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Faiqa Syeed Farooqi
Faculty of Fisheries, SKUAST-K
Shalimar, Rangil,
Jammu and Kashmir, India

Farooz Ahmad Bhat
Faculty of Fisheries, SKUAST-K
Shalimar, Rangil, Jammu and
Kashmir, India

Tasaduq Hussain Shah
Faculty of Fisheries, SKUAST-K
Shalimar, Rangil,
Jammu and Kashmir, India

Oyas Asimi
Faculty of Fisheries, SKUAST-K
Shalimar, Rangil,
Jammu and Kashmir, India

Bilal Ahmad Bhat
Faculty of Fisheries, SKUAST-K
Shalimar, Rangil,
Jammu and Kashmir, India

Adnan Abubakr
Faculty of Fisheries, SKUAST-K
Shalimar, Rangil,
Jammu and Kashmir, India

Anayatullah Chesti
Faculty of Fisheries, SKUAST-K
Shalimar, Rangil,
Jammu and Kashmir, India

Corresponding Author:
Faiqa Syeed Farooqi
Faculty of Fisheries, SKUAST-K
Shalimar, Rangil,
Jammu and Kashmir, India

Dietary *Beta vulgaris* enhances growth and improves hemato-physiological responses in common carp *Cyprinus carpio* var. *communis*

Faiqa Syeed Farooqi, Farooz Ahmad Bhat, Tasaduq Hussain Shah, Oyas Asimi, Bilal Ahmad Bhat, Adnan Abubakr and Anayatullah Chesti

Abstract
This study was conducted to evaluate the effect of *Beta vulgaris* on growth and blood physiology of common carp (*Cyprinus carpio*). The fish with an initial average weight of 50 ± 05g were divided into four groups (three treatments and a control); and fed daily with the plant-based diet for an eight-week period. Each treatment was tested in three replicates and the fish were hand fed twice a day. The results show that incorporating *Beta vulgaris* in the diet of common carp significantly increased ($p<0.05$) the final weight, weight gain ratio, and specific growth rate as compared to the control group, but significantly decreased ($p<0.05$) the feed conversion ratio. The treated groups had significantly higher red blood cell count, white blood cell count, haematocrit (Hct), haemoglobin (Hb) values as compared to the control. It was also observed that feeding the fish with supplemented diets markedly increased total protein, albumin, and globulin values; but decreased the glucose value in the treated groups. However, triglyceride, cholesterol and urea values did not vary significantly among the various treatments. From the study, it may be concluded that dietary *Beta vulgaris* improves growth and blood physiological parameters in common carp and is consequently recommended as a feed additive in its diet.

Keywords: *Cyprinus carpio*, *Beta vulgaris*, feed additive, growth, phenols, flavanoids

1. Introduction
The fish health depends on what they eat or what they are fed with. Nutritious feed and appropriate feeding regime ensures optimum health of fish. The nutrition of fish is regarded as a complex and multidimensional area which interrelates with the immune system, hence fish health, through a broad array of direct and indirect mechanisms (Oliva-Teles-2012) [36]. Plants have been used as immunity boosters for humans for thousands of years throughout the world, as most of them are rich sources of volatile oils, saponins, phenols, tannins, alkaloids, polysaccharides and polypeptides which are responsible for various activities e.g. anti-stress, appetizer, tonic, anti-microbial and immune-modulatory. The use of plants and herbs in the aquaculture sector may have various beneficial effects (Citarasu, 2010) [14]. The application of medicinal herbs and plants in aquaculture has been recommended by many researchers (Herken *et al*., 2007) [24] because herbs and their derivatives optimally improve the immunity and growth in fish (Awad & Awad, 2017) [4]. The use of medicinal plants is considered as an alternative to the use of antibiotics in aquaculture (Chakraborty & Chattopadhya, 1998) [11].
The beneficial properties of plant constituents in fish nutrition may include the stimulation of appetite and feed intake, stimulation of digestion, stimulation of immune response, antibacterial, antiviral, and antioxidant action (Dorman, 2000) [16]. The effect of various herbal products on different species of fish show that the use of natural food additives in the form of medicinal herbs or herbal extract stimulates haematological and biochemical performance, increases the fish growth and also defends from the pathogens (Parrino *et al*., 2019) [38]. Moreover, many reports are available which provide sufficient evidence for linkage between herbal extracts and plant secondary metabolites (PSM) with physiological functions associated with reproduction (Ahmadifar, 2021) [1]. These herbal extracts and PSMs affect fertility by changing the levels and activities of numerous hormones on the HPG axis. This alteration may be in part regulated by induced changes in the expression of reproduction related genes.
Red beetroot (*Beta vulgaris*) is a naturally occurring root vegetable which belongs to the family *Amaranthaceae*. It consists of multiple biologically active phytochemicals including betalains, flavonoids, polyphenols, carotenoids, sterols, saponins and inorganic nitrate (NO_3).

Beetroot is regarded as a potential source of betalains, which are valuable water-soluble nitrogenous pigments (Onkar *et al*., 2013) [37]. Betalains are of two main types: the red betacyanins and the yellow betaxanthins. Beetroot is also a rich source of diverse minerals like potassium, sodium, phosphorous, calcium, magnesium, copper, iron, zinc and manganese (Singh & Hathan, 2014) [52]. Various parts of this plant have been used in traditional medicinal system as anti-oxidant, anti-depressant, anti-microbial, anti-fungal, anti-inflammatory, diuretic, expectorant and carminative (Wettasinghe *et al*., 2002) [56]. In fact, beetroot is classified as one of the ten plants with the highest antioxidant activity on the planet (Baião *et al*., 2017) [5]. Sufficient literature is available on the beneficial effect of *Beta vulgaris* in nutrition of poultry birds (Kumar *et al.*, 2014) [28], while Jha *et al*., (2013) and Amiri *et al*., (2019) [3] report growth promoting effect of *Beta vulgaris* in aquaculture.

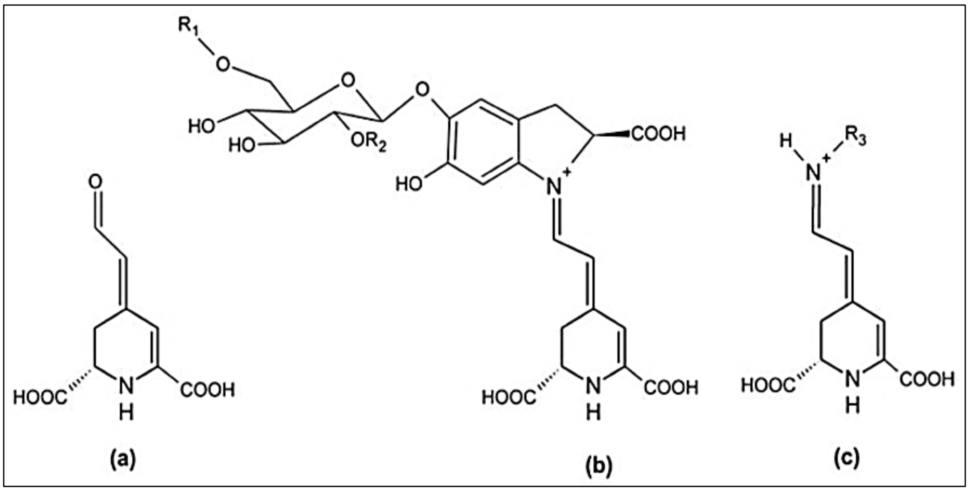

Fig 1: General structures of (a) betalamic acid (b), betacyanins (c) & betaxanthins (Baião *et. al.*, 2017) [5]

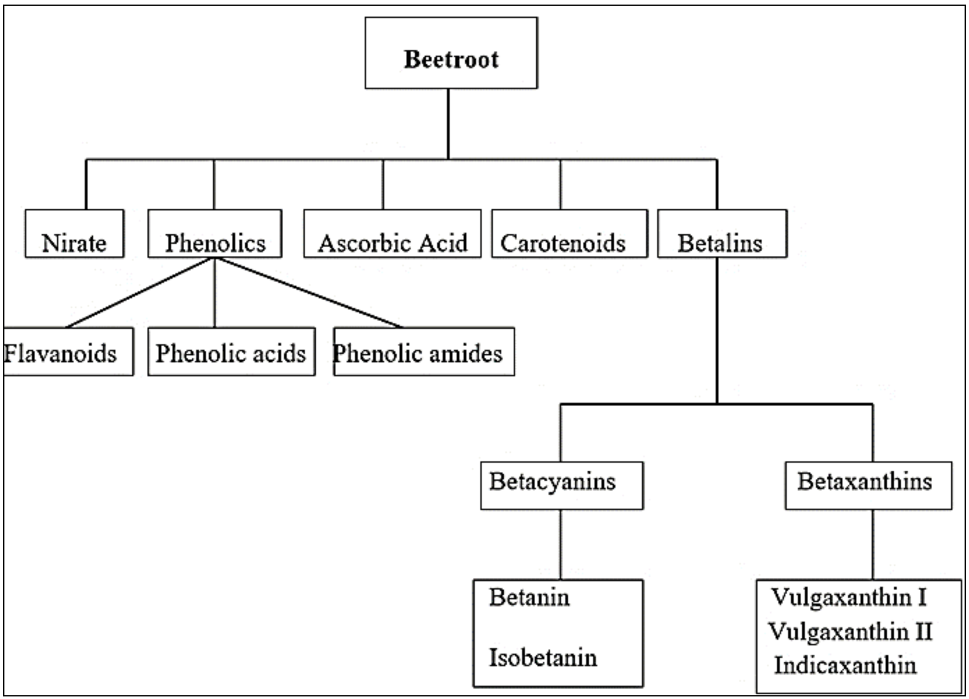

Fig 2: Overview of potentially bioactive compounds in beetroot (Ninfali and Angelino, 2013) [35]

Common carp (*Cyprinus carpio*) is considered as one of the most important freshwater fish species across the world (Shirali *et al*., 2012) [51]. In 2018, the global common carp production reached 4.2 million tonnes, ranked 4th in global fish production (FAO, 2020) [18]. The common carp was first introduced to India from Ceylon in the year 1939 and later again from Bangkok in the year 1957. In the valley of Kashmir, common carp was first introduced in the year 1959 in order to augment the fish yield (Sehgal, 1989) [48]. It has been categorized as a eury-thermal fish *i.e.* it can withstand wide range of temperature fluctuations. Therefore, it is well suited for culture in the climatic conditions of the Kashmir Valley. Common carp is an omnivorous fish feeding on plankton, benthos and detritus in the natural conditions. Augmenting and enhancing carp aquaculture could be a decisive step towards global food security concerns (Liao & Chao 2009) [31]. Therefore, improving the practical diets for common carp is inevitable. Hence, this study was conducted to evaluate the effect of *Beta vulgaris* in common carp diet and understand its impact on the haematological and biochemical parameters of the fish.

2. Materials and Methods

2.1 Fish rearing and feeding protocols

The experimental fish were purchased from the National Fish Seed Farm, Manasbal and transported to the instructional fisheries farm located in FVSc & AH campus in Shuhama, Alesteng. The carp were acclimatized to the system for two weeks. Afterwards, 120 apparently healthy fish (50 ± 06g) were randomly distributed into twelve tanks holding de-chlorinated freshwater at a density of 10 fish in each tank. Each of the experimental groups were tried in three replicate tanks. The health status of the fish during the acclimation and experimental period was good, there was no mortality during the experiment. Fish were manually fed with the experimental diets at a level of 3% of their respective body weight in two equal parts for a period of 60 days. In order to maintain the rearing conditions, tanks were aerated using air stones.

2.2 Diet preparation and experimental design

Beta vulgaris roots were purchased from local market in Srinagar. The beetroots were dried in hot air oven and powered using a household electric grinder and the powder was mixed to the diets just before pelletization at respective concentrations. Four experimental diets (one control and three treatment groups) with 30% crude protein were prepared. The dry ingredients of each diet were thoroughly mixed and dough was prepared by adding distilled water to the mixture. The resultant dough was made into semi moist feed pellets using a hand pelletizer (1 mm diameter mesh). The feed pellets were sun dried for a week. After drying, the pellets were packed in plastic containers and were sealed airtight and labelled according to the treatments.

Table 1: Experimental diets fed to fishes in various treatment groups

Treatment	Dose
Control (C)	Basal diet
Treatment (T_1)	Basal diet + 5% Beetroot powder
Treatment (T_2)	Basal diet + 7.5% Beetroot powder
Treatment (T_3)	Basal diet + 10% Beetroot powder

2.3 Sampling procedure

After 60 days, fish were sampled to evaluate growth, haematological and physiological indices. Five fish per treatment were randomly selected and anesthetized using clove oil (40 mg/l). Blood was collected using a 2 mL sterilized syringe from the caudal vein. For serum preparation, blood samples were immediately transferred into tubes without anti-coagulant and allowed to clot at room temperature for 30 min. The serum samples were subsequently obtained by centrifuging the blood samples at 3500 rpm for 10 min and kept at -80 °C until further analysis.

2.4 Growth parameters

After 60 days of the dietary experiment, the growth performance was evaluated by measuring weight gain, specific growth rate, feed conversion ratio and survival rate according to the following equations (Raissy *et al*., 2022) [42]:

Weight gain (WG) (g) = Final weight-initial weight.

Specific growth rate (SGR) (%) = 100 ({ln final wt. (g)-ln initial wt. (g)}/60 days).

Feed conversion rate (FCR) = Total feed given (g)/weight gain (g).

Survival rate (SR) (%) = (Final numbers/initial numbers) × 100.

The weight was measured using a scale with precision of 0.1 g.

2.5 Haematological Parameters

The blood samples were analysed for the estimation of various haematological parameters *viz*. number of erythrocytes and leucocytes, haemoglobin (Hb), haematocrit (Hct). The number of erythrocytes was determined by the haemocytometer method (Ellis, 1990), haematocrit was estimated by the micro-haematocrit method and haemoglobin measurement was determined by the cyano-metahemoglobin method.

2.6 Biochemical parameters

Determination of total serum protein (TP), albumin (ALB), glucose (GLU), triglyceride (TRIG), cholesterol (CHOL) levels was performed based on the method of Ghafarifarsani *et al.* (2021). The values were measured by using a spectrophotometer.

2.7 Data Analysis

Values were expressed as Mean ± standard deviation (SD). Growth performance, haematological & biochemical parameters were tested using one-way ANOVA and Duncan's multiple range test was used for comparison of the mean values at the 5% level of significance using the software SPSS (Version 16.0).

3. Results and Discussion

3.1 Weight gain and Specific Growth Rate

In the present study, there was a significant difference in the weight gain and specific growth rate among all the treatments. The highest weight gain and specific growth rate was observed in the treatment T2 (Beetroot @ 7.5%), while as lowest weight gain and specific growth rate was found in the control group. This indicates that *Beta vulgaris* has a beneficial effect on the growth performance of common carp which might be due to increased digestive enzyme activity and low metabolic needs induced by the supplementation of beetroot. The enhanced enzyme activity due to the addition of beetroot might have improved the digestion of protein, carbohydrate and fat, which explains the better weight gain observed in the fish. Frankic *et al*., (2009) [19] reports that herbs enhance the secretion of pancreatic enzymes which are important factors in nutrient digestion and assimilation.

Beetroot is rich in alkaloids, phenols and polyphenols. Polyphenols are known to stimulate the activity of digestive enzymes and increase the synthesis of DNA, RNA, and proteins, as well as GH and IGF-1. Besides, they stimulate the expression of genes involved in the appetite and food intake, absorption and transport of nutrients, and exhibit other metabolic functions by enhancing intestinal micro biota, which in turn enhance the growth (Safari *et al.*, 2020) [44]. In addition, phytochemicals stimulate the fish immune system (both specific and non-specific) by enhancing the immune cell functions, increasing cytokine and antibody productions, and improving immune-related gene expression. These beneficial effects of phytochemicals subsequently lead to better health, higher disease resistance and hence faster growth of fish (Chakraborty *et al.*, 2014) [10]. Similar results were observed by Grace *et al.*, (2020) [22] who observed the effect of beetroot juice on growth performance, blood profile and carcass characteristics in broiler chicken. Significant differences (p<0.05) were noted in the average daily feed intake and the average daily weight gain. The hematological parameters and serum biochemical parameters were significantly higher in the treatments as compared to the control.

3.2 Feed Conversion Ratio and Protein Efficiency Ratio

Feed conversion ratio (FCR) is an important variable used to assess the ability of fish to convert feed into flesh. It is a measure of fish's efficiency to convert feed into increased body mass. The lower the value of FCR, the lower amount of feed is needed to increase a unit of body weight. Our study reveals that *Cyprinus carpio* fed with different doses of *Beta vulgaris* show lower FCR as compared to the control. Lowest FCR was recorded in the treatment T2 (Beetroot @ 7.5%), while as highest FCR was recorded in the control. This can be attributed to active compounds in beetroot, which may have improved fish performance by stimulating the secretion of the digestive enzyme, increasing digestibility and stimulating appetite (Platel, 2004) [41]. Similar results have been ascertained by Amiri *et al*., (2019) [3] who studied the effect of *Beta vulgaris* powder on feeding, growth and survival of common carp. This is also in agreement with Grace *et al.* (2020) [22], who evaluated the effect of beetroot juice on growth performance, blood profile and carcass characteristics of broiler chicken. It was observed that the supplementation of beetroot juice significantly lowered the FCR in the treated groups as compared to the control.

Protein Efficiency Ratio reflects the efficiency of protein in the fish feed and its subsequent conversion as meat. De Silva and Anderson (1995) [15] opine that the Protein Efficiency Ratio is a measurement of how the protein sources in a diet can meet the essential amino acids requirement of the fish feed. The Protein Efficiency Ratio (PER) varied significantly among the various treated groups. Highest PER was recorded in the treatment T2 (Beetroot @ 7.5%), while as lowest PER was found in the control group. These results are in agreement with Turan & Akuyurt (2005) [55], who observed that feeding red clover to African catfish (*Clarias gariepenus*) at different concentrations (25, 50 and 75 mg/kg) increased feed efficiency and protein efficiency ratio in the treated groups as compared to the control. Similar results were obtained by Shalaby *et al*., (2006) [49] who report significantly higher protein efficiency ratio (PER) in Nile tilapia (*Oreochromis niloticus*) which were fed diet containing garlic powder @ 30 g/kg diet.

Table 2: Weight gain, specific growth rate, feed conversion ratio & protein efficiency ratio of common carp fed with *Beta vulgaris* supplemented diets at different levels for 60 days

Treatment	Weight Gain (%)	SGR (%)	FCR	PER
Control	24.92±2.50	0.45±0.03	2.02±0.02	1.12±0.02
T_1 (5%)	36.84±2.66	0.72±0.02	1.44±0.02	1.68±0.05
T_2 (7.5%)	38.50±3.54	0.80±0.08	1.40±0.05	1.84±0.04
T_3 (10%)	32.49±2.78	0.64±0.03	1.65±0.05	1.46±0.01

3.3 Haemoglobin (Hb), Red blood cells (RBCs) and Haematocrit (HCT) values

It was observed that the hemoglobin level and RBC count were significantly elevated in the treated groups as compared to the control. The erythrocyte count and haemoglobin level was significantly higher in the treatment T2 (Beetroot @7.5%). Higher RBC count is associated with higher hematocrit value; therefore HCT values were significantly higher in the treatments as compared to the control. The highest haematocrit value was observed in T2 (Beetroot @7.5%). The supplementation of *Beta vulgaris* in the treated groups significantly enhanced the RBC count vis-à-vis the control with the highest RBC count in T2 & T3. Similar results were elucidated by Cho *et al.* (2017) [12] who observed that beetroot enhanced the level of haematocrit and haemoglobin, as well as increased RBC count in mice. This may be attributed to the high iron and folic acid content in beetroot. The significant increase in RBC count of treated groups in the present experiment strengthens the view that *Beta vulgaris* influence the hematopoietic system of common carp.

Table 3: Haemoglobin, RBC count, Haematocrit value of common carp fed with *Beta vulgaris* supplemented diets at different levels for 60 days

Treatment	Hb (g/dl)	RBC Count (10^6/mm^3)	Haematocrit value (%)
Control	6.01±0.40	0.92±0.01	25.20±1.80
T_1 (5%)	7.24±0.30	1.92±0.01	34.10±1.70
T_2 (7.5%)	8.80±0.12	1.92±0.02	36.50±1.60
T_3 (10%)	7.41±0.49	1.34±0.01	32.14±1.34

3.4 Leucocyte Count

The white blood cells or leucocytes are non-pigmented cells which protect body from infections and foreign particles. They are broadly classified as granulocytes and agranulocytes based on the presence or absence of granules in the cytoplasm. The granulocytes include neutrophils, eosinophils and basophils; whereas agranulocytes include monocytes and lymphocytes. White blood cells in fish share functional and morphological similarities with the mammalian lymphocytes, granulocytes and monocytes. They play an important role in enhancing the non-specific or innate immunity, which is regarded as an indicator of fish health (Roberts, 1978) [43]. In the present study, the level of circulatory leucocytes was found to be significantly elevated in the fishes of treatment T2 (Beetroot @7.5%). On the other hand, lowest leucocytes were found in the fishes of control group. Lymphocytes, granulocytes and monocytes were also significantly higher in the fishes of the treated groups as compared to the control. The total leucocyte count (TLC) in the various treated groups is depicted below.

Table 4: Total Leucocyte count of common carp fed with *Beta vulgaris* supplemented diets at different levels for 60 days

Treatment	Total Leucocyte Count (10^3/mm^3)
Control	84.80±3.40
T_1 (5%)	98.25±3.21
T_2 (7.5%)	102.00±2.40
T_3 (10%)	96.32±3.82

The total leucocyte count (TLC) was highest in the treatment T2 (Beetroot @7.5%) at the end of 60th day. This could be an indication that carp fed the herb-based diets show signs of an altered immune response as compared to the control. Herbal bioactive compounds act as immunostimulants and affect several immune-related pathways. The results are in agreement with Grace *et al*. (2020) [22], who observed that the supplementation of beetroot juice significantly increased the WBC count in the treated groups as compared to the control. Also, Beshel *et al*. (2018) [8] reported that the ethanolic extract of beetroot significantly elevates the total leucocyte count in wistar rats. Similar results were obtained by Talpur *et al*. by inclusion of peppermint in the diet of Asian sea bass *Lates calcarifer* and Salah *et al.* (2008) [47] by feeding *Echinacea purpurea* based diet in Nile tilapia (*Oreochromis niloticus*).

3.5 Serum glucose

In the present study, the highest serum glucose was found in the control group (142±4.20 mg/dL) vis-à-vis the groups fed with *B. vulgaris* based diet. The lowest serum glucose was found in the treatment T2 (96±2.40mg/dL). This is in agreement with the work of Sahu *et al*. (2007) [45] and Citarasu *et al*. (2010) [14] who report that glucose levels are reduced in the aquatic animals fed with herbal based diets.

Table 6: Serum glucose level of common carp fed with *Beta vulgaris* supplemented diets at different levels for 60 days

Treatment	Serum glucose (mg/dL)
Control	142±4.20
T_1 (5%)	108±3.15
T_2 (7.5%)	96±2.40
T_3 (10%)	100±4.18

The metabolism of glucose and its homeostasis are highly dependent on the feeding status in fish. Stress has a substantial impact on the welfare and productivity of fish, and blood glucose level of fish is considered as a factor modulating stress response. The elevation of blood glucose in fish takes place as a result of increased secretion of corticosteroids and catecholamines, on activation of chromaffin tissue and internal tissues. Our results are in agreement with Grace *et al.* (2020) [22], who evaluated the effect of beetroot juice on growth performance, blood profile and carcass characteristics of broiler chicken. It was observed that the supplementation of beetroot juice significantly lowered the glucose level in the treated groups as compared to the control. Beals *et al.* (2017) [7] report that six weeks beetroot juice consumption significantly reduced blood glucose levels in young healthy people.

3.6 Total protein, Albumin, Globulin, A/G Ratio

In our study, it was found that lowest albumin, globulin and total protein was found in the control group. On the other hand, highest globulin and total protein was found in the treatment T2, while highest albumin was found in treatment T1. It was found that the total protein, albumin & globulin level varied significantly among all the treatments.

Table 6: Serum protein of common carp fed with *Beta vulgaris* supplemented diets at different levels for 60 days

Treatment	Serum albumin (g/dl)	Serum globulin (g/dl)	Total protein (g/dl)
Control	0.66±0.18	1.40±0.28	2.06±0.50
T_1 (5%)	1.16±0.14	1.40±0.28	2.41±0.24
T_2 (7.5%)	1.02±0.20	2.38±0.10	3.40±0.26
T_3 (10%)	1.15±0.11	2.00±0.30	3.15±0.29

Since serum protein includes humoral elements of the non-specific immune system, high concentrations of total serum protein, albumin and globulin might be due to the enhanced non-specific immune response due to the addition of *Beta vulgaris*. Sahu *et al*. (2007) [45] reported that fish fed with mango kernel showed increased serum protein, albumin (p<0.05) as compared to the control group. Generally increase in the levels of plasma total protein, albumin and globulin in fish is thought to be associated with a stronger innate immune response (Wiegertjes *et al*., 1996). The increase in serum protein may be correlated to increase in serum lysozyme, complement, acute phase proteins, cytokines, lectins and bactericidal peptides.

3.7 Other biochemical parameters

Cholesterol is an important compound which is essential for structuring of the cell membrane. The measurement of cholesterol levels reflect the food status in animals. Triglycerides are considered as the most important forms of fat in the body, which are stored in adipose cells and between muscles and skin (Banaee *et al*., 2011) [6]. They may be used as an energy source by fish under stress (Mattioli *et al*., 2017) [33]. In the present study, it was observed that the cholesterol and triglyceride level did not vary significantly among the treated groups, which differs from the findings of Grace *et al*., (2020) [22] who report cholesterol and triglyceride lowering effect of beetroot juice in broiler chickens.

The physiological role of urea, uric acid and creatinine is ambiguous. Nevertheless, they are regarded as useful indices of overall health of gill and kidney (Campbell, 2004) [9]. Creatinine represents more than 50% of the nitrogenous waste excreted through the fish kidney (Campbell, 2004) [9]. The supplemented diets did not change serum urea and creatinine levels significantly among the various treatments.

Table 6: Other biochemical parameters of common carp fed with *Beta vulgaris* supplemented diets at different levels for 60 days

Treatment	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Urea (mg/dl)
Control	23.20±1.04	232.20±38.50	4.12±0.22
T_1 (5%)	23.28±0.92	233.20±40.23	4.18±0.20
T_2 (7.5%)	23.20±0.40	229.50±30.22	3.80±0.55
T_3 (10%)	22.18±1.50	230.00±28.20	4.00±0.12

Conclusion

Beetroot is a potent vegetable containing significant phenols in addition to the betalain content, which contributes to the plant's antioxidant activity. It may be concluded that dietary *Beta vulgaris* (root) improves growth and enhances the haematological and physiological responses in common carp. In this regard optimum results were obtained primarily on fish supplemented with 7.5% *Beta vulgaris*. Therefore, *Beta vulgaris* may be considered as a potential dietary supplement to be incorporated in the diet of the fish for better production.

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