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Estimation of variations in the haematological and serum-biochemical parameters of *Labeo rohita* (Hamilton, 1822) fingerlings due to mold infested feed

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Abstract

The effect of mold infested feed on haematological and serum biochemical parameters in the fingerlings of *Labeo rohita* was investigated. Three different experimental groups were set up as G1 (0% molded feed), G2 (50% mold infested feed and 50% good feed) and G3 (100% mold infested feed) respectively. Fish fed with G2 and G3 diet showed significant ($p < 0.05$) changes in the composition of blood and serum in comparison to the control group G1. Hb content, TEC and TLC count decreased with the increased intake of mold infested feed. Total serum protein, serum glucose and serum creatinine showed a significant decrease with the increase in the time of intake. SGPT and SGOT increased with the progress of experiment till the end. The present study concluded that the mold infested feed negatively affects the fish health.

Keywords: hematology, *Labeo rohita*, mold, serum-biochemical

Introduction

Fisheries is the fastest growing food sector which is playing a vital role in bridging the gap between increasing population and their nutritional requirement. The present status of aquaculture industry poses challenges and opportunities for improvement of production further to meet the growing demand in coming years ^[1]. This demand may be fulfilled by increasing the quality of aqua feeds through improving nutrient content as well as the digestibility of low quality feed by the use of efficient feed additives ^[2]. In aquaculture, maximum share of input cost is due to enriched fish feed.

Lack of storage facilities or ignorance lead to the deterioration of fish feed which will ultimately affect the fish health and growth. Fish is the preferred source of animal protein by human beings. By entering the food chain, the toxins or any other compounds present in the fish body enters the human body and ultimately affects their health also by the process of biomagnification. The ingested mycotoxins affect the health of man varying from acute to chronic. Researchers ^[3] elegantly discussed various aspects of health hazards of man caused by mycotoxins. The inhalation of dust containing mycotoxins can also cause a variety of toxic effects humans ^[4]. Mycotoxins also cause severe damage to the vital organs.

Mold is a simple, common type of fungi that can be found virtually everywhere. Mold can grow on nearly every material and surface, even on the dust in the air we breathe. Any material that remains wet or moist for 24 to 48 hours can be a home for mold. Mold gives off tiny, lightweight particles, called spores, which can travel through the air and settle where the air is calm. The most common fungi involved in the spoilage of feeds belong to the *Aspergillus* species and *Penicillium* species. They are most destructive when the temperature exceeds 25°C and relative humidity exceeds 85 percent ^[5].

Labeo rohita a common Indian carp is widely distributed in Indian rivers and ponds. It is very important as a human food for its high quality flesh. In the present investigation, effect of mold infested feed on haematological and serum-biochemical parameters of *Labeo rohita* has been evaluated in order to explore the effect of toxin in the fish.

Materials and Methods

Experimental fish

The experiment was conducted at College of Fisheries, GB Pant University of Agriculture and

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Technology, Pantnagar (Uttarakhand). The total duration of the experiment was 60 days. Experimental fishes were obtained from the Instructional Fish Farm, College of Fisheries, Pantnagar. 90 fishes of average weight 100g of both sexes were randomly divided into 3 groups of 30 fishes in each group. Each group was set in triplication with 10 fishes per tank per group and were acclimatized for one week before the start of experimental feeding.

Experimental fungal growth

Mold infested feed was prepared in the laboratory. The commercial fish feed was first sprinkled with a small amount of tap water to make the feed moist. The feed was kept in suitable condition for few days which is favourable for the growth of mold. Water was added to the feed whenever required. After the growth of mold, sample of mold infested feed was tested in the department of microbiology of College of Basic Sciences and Humanities, GBPUA&T for the identification of mold species. The identified species was *Aspergillus flavus*. Required amount of mold infested feed and good feed were weighed carefully for each group and then mixed thoroughly for the group G₂. The fish were fed daily at the rate of 5% of their body weight, in two split portions (the morning (9.00hr) and evening (16.00hr)).

Formulation of experimental diets

Three different compositions of feeds were employed as follows:

G₁ (Control group) - Feed I contained 0% molded feed.

G₂ - Feed II contained 50% mold infested feed and 50% good feed.

G₃ - Feed III contained 100% mold infested feed.

Haematological study

Sampling was done at 0th, 15th, 30th, 45th and 60th days post treatment (DPT). Blood samples were collected within 35-40 s through a cardiac puncture into 2 ml disposable heparinised syringes, with 21 gauge needles after stunning fish by a blow to the head. From syringes the collected blood was transferred to heparinised blood collecting tubes then stored at 4 °C until the blood parameter studies were completed. Haemoglobin estimation was done by using the Sahli's method. Total numbers of red blood cells (RBCs) and white blood cells (WBCs) were counted under a microscope at 640 \times using an improved Neubauer haemocytometer. Blood was diluted 1:200 with Hayem's solution and 1:20 with Turk's diluting fluid for RBCs and WBCs respectively.

Serum Biochemical estimation

At 0th, 15th, 30th, 45th and 60th days post treatment (DPT), blood samples of 5 fish from each experimental group was drawn and serum was separated. The separated serum was stored in the refrigerator at 4°C until further use. Separated serum was used for estimation of total serum protein, albumin, globulin, creatinine and serum enzymes such as aspartate amino transferase and alanine amino transferase (Transasia Bio-medicals Ltd., Solan, HP, India) and total serum glucose (Span Diagnostics Ltd., Surat, India).

Statistical analysis

Data obtained during the course of investigation was statistically analysed by 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 $P < 0.05$.

Results

Hemoglobin

Fig. 1 shows the variation in the haemoglobin content in different groups of experimental fishes at varying time intervals. The mean Hb content in group G₁, varied between 9.4 ± 0.25 to 10.5 ± 0.23 g% and did not show significant variation. In groups, G₂ and G₃ average values of Hb concentration between 6.8 ± 0.20 to 9.7 ± 0.15 and from 5.8 ± 0.15 to 10.4 ± 0.22 g% respectively. The values of Hb showed significant ($p < 0.05$) decrease in the groups G₂ and G₃ as compared to G₁ from 0th DPT to 60th DPT. When these values in different groups were compared at different time intervals, significant variation was recorded at 30th, 45th and 60th DPT between any groups.

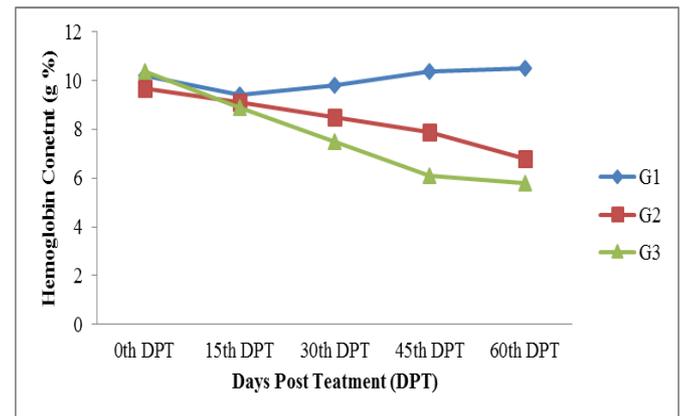


Fig 1: Average (Mean±SE) hemoglobin concentration (g%) in different groups of experimental fishes at different time intervals

Total Erythrocyte Count (TEC)

The variation in TEC in different groups at various time intervals is shown in Fig. 2. In group G₁, the mean TEC ranged from 821.3 ± 0.12 to 823.6 ± 0.26 $10^3/\text{mm}^3$ with no significant variation from 0th DPT to 60th DPT. In groups, G₂ and G₃, the values ranged between 779.5 ± 0.21 to 820.6 ± 0.25 and 721.0 ± 0.18 to 822.4 ± 0.23 $10^3/\text{mm}^3$ respectively. In the group G₂ and G₃, TEC level was decreased significantly ($p < 0.05$) from 0th DPT to 60th DPT. When these values were compared between different groups, no significant variation was recorded at 0th DPT between any groups. There was a significant ($p < 0.05$) decrease in the TEC level in all the groups from 15th to 60th DPT.

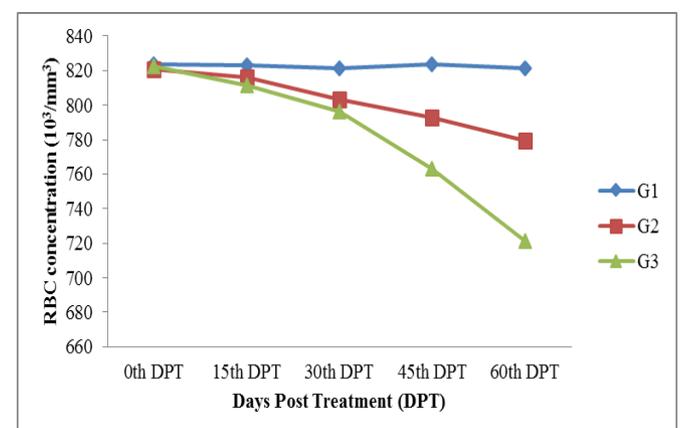


Fig 2: Average (Mean±SE) total erythrocyte count ($10^3/\text{mm}^3$) in different groups of experimental fishes at different time intervals

Total Leucocyte Count (TLC)

The variation in TLC in different groups at various time

intervals is shown in Fig. 3. In group G1, the mean TLC ranged from 3.49 ± 0.14 to 3.71 ± 0.11 $10^3/\text{mm}^3$ with no significant variation from 0th DPT to 60th DPT. In groups, G2 and G3, the values ranged between 2.83 ± 0.16 to 3.76 ± 0.23 and 2.13 ± 0.12 to 3.81 ± 0.21 $10^3/\text{mm}^3$ respectively. In the group G2 and G3, TLC level was decreased significantly ($p < 0.05$) from 0th DPT to 60th DPT. When these values were compared between different groups, no significant variation was recorded at 0th DPT between any groups. There was a significant ($p < 0.05$) decrease in the TLC level in all the groups from 15th to 60th DPT.

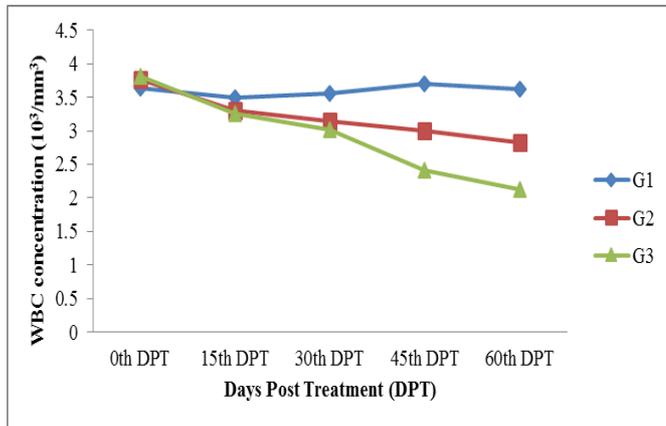


Fig 3: Average (Mean±SE) total leucocyte count ($10^3/\text{mm}^3$) in different groups of experimental fishes at different time intervals

Total serum protein (g/dl)

Fig 4 shows the variation in the concentration of total serum protein in different groups of experimental fishes at varying time intervals. The mean total serum protein levels in group G1, varied between 3.61 ± 0.12 to 3.78 ± 0.24 g/dl and did not show significant variation. In groups, G2 and G3 values of total serum protein ranged between 2.77 ± 0.11 to 3.68 ± 0.14 and from 2.56 ± 0.20 to 3.71 ± 0.19 g/dl respectively. The values of total serum protein showed insignificant ($p < 0.05$) decrease in the groups G2 and G3 as compared to G1 from 0th DPT to 60th DPT. When these values in different groups were compared at different time intervals, significant variation was recorded at 45th DPT and 60th DPT between any groups.

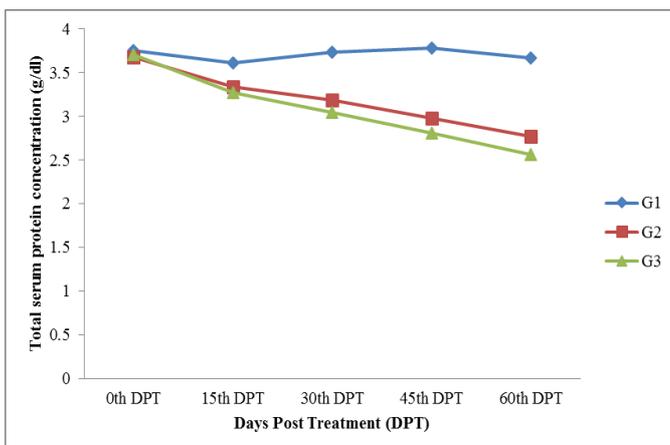


Fig 4: Average (Mean±SE) total serum protein (g/dl) in different groups of experimental fishes at different time intervals

Serum albumin (g/dl)

The variation in serum albumin values in different groups at various time intervals is shown in Fig 5. In group G1, the mean serum albumin ranged from 2.30 ± 0.18 to 2.61 ± 0.07 g/dl

with no significant variation from 0th DPT to 60th DPT. In groups, G2 and G3, the values ranged between 1.62 ± 0.10 to 2.49 ± 0.19 and 1.37 ± 0.22 to 2.56 ± 0.13 g/dl respectively. In the groups G2 and G3, serum albumin was decreased insignificantly ($p < 0.05$) from 0th DPT to 60th DPT. When these values were compared between different groups, no significant variation was recorded at 0th DPT between any groups. There was a significant ($p < 0.05$) decrease in the serum albumin level in all the groups at 15th, 30th and 60th DPT.

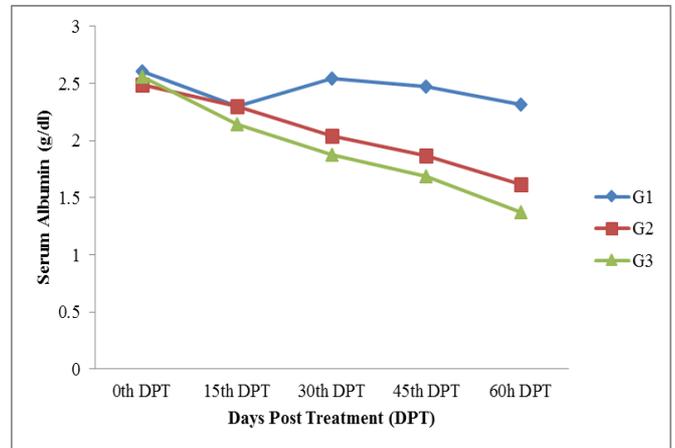


Fig 5: Average (Mean±SE) serum albumin (g/dl) in different groups of experimental fishes at different time intervals

Serum globulin (g/dl)

The variation in serum globulin values in different groups at various time intervals are presented in Fig 6. In group G1, the mean serum albumin ranged from 1.14 ± 0.17 to 1.35 ± 0.16 g/dl with no significant variation from 0th DPT to 60th DPT. In groups, G2 and G3, the values ranged between 1.04 ± 0.07 to 1.19 ± 0.11 and 1.13 ± 0.26 to 1.19 ± 0.07 g/dl respectively. In all the groups (G1, G2 and G3), serum globulin varied insignificantly ($p < 0.05$) from 0th DPT to 60th DPT.

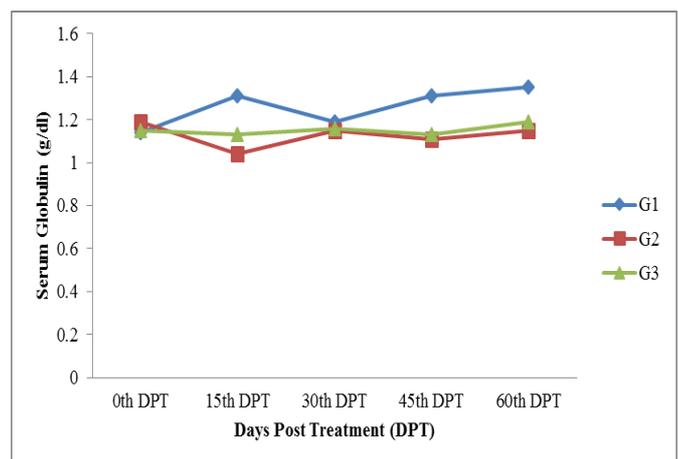


Fig 6: Average (Mean±SE) serum globulin (g/dl) in different groups of experimental fishes at different time intervals

Total serum glucose (mg/l)

The variation in total serum glucose level in different groups at varying time intervals is shown in Table 7. In group G1, the mean serum glucose content ranged from 80.23 ± 0.09 to 80.25 ± 0.18 mg/l and there was no significant variation from 0th DPT to 60th DPT. In the groups G2 and G3, the serum glucose content from 0th DPT to 60th DPT varied from 76.11 ± 0.14 to 80.25 ± 0.10 and 75.83 ± 0.19 to 80.26 ± 0.11 mg/l

respectively. When these values were compared between different groups, no significant variation was recorded at 0th and 15th DPT but there was a significant ($p < 0.05$) decrease in the serum glucose level at the time intervals 30th, 45th and 60th DPT where groups G2 and G3 showed minimum and maximum decrease in the values, respectively.

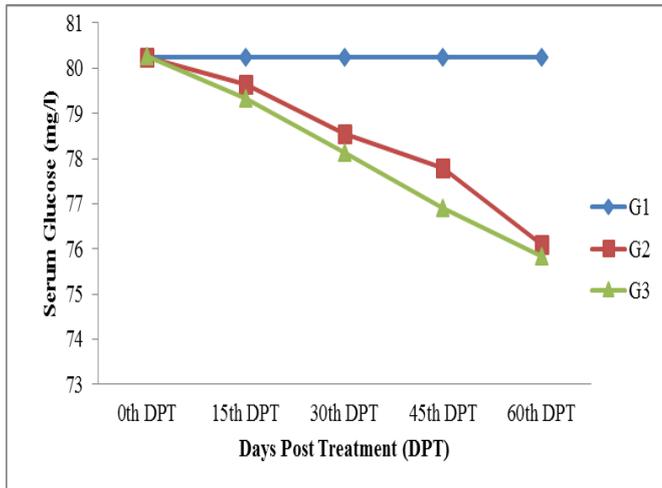


Fig 7: Average (Mean±SE) serum glucose (mg/l) in different groups of experimental fishes at different time intervals

Serum creatinine (mg/l)

Fig. 8 shows the variation in serum creatinine content in different groups at varying time intervals. In group G1, the mean concentration of creatinine ranged from 0.561 ± 0.12 to 0.573 ± 0.11 mg/l and there was no significant variation from 0th DPT to 60th DPT. In groups G2 and G3, the serum creatinine content ranged from 0.487 ± 0.17 to 0.576 ± 0.09 and 0.426 ± 0.18 to 0.575 ± 0.14 mg/l respectively. When the mean creatinine values in different groups were compared at varying time intervals no significant difference could be noted at 0th and 15th DPT between any groups. From 30th DPT to 60th DPT, groups G2 and G3 showed significant ($p < 0.05$) decrease in serum creatinine.

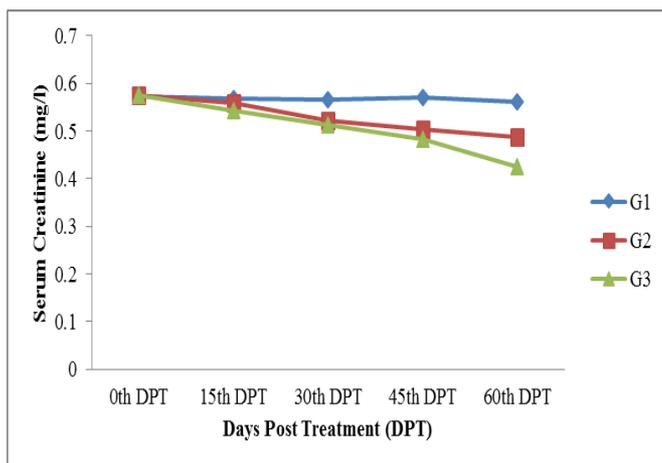


Fig 8: Average (Mean±SE) serum creatinine (mg/l) in different groups of experimental fishes at different time intervals

Serum Aspartate aminotransferase (AST)/ Serum glutamic oxaloacetic transaminase (IU/L)

The variation in serum AST activity in different groups of experimental fishes at varying time intervals is shown in Fig. 9. In group G1, the mean AST activity ranged between 55.24 ± 0.23 to 55.36 ± 0.11 IU/L and there was no significant variation from 0th DPT to 60th DPT. In the groups G2 and G3, there was a significant ($p < 0.05$) increase in the AST activity from 30th DPT to 60th DPT, with the values varying between 55.28 ± 0.13 to 57.44 ± 0.12 and 55.29 ± 0.10 to 58.13 ± 0.17 IU/L respectively. When the mean AST values in different groups were compared at different time intervals, no significant variation was recorded at 0th DPT between any groups. The groups G2 and G3 showed significant ($p < 0.05$) increase in the values from 15th DPT to 60th DPT where maximum increase in the values of serum AST was recorded in group G3 followed by group G2.

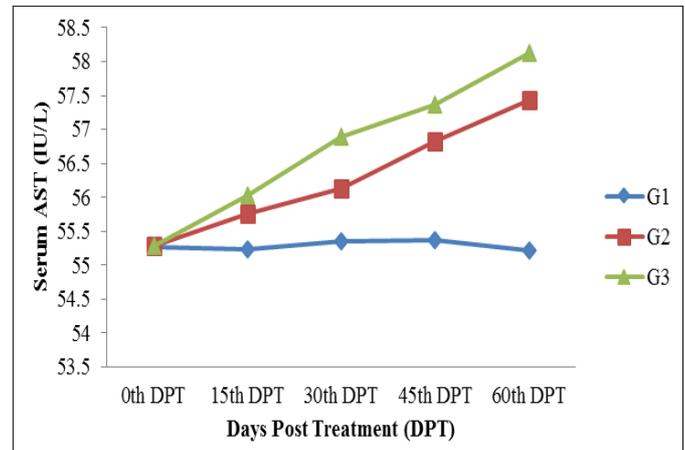


Fig 9: Average (Mean±SE) serum AST (IU/L) in different groups of experimental fishes at different time intervals

Serum Alanine aminotransferase (ALT)/ Serum glutamic pyruvic transaminase (IU/L)

Fig. 10 shows the variation in serum ALT activity in different groups of experimental fishes at varying time intervals. In group G1, the mean ALT activity ranged between 21.23 ± 0.14 to 21.31 ± 0.22 and there was no significant variation from 0th DPT to 60th DPT. In all the groups, G2 and G3 there was a significant ($p < 0.05$) increase in the ALT activity with the values ranging between 21.26 ± 0.10 to 23.56 ± 0.09 and 21.25 ± 0.14 to 24.47 ± 0.11 IU/L respectively. When the mean ALT values in different groups were compared at different time intervals, no significant variation was recorded at 0th DPT between any groups. All the groups showed significant ($p < 0.05$) increase in the values of ALT from 30th DPT to 60th DPT.

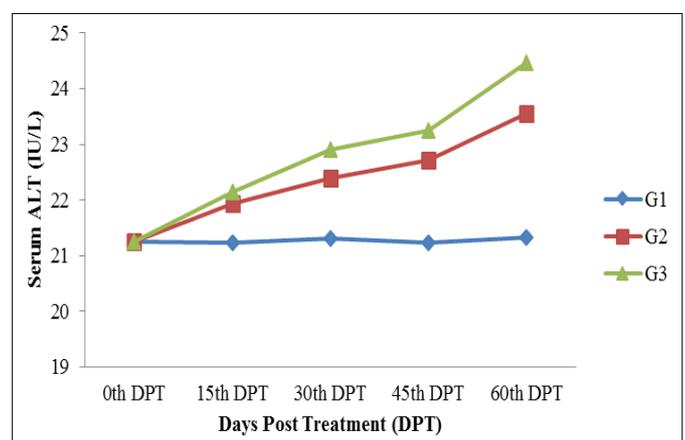


Fig 10: Average (Mean±SE) serum ALT (IU/L) in different groups of experimental fishes at different time intervals

Discussion

The results of this study are in agreement with observations of Shah [6] who observed a significant ($p < 0.05$) decrease in

Hb, TEC and TLC of tench (*Tinca tinca*) receiving *Saprolegnia* infected feed. Hyben and his co-workers [7] observed that fish fed the WA (60% replacement with *Wickerhamomyces anomalus* and *S. cerevisiae* mix) diet had significantly reduced erythrocyte area in comparison to the control group and fish fed SC (60% replacement of fish meal protein, on a digestible basis, with *Saccharomyces cerevisiae*) and WA diets had increased mean corpuscular haemoglobin levels, indicating haemolytic anaemia. Scientists [8] have also recorded decrease in haemoglobin, albumins, proteins and sugar of the blood of chicks under the influence of gliotoxin. A significant decrease ($p < 0.01$) in plasma protein was observed in Gilthead Sea Bream (*Sparus aurata*) by replacing fish meal by yeast (*Saccharomyces cerevisiae*) [9]. Urea, calcium and albumin levels were decreased with the increase of intake of infested feed. Significant increase in Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT) has been recorded in the blood of chick under the influence of gliotoxin produced by *Trichoderma viride* [8]. Significant changes ($p < 0.01$) were observed in plasma glucose level in Gilthead Sea Bream (*Sparus aurata*) by replacing fish meal by yeast (*Saccharomyces cerevisiae*) [9]. Various researchers [10-12] have reported decreased serum protein in chicks fed with aflatoxin contaminated corn. Kiran and her co-workers [13] reported a significant ($p < 0.05$) increase in SGOT and SGPT level and the decrease in total serum protein content under the influence of *Aspergillus terreus* infested feed.

Conclusion

Feeding of fishes with mold infested feed has a significant influence on the health of the experimental fishes. Significant changes in the composition of blood and serum were noticed. Total serum protein, serum glucose and serum creatinine showed a significant decrease with the increase in the time of intake. SGPT and SGOT increased with the progress of experiment till the end. Similarly, Hb content, TEC and TLC count also decreased with the increased intake of infested feed. From these observations, it can be concluded that the mold infested feed negatively affects the fish health.

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