RESEARCH ARTICLE

# Effect of Dietary Administration of Mineral Mixture Containing Amino Acid Tryptophan and Vitamins for Growth Performance and immunity of *Catla catla* (Hamilton, 1822)

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## Abstract

The commenced investigation was conducted to assess the implication of tryptophan in diets of *Catla catla*. The fingerlings were fed with two different diets C1 and C2; in diet C1, mineral mixture (A) without L-tryptophan and in diet C2 mineral mixture (B) containing L-tryptophan, in addition to vitamins was used. After 90 days of the feeding trial, high growth performance, nutrition retention, and better immunity were observed in fingerlings fed on diet B signifying that tryptophan influences the growth performance and immune response. Excretion of metabolites (total ammonia excretion and orthophosphate production) was significantly low in the holding water when *C. catla* fingerlings fed on diet C2. Better growth performance and better water quality in treatment C2 may be attributed to the presence of essential vitamins and amino acid tryptophan. Hence, the incorporation of a mineral mixture containing L-tryptophan and vitamins in the fish diet appears to be significant for better growth, nutrition retention, digestibility and immunity.


**Keywords:** *Catla catla*, Growth performance, Immune response, Serotonin, Tryptophan.

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## Introduction

Increased demand for fishes and high-speed development of aquaculture leads to intensification in fish culture system (Naylor *et al*., 2000; Easterling, 2007 and Smith *et al*., 2011). In fish culture due to intensification, many stress factors are involved such as overcrowding, poor handling, poor quality of water, a poor nutritional status which can be reduced by providing good quality feed, suitable environment, and disease free seeds for fish welfare. Furthermore, a nutritional attribute of diet can inflect the proneness of fish to virulent diseases. Protein is a very pivotal ingredient in fish diets and provides many amino acids that increase growth performance, strength and act as substrates for leading metabolic pathways. Various studies (Wilson *et al*., 1978; Coloso *et al*., 2004; Abidi and Khan, 2010 and Painesso *et al*., 2015) have concluded the inclusion of proper amino acids in fish diets provides better growth, health benefits and improved immunity as these amino acids administrate various crucial metabolic pathways. Amino acid L-tryptophan in fishes is the metabolic predecessor of serotonin and vitamin niacin that subsidize the growth hormone and assist adequate protein utilization that impacts on food intake. Tryptophan deficiency has been shown to provoke morphological abnormalities like scoliosis and lordosis in salmon (Halver and Shanks, 1960) and rainbow trout (Shanks *et al*., 1962 and Kloppel and Post, 1975). Tryptophan may have anti-stress and immunomodulatory effects in different animals like chickens (Erwan *et al*., 2014). Likewise, vitamins are also very requisite for different biochemical, physiological, and metabolic functions. Vitamin deficiency may cause various cellular and organ damage. Vitamins are highly recommended during earlier developmental stages in fish growth. Their scarcity in fish diet weakens immunity that makes fish more responsive to diseases. Vitamins E and C are required to a great extent in the fish diet for better immunity as they reduce the production of oxidative free radical that constrains the immunity by destroying immune cells. Vitamin C absorption in high dosage makes fish more vulnerable to bacterial and virus resistance (Waagbo, 1997; Verlhac and Gabaudan, 1997). Ammonia and phosphate excretion is of great concern in fish nutrition for determining the pollution caused by undigested food, i.e., improper utilization of protein present in feed-in holding water which retards the growth by provoking various diseases which ultimately leads to poor fish health. Commercially, several ranges of mineral mixtures are available in the market; out of which, some mineral mixture containing different minerals without amino acids, some containing different minerals with amino acids, some containing different minerals with amino acids, vitamins, and essentials. In our study, we have taken two different commercially available mineral mixtures to study the impact of different amino acids, vitamins and different essentials for

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fish growth, nutritional physiology and for immunity. In the present study, we centralized our intentness towards amino acid tryptophan and vitamins supplemented in the fish diet for growth performance and immunity of *Catla catla*.

## Materials and Methods

### Collection of Experimental Fish

The experimental fish fingerlings of *Catla catla* were procured from Sultan Fish Farm, Village Butana, Nilokheri, Kurukshetra, India and were acclimated for 10 days.

### Feed Preparation

Two diets were prepared (C1 and C2) by using groundnut oil cake (650 g), rice bran (32 g), duckweed (266 g), wheat flour (32 g), chromic oxide (10 g) and mineral mixture (10 g). In diet C1, mineral mixture A was used which contained calcium, phosphorus, magnesium, manganese, zinc, ferrous, copper, cobalt, sodium, potassium, sulfur and iodine along with two amino acids L-Lysine, DL-Methionine. In diet C2, mineral mixture B was used which contained all minerals and amino acids as in mineral mixture A along with amino acid tryptophan, various vitamin contents such as vitamin A, vitamin $D_3$, vitamin E, vitamin $K_3$, vitamin $B_1$, vitamin $B_2$, vitamin $B_5$, vitamin $B_6$, vitamin $B_{12}$, vitamin C (Table 1). All ingredients were comprehensively grounded, sieved, mixed and then followed by preparation of dough homogeneously. Feed was prepared in the form of pallets by using pelletizer. Feed was then oven dried at the temperature of 60°C and stored in airtight containers.

**Table 1:** Ingredients of mineral mixture in diet C1 and C2

| *Ingredients* | *Dietary treatments* | |
|---|---|---|
| | *C1 (Mineral premix A )* | *C2 (Mineral premix B)* |
| Minerals | Calcium | Calcium |
| | Phosphorus | Phosphorus |
| | Magnesium | Magnesium |
| | Manganese | Manganese |
| | Zinc | Zinc |
| | Ferrous, | Ferrous |
| | Copper | Copper |
| | Cobalt | Cobalt |
| | Sodium | Sodium |
| | Potassium | Potassium, |
| | Sulphur | Sulphur |
| | Iodine | Iodine |
| Vitamins | | Vitamin A |
| | | Vitamin $D_3$ |
| | | Vitamin E |
| | | Vitamin $K_3$ |
| | | Vitamin $B_1$ |
| | -------- | Vitamin $B_2$ |
| | | Vitamin $B_5$ |
| | | Vitamin $B_6$ |
| | | Vitamin $B_{12}$ |
| | | Vitamin C |
| Amino acids | L-Lysine | L-Lysine |
| | DL-Methionine | DL-Methionine |
| | | L-Tryptophan |

### Experimental Set-up

The experiment was administered in Aquaculture Research Unit, Department of Zoology, Kurukshetra University, Kurukshetra. The acclimated fingerlings were distributed in different aquaria containing 10 fingerlings each under laboratory conditions (24 ± 1°C). All fishes of two different treatments in triplicate were fed daily at 3% BW in two installments at 9:30 and 16:00 hours for 90 days provided 50–60% exchange of dechlorinated water daily along with constant aeration. Uneaten food was collected after 3 hours of feeding for further analysis. Every individual fingerling was weighed at the start of the experiment, after every 15 days and at the end of the experiment for the calculation of growth performance parameters.

### Apparent Protein Digestibility and Proximate Analysis

According to standard procedures of AOAC (2012), proximate analysis of fish feed constituents, fish diets, and fish carcass were done initially as well as after completion of feeding trial. Moisture (by after desiccation in an oven at 100°C for 20 hours), ash (incineration at 550°C for four hours in a muffle furnace), nitrogen (by micro-Kjeldahl method), crude protein (multiplying the nitrogen by a factor of 6.25), crude fiber (by digestion of moisture and fat free sample with dilute acid and alkali) and crude fat (by petroleum benzene/ether extraction using Soxhlet's apparatus) were analyzed. Phosphorus was analyzed after acid digestion by using spectrophotometer in which nitric acid and perchloric acid were taken in the ratio of 10:1. Nitrogen-free extract (NFE %) was estimated by subtraction method; NFE= 100–(crude protein + fat + moisture). Apparent protein digestibility was determined by following Cho *et al.* (1982).

### Water Quality

During experimentation, at every 15th day, water quality parameters such as temperature, pH, DO, chloride, calcium, total alkalinity, electrical conductivity, and total hardness were analyzed following by APHA (1998) to check the effect of feed on water.

### Ammonia Excretion and Orthophosphate Production in holding water:

After completion of the feeding trial, fishes were offered the same diet. Excess of feed was expelled after 2 hours and fixed levels of water of all aquaria were retained for experiments.

Then, water samples from each aquarium were collected at every 2 hours interval to calculate the total ammonia (N-$NH_4$) and reactive orthophosphate (o-$PO_4$) excretion level by following APHA (1998). The quantity of nitrogen excretion and orthophosphate production by fishes in holding water were computed as follows:

$$\text{Total N-NH}_4/o\text{-PO}_4 \text{ excretion (mg kg}_{-1}\text{BW 2h}_{-1}) = [(\text{N-NH}_4/o\text{-PO}_4)_{120} - (\text{N-NH}_4/o\text{-PO}_4)_0] \times a/\text{ Fish biomass/ kg}$$

Where, (N-$NH_4$/*o*-$PO_4$ )$_0$ and ( N-$NH_4$/*o*-$PO_4$ )$_{120}$ = Post feeding concentration at times 0 and 120 min (2 h).

a = amount of holding water (L) in which fishes were kept.

### Growth Parameters

Various growth parameters such as live weight gain, growth percent gain in body weight, growth per day in percentage body weight, specific growth rate (SGR), gross conversion efficiency (GCE), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated for evaluating dietary performances and nutritional indices (Garg *et al*., 2002).

### Intestinal Enzyme Activities

Intestinal enzymes activities were analyzed using standard procedures; protease (Walter, 1984), cellulase (Sadasivam and Manickam, 1996) and amylase (Sawhney and Singh, 2000).

### Hematological Diagnosis

Total leucocytes count (TLC) and total erythrocytes count (TEC) were estimated following Dacie and Lewis (1971).

### Serological Diagnosis

The serum protein was determined according to Gornall (1947).

### Phagocytic Assay

The phagocytosis assay was performed according to the method of Siwicki *et al*. (1994) and Park and Jeong (1996).

Phagocytic ratio (PR; i.e. the percentage of the cell with engulfed bacteria) = Number of phagocytic cells with engulfed bacteria/ Number of phagocytic cells × 100.

Phagocytic index (PI; i.e., number of engulfed bacteria per cell) = Number of engulfed bacteria/number of phagocytic cells.

### Nitroblue Tetrazolium Assay

Respiratory burst activity by blood phagocytes was measured through nitroblue tetrazolium (NBT) assay using spectrophotometer at 540 nm, following Anderson and Siwicki (1995).

### Serum Bactericidal Activity

Serum from blood samples of each treatment was collected for serum bactericidal activity (Kajita *et al*., 1990). The number of viable bacteria was calculated by counting the colonies from the resultant incubated mixture on TSA plates in duplicate (two plates per sample) after 24 h incubation.

### Statistical Analysis

All values were expressed as mean ± SE of the mean. Data were subjected to independent sample't' test to test the significant differences between two dietary treatments. Statistical significance was settled at a probability value of $p < 0.05$. All statistics were performed using suitable Statistical Package for Social Sciences (SPSS) version.

## Results and Discussion

### Growth Performance, Apparent Protein Digestibility and Intestinal Enzyme Activity

A balanced diet with functional constituents is aimed to extensively escalate the growth by reducing the negative impacts of disease and other stressors for an individual well being. Proximate composition of experimental diets is shown in Table 2. Both diets were isoproteinous approximately 40% protein, weight gain (WG), SGR, PER, GCE, FCR and apparent protein digestibility (APD) were high in treatment C2 but significant difference was not observed in growth performance parameters for fingerlings of treatment C1 and C2 (Table 3). While significant ($p$ <0.05) increase in intestinal enzymes activities were observed in treatment C2 in comparison to treatment C1 (Table 3). Protease, amylase and cellulase activities of fishes in treatment C2 were recognized significantly high as compared to fishes in treatment C1. Zehra and Khan (2014) also revealed that supplementation of tryptophan in fish diet boost the protein utilization which impacts on growth performance. Tryptophan is a very essential amino acid and it is the progenitor of hormone serotonin and the vitamin niacin (Ahmed, 2010). Serotonin confers the available protein expenditure and synthesis of other new proteins which may improve its body weight gain, digestibility and bring down FCR ultimately leads to growth promotion. Furthermore, tryptophan prompts the secretion of insulin and growth hormone (Dyer *et al*., 2004). The results associated with the growth of *C. catla* induced by tryptophan supplementation in the diet C2 confirm the

**Table 2:** Proximate composition (% dry weight basis) of experimental diets

| *Proximate composition* | *Dietary treatments* | |
|---|---|---|
| | *C1 (Mineral premix A )* | *C2 (Mineral premix B)* |
| Crude protein (%) | 40.67 ± 0.03 | 40.87 ± 0.04 |
| Crude fat (%) | 8.99 ± 0.03 | 9.05 ± 0.04 |
| Crude fiber (%) | 6.11 ± 0.03 | 6.28 ± 0.04 |
| Total Ash (%) | 7.16 ± 0.03 | 7.34 ± 0.05 |
| Moisture (%) | 4.35 ± 0.06 | 4.47 ± 0.01 |
| Nitrogen free extract (%)* | 32.72 ± 0.02 | 38.89 ± 0.08 |
| Gross energy (kJ $g^{-1}$)* | 18.77 ± 0.01 | 19.99± 0.01 |

All values are mean ± SE of mean

*Values are significantly ($p$ <0.05) different (students't' test)

**Table 3:** Effect of dietary inclusion of different mineral mixtures on growth performance and Intestinal enzyme activities of *Catla catla* when fed on duckweed based diets

| *Parameters* | *Dietary treatments* | |
|---|---|---|
| | *C1 (Mineral premix A )* | *C2 (Mineral premix B)* |
| Initial weight (g) | 1.61 ± 0.01 | 1.04 ± 0.01 |
| Final weight (g) | 2.45 ± 0.03 | 2.50 ± 0.01 |
| Live weight gain (g) | 1.40 ± 0.02 | 1.47 ± 0.02 |
| Growth % gain in BW | 131.81 ± 1.97 | 141.09 ± 4.15 |
| Growth per day in BW | 0.88 ± 0.01 | 0.92 ± 0.03 |
| Specific growth rate (SGR) (% BW $d^{-1}$) | 0.36 ± 0.04 | 0.38 ± 0.01 |
| Feed conversion ratio (FCR) | 3.19 ± 0.06 | 3.13 ± 0.05 |
| Gross conversion ratio (GCR) | 0.23 ± 0.004 | 0.24 ± 0.01 |
| Protein efficiency ratio (PER) | 0.034 ± 0.001 | 0.04 ± 0.001 |
| Apparent protein digestibility (APD) (%) | 73.49 ± 0.56 | 74.32 ± 0.60 |
| Protease activity (mg of tyrosine liberated mg of protein$^{-1}$ $h^{-1}$)* | 1.64 ± 0.01 | 1.77 ± 0.01 |
| Amylase activity (mg of maltose liberated mg of protein$^{-1}$ $h^{-1}$)* | 1.12 ± 0.01 | 1.29 ± 0.02 |
| Cellulase activity (mg of glucose liberated mg of protein$^{-1}$ $h^{-1}$)* | 0.93 ± 0.01 | 0.97 ± 0.01 |

All values are mean ± SE of mean
*Values are significantly ($p$ <0.05) different (students't' test)

significance of this amino acid. Vitamins supplemented in fish diet mitigate the fishes from the physiological and metabolic stress resulting in overall growth promotion.

### Water Quality Parameters

No significant variations were observed in water quality parameters. These parameters do not show any specific trend with the incorporation of different mineral mixtures. But total ammonia excretion and total phosphate production (Table 4) was found to be significantly low in treatment C2 when compare with treatment C1 which concludes the proper utilization of protein in fishes fed on diet C2 which lessens the deamination of feed protein ultimately leading to lower ammonia excretion. Also in the group of fishes fed on diet C2, vitamin C contents were present which lessens the ammonia excretion. According to Lall (2000), different fishes fed on Vitamin C deficient diet showed more inclination towards stress provoked by poor water quality due to high ammonia excretion. Total orthophosphate excretion was found to be

**Table 4:** Effect of dietary inclusion of different mineral mixtures on water quality parameters of *Catla catla* culture system

| *Parameters* | *Dietary treatments* | |
|---|---|---|
| | *C1 (Mineral premix A )* | *C2 (Mineral premix B)* |
| Dissolved oxygen (DO) mg $L^{-1}$ | 6.71± 0.04 | 6.73 ± 0.13 |
| pH | 7.60 ± 0.04 | 7.57 ± 0.13 |
| Temperature (°C) | 26.50 ± 0.20 | 26.23 ± 0.08 |
| Conductivity ( µ mhos $cm^{-1}$ ) | 662.22 ± 0.71 | 664.17 ± 5.17 |
| Alkalinity (mg$L^{-1}$) | 191.11 ± 1.44 | 188.67 ± 2.40 |
| Chloride (mg $L^{-1}$ ) | 39.59 ± 0.50 | 38.43 ± 0.43 |
| Calcium (mg$L^{-1}$) | 18.73 ± 0.68 | 18.33 ± 0.17 |
| Hardness (mg$L^{-1}$) | 83.22 ± 0.73 | 80.11 ± 0.95 |
| Total NH3-N excretion (mg $Kg^{-1}$ BW $day^{-1}$ )* | 383.33 ± 3.17 | 766.67 ± 2.33 |
| Total o-PO4 production (mg $Kg^{-1}$ BW $day^{-1}$ ) * | 412.67 ± 2.17 | 383.33 ± 3.17 |

All values are mean ± SE of mean
*Values are significantly ($p$ <0.05) different (students't' test)

high in treatment C1 in comparison to treatment C2. The reason behind the high orthophosphate production may be due to improper utilization of phosphorus present in the diet.

### Carcass Composition of Experimental Fishes

In our study, initial and final carcass composition of fishes supplemented with two different diets were inspected and revealed the significant increase in carcass protein, carcass fat and gross energy (Table 5) in fishes of treatment C2 in comparison to fishes of treatment C1 concede with the results of Zehra and Khan (2014) and Painesso *et al.* (2015). Nonetheless, no significant difference was observed in Total Ash, Nitrogen-free extract (NFE), Moisture (%) between these two treatments.

### Hematological Parameters:

Blood is one of the most influential body tissues and capable of imitating changes in physiological and nutritional health status (Kader *et al.*, 2012). Blood samples of fishes of these two treatments were collected to evaluate the hematological and found that total leucocytes and erythrocytes count (Graph 1) of fishes of treatment C2 fishes were significantly ($p$ <0.05) high as compared to fishes of treatment C1, as diet C2 contained different vitamins along with amino acid tryptophan which influenced the hematological responses.

### Immunological Parameters

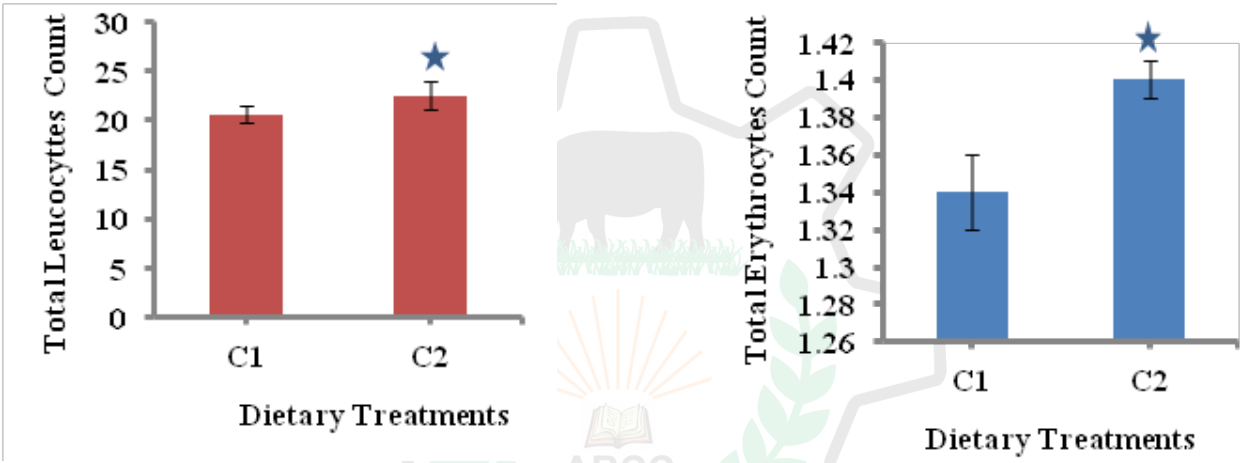
The innate immune system of fishes has various mechanisms to restraint the endogenous and exogenous bacteria and thus prohibits infection by these microorganisms. These mechanisms vary from physical barriers to a highly refined

**Table 5:** Proximate carcass composition of experimental fishes

| *Proximate composition* | *Dietary treatments* | | |
|---|---|---|---|
| | *Initial value* | *C1 (Mineral premix A )a* | *C2 (Mineral premix B)b* |
| Crude protein (%) | 12.26 ± 0.02 | 13.39 ± 0.02* | 15.33 ± 0.02* |
| Crude fat (%) | 4.95 ± 0.01 | 7.13 ± 0.01* | 6.48 ± 0.01* |
| Total ash (%) | 2.79 ± 0.01 | 3.97 ± 0.01 | 3.82 ± 0.02 |
| Moisture (%) | 68.28 ± 0.02 | 64.95 ± 0.02 | 63.72 ± 0.03 |
| Nitrogen free extract (%) | 11.72 ± 0.04 | 14.53 ± 0.04 | 14.46 ± 0.04 |
| Gross energy (kJ $g^{-1}$) | 6.84 ± 0.01 | 8.19 ± 0.002* | 8.62 ± 0.01* |

All Values are mean ± SE of mean

*Values are significantly ($p$ <0.05) different (Students't' test, a *vs.* b)




**Graph 1:** Total leucocytes count ($10^6$ $mm^{-3}$) and Total erythrocytes count ($10^6$ $mm^{-3}$), (mean ± SE of mean) of *Catla catla* in different dietary treatments

pattern of molecules and cells that operate to abolish or avoid the microbial infection (Magnadottir, 2010). Phagocytosis and respiratory burst activity are considered as a foremost mechanism to resist the bacterial infection. Phagocytic index, phagocytic ratio and respiratory burst activity (Table 6) of diet C2 fed group fishes showed a significant increase when compared with diet C1 fed group fishes. This may be attributed to the tryptophan which when act with serotonin stimulates the immunological responses. Serotonin is also a neurotransmitter and has a lead aspect to lessen anxiety and aggression and also governs osmoregulatory, hematological, immunological responses (Gonzalez-Silvera *et al.*, 2018). Wen *et al.* (2014) revealed the presence of amino acid tryptophan in fish diet can make fishes more tolerant to stress induced by any bacterial, viral and pathogen infection. Various amino acids such as tryptophan and aspartate can modulate the immunity in culture medium (Azeredo *et al.*, 2017). Lall (2010) stated that during the establishment of a strong immune system in early developmental stages as well as in growing stages, fish requires an optimum quantity of vitamins in the diet. Vitamins exhibited to trigger the serum hemolytic complement activity, immune cells proliferation,

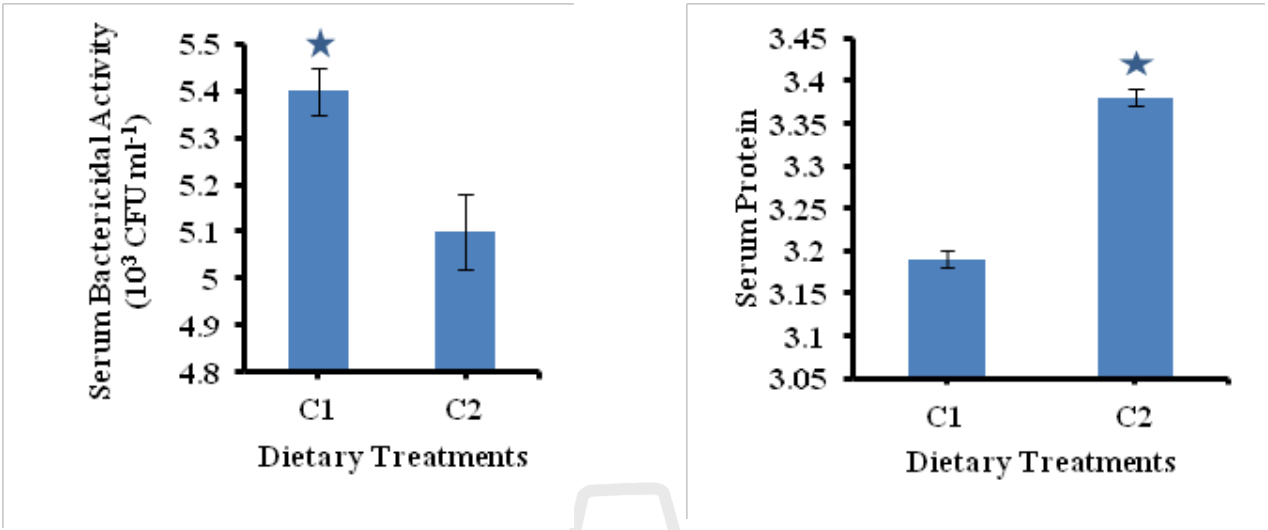
**Table 6:** Effect of dietary inclusion of different mineral mixtures on immunological parameters of *Catla catla* when fed on duckweed based diets

| *Parameters* | *Dietary treatments* | |
|---|---|---|
| | *C1 (Mineral premix A )* | *C2 (Mineral premix B)* |
| Phagocytic ratio* | 64.19 ± 0.25 | 67.13 ± 0.54 |
| Phagocytic index* | 1.32 ± 0.003 | 1.43 ± 0.003 |
| Respiratory burst activity (NBT)* | 0.43 ± 0.01 | 0.48 ± 0.01 |

All values are mean ± SE of mean

*Values are significantly ($p$ <0.05) different (students't' test)

and phagocytosis (Verlhac and Gabaudan, 1997). Serum bactericidal activity (Graph 2) is the measure of total bacterial count (103 CFU/mL). The serum bactericidal activity and serum protein of treatment C1 group fishes revealed significant decrease when we compared with treatment C2 group fishes. The serum of fish has a variety of enzymes that personify the immunity (Guardiola *et al.*, 2014). The results on serum protein of present study are in concede with the




**Graph 2:** Serum Bactericidal Activity (CFU ml-1) and serum protein, (Mean ± S.E of mean) of *Catla catla* in different dietary treatments

study conducted by Pianesso (2015) in silver catfish (Rhamdia quelen) fed with different doses of tryptophan in fish diet and found the maximum serum protein level in dietary tryptophan level of 3.4 g/kg as compared to control.

## Conclusion

Nutrition, immunological function, and disease resistance in fishes is an extensive field of research. So in this reference, investigation of the present study revealed the incorporation of the mineral mixture is essential for optimum growth performance, and when mineral mixture with amino acid tryptophan, vitamins, and other essentials is incorporated in fish feed it improves the health, growth performance, associated nutritional physiology and immunity of *Catla catla*. However, there is a need to determine the dosage of each constituent, which is an extended process. There are several mineral mixtures available commercially containing minerals, vitamins, trace elements, amino acids, etc. in different combinations. Therefore, while selecting mineral mixture, it is also suggested that the mineral mixture with an adequate amount of essential minerals, amino acids and vitamins should be preferred for the efficiency of fish towards better growth, curtailment of stress and disease resistance leading to sustainable aquaculture.

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