries, Pantnagar. Seventy-two fish of average weight 250 g of both sexes were randomly divided into 6 groups of 12 fish each. Fishes were acclimatized for a period of one week before the start of feeding trial.

Collection and preparation of experimental plant material:

Fresh leaves of pointed gourd were collected from Instructional Fish Farm, College of Fisheries, GBPUA&T, Pantnagar and TDF were purchased from the local vegetable market of Pantnagar and were identified by the Department of Vegetable Sciences, College of Agriculture, GBPUAT, Pantnagar. It was washed thoroughly with tap water to remove dirt. The leaves and fruit were grinded using a laboratory grinder and was kept in the fridge at 4°C till use.

Formulation of Experimental Diets: The basal fish feed included Rice bran and Mustard Oil cake. Both leaf and fruit paste was given @ 5%, 10% and 15% of the daily fish diet.

Mortality: Mortality, if any, was recorded in any of the groups.

Biochemical Analysis: Different biochemical parameters were measured at 0th, 3rd, 6th, 9th and 15th days post treatment (DPT). Blood samples were collected by cardiac puncture. Collected blood samples were centrifuged at 2200 rpm for 8-9 minutes and serum was separated. Separated serum was stored in refrigerator at 4°C till further use. Some serum biochemical parameters viz. total serum protein, albumin, globulin, aspartate amino transferase, alanine amino transferase and creatinine were measured spectrophotometrically using kits of Transasia Bio-medicals Ltd., Solan, HP, India and the total serum glucose was estimated using commercial kit manufactured by Span Diagnostics Ltd., Surat, India.

Statistical Analysis: Biochemical data of the present

study were statistically analyzed through two-way analysis of variance (ANOVA) using Statistical Package for Social Sciences 2006 version 16.0 (SPSS). Means of the samples were compared by Duncan multiple range test and the level of significance were tested at P<0.05 (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Mortality: Groups (G1, G2 and G3) fed with TDF showed no mortality at any DPT where as in the groups (G4, G5 and G6) fed with TDL, high rate of mortality was observed within first 5 days of feeding and it increased with the increase in the dose of TDL paste and the days of feeding (Table 1). Highest and lowest mortality rate was observed in groups G6 (91.67%) and G4 (33.34%) respectively. TDL poses a negative impact on the fish as heavy mortality occurred.

Effect of pointed gourd fruit on biochemical parameters

Total serum protein: Total serum protein content in different groups at different time intervals is given in Table 2 which reveals that, the average total serum protein content in groups G1, G2 and G3, ranged from 2.442±0.003 to 2.816±0.003 (g/dl), 2.444±0.003 to 2.891±0.005 (g/dl) and 2.447±0.004 to 3.124±0.004 (g/dl) respectively showing significant increase in the values in all the groups from 0 DPT to 15th DPT.

When these values were compared between different groups at various time intervals, no significant difference was recorded at 0th DPT between any groups. From 3rd DPT to 15th DPT, all groups showed significant increase with maximum increase in group G3 followed by groups G2 and G1.

Serum albumin: The serum albumin values in different groups at various time intervals are presented in Table 3.

Table 1: Mortality pattern in fishes fed with different concentrations of pointed gourd fruit and leaf paste

Days Post Treatment (DPT)	No. of fishes died in different feed groups					
	TDF			TDL		
	G1	G2	G3	G4	G5	G6
1 st DPT	-	-	-	0	0	1
2 nd DPT	-	-	-	0	1	1
3 rd DPT	-	-	-	1	2	2
4 th DPT	-	-	-	1	2	3
5 th DPT	-	-	-	2	3	4
Mortality rate (%)	0.00	0.00	0.00	33.34	66.67	91.67

Table 2: Average (Mean±SE) total serum protein (g/dl) in different groups of experimental fishes at different time intervals

DPT	Groups		
	G1	G2	G3
0 th DPT	2.442±0.003 ^{Fa}	2.444±0.003 ^{Fa}	2.447±0.004 ^{Fa}
3 rd DPT	2.523±0.006 ^{Ec}	2.594±0.003 ^{Eb}	2.637±0.007 ^{Ea}
6 th DPT	2.582±0.004 ^{Dc}	2.635±0.005 ^{Db}	2.777±0.004 ^{Da}
9 th DPT	2.655±0.004 ^{Cc}	2.684±0.011 ^{Cb}	2.873±0.003 ^{Ca}
12 th DPT	2.721±0.004 ^{Bc}	2.735±0.003 ^{Bb}	2.924±0.003 ^{Ba}
15 th DPT	2.816±0.003 ^{Ac}	2.891±0.005 ^{Ab}	3.124±0.004 ^{Aa}

Different small letters (a, b and c) indicate significant (P<0.05) difference between groups at a particular day, whereas different capital letters (A, B, C, D, E and F) indicate significant (P<0.05) difference between days within a particular group.

Table 3: Average (Mean±SE) serum albumin (g/dl) in different groups of experimental fishes at different time intervals

DPT	Groups		
	G1	G2	G3
0 th DPT	1.354±0.003 ^{Fb}	1.351±0.008 ^{Fa}	1.355±0.003 ^{Fb}
3 rd DPT	1.384±0.002 ^{Ec}	1.465±0.005 ^{Eb}	1.548±0.005 ^{Ea}
6 th DPT	1.467±0.005 ^{Dc}	1.577±0.004 ^{Db}	1.697±0.006 ^{Da}
9 th DPT	1.506±0.004 ^{Cc}	1.678±0.005 ^{Cb}	1.737±0.004 ^{Ca}
12 th DPT	1.657±0.006 ^{Bc}	1.705±0.004 ^{Bb}	1.864±0.004 ^{Ba}
15 th DPT	1.788±0.005 ^{Ac}	1.868±0.004 ^{Ab}	1.968±0.005 ^{Aa}

Different small letters (a, b and c) indicate significant (P<0.05) difference between groups at a particular day, whereas different capital letters (A, B, C, D, E and F) indicate significant (P<0.05) difference between days within a particular group.

In groups G1, G2 and G3, the values increased from 1.354±0.003 to 1.788±0.005 (g/dl), 1.351±0.008 to 1.868±0.004 (g/dl) and 1.355±0.003 to 1.968±0.005 (g/dl) respectively, from 0th DPT to 15th DPT. In all the groups, serum albumin increased significantly from 0th DPT to 15th DPT.

When the mean albumin values in different groups were compared at varying time intervals, the values did not show any significant variation at 0th DPT between any groups. From 3rd to 15th DPT, all groups showed significant increase in the values where group G3 had highest values followed by groups G2 and G1.

Serum globulin: Serum globulin level in different groups at varying time intervals is presented in Table 4. In groups, G1, G2 and G3, the mean serum globulin ranged from 1.067±0.004 to 1.278±0.005 (g/dl), 1.064±0.003 to 1.367±0.005 (g/dl) and 1.064±0.003 to 1.485±0.006 (g/dl) respectively. Serum globulin content increased significantly from 0th DPT to 15th DPT in all the groups.

When the mean globulin values in different groups were compared at varying time intervals, no significant

difference could be noted between any groups at 0th DPT. From 3rd DPT to 15th DPT there was a significant increase in the values of globulin between different groups with highest and lowest increase was in groups G1 and G3 respectively.

Total serum glucose: Total serum globulin level in different groups at varying time intervals is presented in Table 5. In all the groups G1, G2 and G3, there was a significant decrease in the serum glucose content from 0th DPT to 15th DPT with the values varying from 80.246±0.016 to 78.274±0.011 (mg/dl), 80.247±0.014 to 77.690±0.012 (mg/dl) and 80.247±0.008 to 75.245±0.011 (mg/dl) respectively.

When these values were compared at different time intervals between different groups, no significant variation could be recorded at 0th DPT. There was a significant decrease in the values of total serum glucose between different groups from 3rd DPT to 15th DPT with minimum and maximum decrease in the serum glucose content showed by groups G1 and G3 respectively.

Serum creatinine: Serum creatinine content in different

Table 4: Average (Mean±SE) serum globulin (g/dl) in different groups of experimental fishes at different time intervals

DPT	Groups		
	G1	G2	G3
0 th DPT	1.067±0.004 ^{Fa}	1.064±0.00 ^{3Fa}	1.064±0.003 ^{Fa}
3 rd DPT	1.138±0.004 ^{Ec}	1.175±0.005 ^{Eb}	1.236±0.006 ^{Ea}
6 th DPT	1.163±0.003 ^{Dc}	1.235±0.005 ^{Db}	1.298±0.006 ^{Da}
9 th DPT	1.197±0.005 ^{Cc}	1.286±0.006 ^{Cb}	1.334±0.004 ^{Ca}
12 th DPT	1.227±0.006 ^{Bc}	1.328±0.005 ^{Bb}	1.406±0.005 ^{Ba}
15 th DPT	1.278±0.005 ^{Ac}	1.367±0.005 ^{Ab}	1.485±0.006 ^{Aa}

Different small letters (a, b and c) indicate significant (P<0.05) difference between groups at a particular day, whereas different capital letters (A, B, C, D, E and F) indicate significant (P<0.05) difference between days within a particular group

Table 5: Average (Mean±SE) total serum glucose (mg/dl) in different groups of experimental fishes at different time intervals

DPT	Groups		
	G1	G2	G3
0 th DPT	80.246±0.016 ^{Fa}	80.247±0.014 ^{Fa}	80.247±0.008 ^{Fa}
3 rd DPT	79.948±0.020 ^{Ea}	79.525±0.023 ^{Eb}	79.381±0.020 ^{Ec}
6 th DPT	79.530±0.021 ^{Da}	78.867±0.020 ^{Db}	78.846±0.020 ^{Dc}
9 th DPT	79.164±0.005 ^{Ca}	78.247±0.014 ^{Cb}	77.736±0.014 ^{Cc}
12 th DPT	78.752±0.006 ^{Ba}	78.215±0.008 ^{Bb}	76.564±0.011 ^{Bc}
15 th DPT	78.274±0.011 ^{Aa}	77.690±0.012 ^{Ab}	75.245±0.011 ^{Ac}

Different small letters (a, b and c) indicate significant (P<0.05) difference between groups at a particular day, whereas different capital letters (A, B, C, D, E and F) indicate significant (P<0.05) difference between days within a particular group.

groups at varying time intervals is presented in Table 6. There was a significant decrease in the creatinine values in all the groups with values ranging between 0.612±0.002 to 0.568±0.003 (mg/dl), 0.615±0.014 to 0.529±0.003 (mg/dl) and 0.618±0.008 to 0.493±0.003 (mg/dl), respectively.

When the mean creatinine values in different groups were compared at varying time intervals no significant difference could be recorded between any groups at 0th DPT. From 3rd DPT to 15th DPT, all groups showed significant decrease with maximum decrease in the level of serum creatinine was in group G3 followed by groups G2 and G1.

Serum Aspartate aminotransferase (AST)/ Serum Glutamate Oxaloacetate Transaminase (SGOT): Serum AST activity in different groups of experimental fishes at varying time intervals is depicted in Table 7. In groups, G1, G2 and G3 there was a significant decrease in the AST activity with the values varying from 55.942±0.020 to 53.726±0.012 (IU/L), 55.949±0.014 to 51.833±0.008 (IU/L) and 55.946±0.008 to 49.526±0.012 (IU/L), respectively. All the groups showed significant decrease in the serum AST activity from 0th DPT to 15th DPT.

When the mean AST values in different groups were compared at varying time intervals, the values did not show any significant variation at 0th DPT between any groups. From 3rd DPT to 15th DPT, all groups showed significant decrease. These values were highest in G3 followed by G2 and G1.

Serum Alanine aminotransferase (ALT)/ Serum Glutamate Pyruvate Transaminase (SGPT): Serum ALT activities in different groups of experimental fishes at varying time intervals are depicted in Table 8. In all the groups, G1, G2 and G3 there was a significant decrease in the ALT activity from 0th DPT to 15th DPT with the values varying from 21.382±0.020 to 20.440±0.017 (IU/L), 21.385±0.014 to 19.015±0.16 (IU/L) and 21.386±0.008 to 18.226±0.020 (IU/L), respectively.

When the mean ALT values in different groups were compared at varying time intervals, the values did not show any significant variation at 0th DPT between any groups. From 3rd DPT to 15th DPT, all groups showed significant decrease. These values were highest in G3 followed by G2 and G1.

Groups fed with TDL paste in the fish diet showed

Table 6: Average (Mean±SE) serum creatinine (mg/dl) in different groups of experimental fishes at different time intervals

DPT	Groups		
	G1	G2	G3
0 th DPT	0.612±0.002 ^{Fa}	0.615±0.014 ^{Fa}	0.618±0.008 ^{Fa}
3 rd DPT	0.603±0.003 ^{Ea}	0.595±0.003 ^{Eb}	0.586±0.003 ^{Ec}
6 th DPT	0.591±0.003 ^{Da}	0.583±0.003 ^{Db}	0.560±0.005 ^{Dc}
9 th DPT	0.584±0.003 ^{Ca}	0.566±0.003 ^{Cb}	0.546±0.003 ^{Cc}
12 th DPT	0.572±0.003 ^{Ba}	0.541±0.005 ^{Bb}	0.523±0.006 ^{Bc}
15 th DPT	0.568±0.003 ^{Aa}	0.529±0.003 ^{Ab}	0.493±0.003 ^{Ac}

Different small letters (a, b and c) indicate significant (P<0.05) difference between groups at a particular day, whereas different capital letters (A, B, C, D, E and F) indicate significant (P<0.05) difference between days within a particular group.

Table 7: Average (Mean±SE) serum AST (IU/L) in different groups of experimental fishes at different time intervals

DPT	Groups		
	G1	G2	G3
0 th DPT	55.942±0.020 ^{Fa}	55.949±0.014 ^{Fa}	55.946±0.008 ^{Fa}
3 rd DPT	54.853±0.020 ^{Ea}	54.656±0.008 ^{Eb}	54.376±0.102 ^{Ec}
6 th DPT	54.550±0.017 ^{Da}	54.226±0.014 ^{Db}	53.167±0.012 ^{Dc}
9 th DPT	54.173±0.012 ^{Ca}	53.430±0.011 ^{Cb}	52.656±0.008 ^{Cc}
12 th DPT	53.946±0.008 ^{Ba}	52.663±0.017 ^{Bb}	51.346±0.008 ^{Bc}
15 th DPT	53.726±0.012 ^{Aa}	51.833±0.008 ^{Ab}	49.526±0.012 ^{Ac}

Different small letters (a, b and c) indicate significant (P<0.05) difference between groups at a particular day, whereas different capital letters (A, B, C, D, E and F) indicate significant (P<0.05) difference between days within a particular group.

Table 8: Average (Mean±SE) serum ALT (IU/L) activity in different groups of experimental fishes at different time intervals

DPT	Groups		
	G1	G2	G3
0 th DPT	21.382±0.020 ^{Fa}	21.385±0.014 ^{Fa}	21.386±0.008 ^{Fa}
3 rd DPT	21.123±0.014 ^{Ea}	20.046±0.012 ^{Eb}	19.960±0.015 ^{Ec}
6 th DPT	20.850±0.011 ^{Da}	19.840±0.017 ^{Db}	19.526±0.008 ^{Dc}
9 th DPT	20.640±0.011 ^{Ca}	19.656±0.024 ^{Cb}	19.140±0.015 ^{Cc}
12 th DPT	20.546±0.014 ^{Ba}	19.237±0.014 ^{Bb}	18.746±0.012 ^{Bc}
15 th DPT	20.440±0.017 ^{Aa}	19.015±0.16 ^{Ab}	18.226±0.020 ^{Ac}

Different small letters (a, b and c) indicate significant (P<0.05) difference between groups at a particular day, whereas different capital letters (A, B, C, D, E and F) indicate significant (P<0.05) difference between days within a particular group.

high rate of mortality due to the presence of saponin in the leaf of *T. dioica* (Chopra *et al.*, 2002). Saponin when dissolve in water acts as a poison which is commonly being used to kill weed or predatory fishes in the fish pond (Howes, 1930). TDF paste diet resulted in significant increase in total serum protein, albumin and globulin level. Rai *et al.* (2008a) fed the aqueous extract of *Trichosanthes dioica* fruits to rats at a dose of 1000mg/kg body weight daily once for 28 days resulted in increase in total protein. The values of serum glucose, creatinine, AST and ALT showed decreased with increase in concentration of the pointed gourd fruit paste

supplementation. Results of this study showed that serum AST and ALT activities decreased significantly in the fish group fed on all levels of TD. These observations are in agreement with those of Rai *et al.* (2008b) and Ghaisas *et al.* (2008) who found that the enzyme activities (AST, ALT, and ALP) in serum of rats decreased significantly when were fed pointed gourd fruit extract. Decreased serum activity of AST and ALT might be due to decreased hepatocellular production or release of the enzymes (PSD, 2007). It acts as plant protein source which increases the blood protein level.

In the present study, total plasma glucose concentration reduced significantly in fish fed diets containing the different levels of PGF paste. These results are in accordance with those of Rai *et al.* (2008b) and Banu *et al.* (2007), as they also observed reduction of blood glucose in normal and STZ diabetic rats fed aqueous fruit extract of *T. dioica*. Chandrasekhar *et al.* (1988) have also reported that pointed gourd (whole plant and aerial part) possesses the medicinal property of lowering blood sugar level in rats. The values of serum glucose decreased significantly ($P \leq 0.05$) which may be due to hypoglycemic activity of pointed gourd and probably due to phyto-chemical constituents demonstrated in the fruit i.e. flavonoids, saponins, polyphenols, alkaloids and tannins (Ghaisas *et al.*, 2008). Although the results of the present study showed a decrease in serum AST, ALT, creatinine and glucose levels but these values ranged within the normal limits of parameters in carps (Field *et al.*, 1943).

CONCLUSION

It is concluded that *T. dioica* fruit can be used in the diet of common carp up until 15% inclusion without any adverse effect on the growth and health.

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