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INFLUENCE OF FEEDING WITH DIFFERENT TYPES OF PROTEIN DIETS ON HAEMATOLOGICAL PROFILE OF ASIAN CATFISH, *CLARIAS BATRACHUS* (LINN.)

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ABSTRACT

Haematological parameters have been recognized as valuable tools for monitoring fish health. So, the nature and quality of nutrients in most animals depend largely on their food type and protein which is the most important component of the diet of fish for energy requirement. Present paper dealt the variation in the level of seventeen haematological parameters of freshwater catfish, *Clarias batrachus*, maintained on three different fish meals i.e. earthworm (*P. sansibaricus*), goat liver and *Pila bengalensis* for 15 days under ideal and identical laboratory condition. Physico-chemical parameters of aquaria water and no mortality as well as a significant ($p < 0.05$), more significant ($p < 0.01$) and highly significant ($p < 0.001$) increase and decrease in haematological parameters were recorded. Haematological changes for different protein diets at different time intervals was in order : earthworm > pila > goat liver. The range of leucocytes ($10^3/\text{ul}$) varied from 5.2 ± 2.8 to 7.4 ± 4 ; neutrophils (%) was in the range 52 ± 0.6 to 58.2 ± 1.25 ; lymphocytes (%) from 32.5 ± 0.6 to 38.8 ± 0.5 ; eosinophils (%) was 3.1 ± 0.5 to 1.4 ± 0.4 and basophils (%) varied from 0.7 ± 0.2 to 0.2 ± 0.1 . Total RBC ($10^6/\text{ul}$) varied from 1 ± 0.25 to 3.85 ± 0.1 ; haemoglobin (g/dl) from 7.6 ± 2 to 10.1 ± 2.45 ; haematocrit (%) was 6.88 ± 1 to 8.5 ± 0.6706 and MCV (fl) was in the range 91.35 ± 11.4 to 100.1 ± 7.245 ; MCH (pg) from 30.57 ± 6.244 to 34.1 ± 5 and ESR varied from 8 ± 3 to 10 ± 2 in earthworm fed group, pila fed group and goat liver fed group. A non-significant variation in monocytes (%), MCHC (%), RDW (fl), PDW (%), platelet ($10^3/\text{uL}$) and MPV (%) in all three fed groups was observed. Present investigation showed increase in haematological parameters indicate rich production of blood in the body and no anemic conditions which indicate the good quality of provided food materials (earthworm, pila and goat liver) to fish. So, these meals are beneficial for the fish culture and provide rich nutrition to fish.

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INTRODUCTION

Knowledge of the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology (Rambhaskar and Srinivasa Rao, 1986; Xiaoyun *et al.*, 2009). The analysis of blood indices has proven to be a valuable approach for analyzing the health status of farmed animals as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting (Bahmani *et al.*, 2001). Clinical haematological studies are of great importance in the epidemiology, management and husbandry practices, veterinary diagnosis; treatment and prognosis in many disease conditions. Undoubtedly, these haematological characteristics of fish are affected by age, sex, nutrition, seasons, cycle of sexual maturity as well as other physiological conditions of blood such as composition of serum protein and pathological state.

Proteins is the main components of fish, are essential for growth and reproduction of fish species. Numerous studies have highlighted the special role of ensuring and maintaining feed quality in a normal physiological state. Both quantity and proportion of nutrients in fish feed can influence susceptibility to disease. The researches in fish nutrition have emphasized the importance of nutrients in maintaining normal immune functions and resistance to diseases (Maita, 2007). Any disturbance of environmental condition, extent or duration of certain activities, including the quantity and quality of food availability, may become a fish stress factor, diagnosed as such, by studying the fish main haematological (index) value (Bucur, 2009). The haematological indices, such as the red blood cells number (RBC- $\times 10^6$ cells/ μL blood), haemoglobin (Hb- g/dL), hematocrit (Ht-%) and the erythrocyte constants: the mean corpuscular volume (MCV- μm^3), the mean corpuscular haemoglobin (MCH-pg), the mean corpuscular haemoglobin concentration (MCHC - g/dL), are important parameters for the evaluation of general physiological condition of the fish.

It is well-known that blood sampling, laboratory techniques, seasonal variations, fish size, genetics patterns, stocking density, food privation, social stress, physico-chemical quality of water, handling and transport can influence the haematological values (Rey Vazquez, 2007 and . Docan, 2010). In the present investigation mollusks (*Pila bengalensis*), goat liver and earthworm (*P. sansibaricus*) these three different types of food was provided to experimental fish because they are excellent sources of protein which play an pretty role in proper growth, stay healthy and development of fish as well as can also be used as high-nutrient supplementary feed for domestic animals, birds and even for fish culture. Keeping all above thing in mind, the present research work was conduct in rainy season of 2011. The goal of the present study was the influence of feeding with different types of protein diets on haematological profile of Asian catfish, *Clarias batrachus* (Linn.) in ideal and identical laboratory condition.

The findings of the study may help the fish farmers for fish production and reduce the cost of fish feed.

MATERIALS AND METHODS

Selection and identification of species

Species were selected on the basis of abundance and use pattern in the study area. Species having economic importance were selected for the analysis of haematological profile fed with protein diets. *Clarias batrachus* has an elongate body that is broader at the head, tapering toward the tail and recognizable as a catfish with four pairs of barbels (whiskers) and fleshy, papillated lips. The teeth are villiform (small and bristle-like), occurring in patches on the jaw and palate, eyes are small, pectoral spines are large and robust and finely serrate along the margins. There is no dorsal spine. The dorsal fin is continuous and extends along the back two-thirds of the length of the body. The dorsal, caudal, and anal fins together form a near-continuous margin; the caudal fin is rounded.

Species collection and management

Live and healthy *Clarias batrachus* weighting 500-600g and body length measuring 20-25cm were purchased from local fish market, Lalpur bazar, Ranchi, Jharkhand and were acclimated to laboratory condition for 15 days. Fishes were selected for experiment and were divided into three groups and each group consisted of five sets (five individuals in each set). Each set of individuals was introduced into rectangular glass aquaria (60 × 30cm capacity) containing 15L of water. The first group was fed on an ad libitum diet of chopped muscle of earthworm (*P. sansibaricus*) twice a day. Prior to feeding, the gut of earthworms were evacuated by keeping 24h in water (Das and Patra, 1977) and then washed carefully in tap water to remove sand particles present (if any). Similarly second and third group were fed on ad libitum diet of chopped muscle of *Pila bengalensis* and liver of goat respectively. Food remains were collected with least disturbance to the fish, using pipette. Aquarium water was aerated continuously and changed daily. Fishes were sacrificed and blood was collected by puncturing the heart for analysis of haematological parameters.

Water quality

During the period of investigation water was exchange every day. Water was well aerated with the help of air pumps to maintain the dissolve oxygen (DO). Various physico-chemical parameters such as water temperature, dissolve oxygen, total alkalinity, pH, hardness and free carbon dioxide were analyzed by using standard methods.

Statistical analysis

Haematological parameters were statistically analyzed using Microsoft Excel 2010 statistical computer program, from which all values were represented as mean ± SD of n=5 and the values were significant at P < 0.01, P < 0.001 and P < 0.02 by performing student t-test between vermin meal, goat liver meal and molluscan meal.

RESULTS AND DISCUSSION

Throughout the experiment, water quality remained within

the favorable range required by *Clarias batrachus* indicating that provided feed could be utilized in the *Clarias* farming pond. Water quality parameters such as temperature, pH, dissolved oxygen, free CO₂, total alkalinity, total hardness shown in Fig. 1. Water is very important and precious for every living organism on this earth. It functions as a habitat for large number of aquatic organism ranging from microscopic planktons to large aquatic animals and macrophytes. There is an intricate relationship between metabolism of aquatic organisms and hydrobiological parameters in freshwater body (Desmukh and Ambore, 2006). Temperature, pH, dissolve oxygen, alkalinity, hardness and free CO₂ are the significant parameters used to study of the water quality (Shaikh and Yegari, 2003). In the present study temperature was 23.5°C, pH 7.4, total hardness 66ppm, total alkalinity 105ppm, dissolve oxygen 6.3ppm and free carbon dioxide 0.5ppm recorded during 15 day of experiment.

Dissolved oxygen is an important aquatic parameter whose measurement is vital in the context of culture of any aquatic animal as oxygen play a crucial role in its life processes. Free CO₂ is also one of the important factors in aquatic habitat. It is highly soluble in water and is main source of carbon pathway in the nature. CO₂ in water bodies is contributed by the respiratory activity of animals.

The hardness of water is not a pollution parameter but indicates water quality. Water is categorized according to degree of hardness as follows:

0 – 75 mg/L = soft

75 – 150 = moderately hard

150 – 300 mg/L = hard

Above 300 mg/L = very hard.

So, finding of total hardness of water in the present study was soft which indicate favorable condition for experimental fish.

Because of the dynamism and the functions that are fulfilled in the organism, the blood represents the “mirror” of general physiological condition of the fish. Thus, the haematological modifications of the studied fish were analyzed collaboration with the technological factors (fed with different protein diets) which can influence the metabolic processes. Changes in the haematological parameters due to proteineous food are presented in Table 1. The value of total leucocytes (10³/ul) of pila fed group 5.2 ± 2.8 was significantly (p < 0.05) decrease from goat liver fed group 7.4 ± 1.4 by 29.72% and 42.3% from earthworm fed group 7.4 ± 4 while neutrophils (%) of experimental fish fed with earthworm meal was 58.2 ± 1.25 which highly significant (p < 0.001) increased from molluscan fed group 52 ± 0.6 by 6.2% and non-significant increased from meat meal fed group 54.47 ± 1.89 by 1.5%. Similarly, the number of lymphocytes (%) of *Clarias batrachus* fed with earthworm meal was 38.8 ± 0.5 significant (p < 0.05) increased from fish fed with liver of goat 34.6 ± 1.7538 by 4.2% but it was more significant (p < 0.01) increase from molluscan fed group 32.5 ± 0.6 by 6.3%. A sharply more significant (p < 0.01) increase in eosinophils (%) of molluscan meal fed group was 3.1 ± 0.5 from earthworm meal fed group 1.4 ± 0.4 by 1.7% and 0.6% from goat liver fed group 2.5 ± 0.5 while basophils (%) of molluscan fed group was 0.7 ± 0.2 also significantly (p < 0.05) higher than goat liver fed group

Table 1: Changes in haematological parameters of *Clarias batrachus* fed with three different type of proteineous meal, ± SD, n = 5

Sl.no	Parameters	Goat liver fedgroup ,± SD	Meals <i>Pila bengalnesis</i> fed group,± SD	Earthworm (<i>P.sansibaricus</i>)fed group ,± SD
1	Total leucocytes (10 ³ /uL)	7.4 ± 1.4 ^b	5.2 ± 2.8 ^b	7.4 ± 4
2	Neutrophils (%)	54.47 ± 1.89 ^d	52 ± 0.6 ^{cd}	58.2 ± 1.25 ^c
3	Lymphocytes (%)	34.6 ± 1.7538 ^b	32.5 ± 0.6 ^a	38.8 ± 0.5 ^{ab}
4	Monocytes (%)	0.6 ± 0.5	0.9 ± 0.4	1.1 ± 0.5
5	Eosinophils (%)	2.5 ± 0.5	3.1 ± 0.5 ^a	1.4 ± 0.4 ^a
6	Basophils (%)	0.2 ± 0.1 ^b	0.7 ± 0.2 ^b	0.5 ± 0.4
7	Total RBC (10 ⁶ /ul)	1 ± 0.25 ^b	1.9 ± 0.6 ^{ab}	3.85 ± 0.1 ^a
8	Haemoglobin (g/dl)	7.6 ± 2 ^d	8.5 ± 2 ^{ad}	10.1 ± 2.45 ^a
9	Haematocrit (%)	6.88 ± 1 ^b	7.65 ± 0.9 ^{ab}	8.5 ± 0.6706 ^a
10	Mean corpuscular volum(fl)	91.35 ± 11.4 ^b	95 ± 2.5	100.1 ± 7.245 ^b
11	Mean corp.haemoglobin (pg)	30.57 ± 6.244a	32.25 ± 3.49	34.1 ± 5a
12	Mean corp.haemo.Conc. (%)	32 ± 5	33.3 ± 4.5	34 ± 6
13	Red cell distribution width (fl)	14.4 ± 7.55	15 ± 2	16.9 ± 7.63
14	Platelet distribution width (%)	13.2 ± 4	13.9 ± 0.4	14.4 ± 1.4
15	Platelet (10 ³ /ul)	249 ± 10	249.93 ± 3.5	251 ± 0.6
16	Mean platelet volume (%)	6.2 ± 2.2	6.9 ± 0.5	8.7 ± 2.5
17	Erythrocytes sedimentation rate	8 ± 3 ^b	9 ± 3	10 ± 2 ^b

Alphabet in superscript represent significant difference between triplicate sets of feeding trial. a = more significant; p < 0.01, b = significant p < 0.05, c = highly significant p < 0.001, d = significant p < 0.02. Absence of alphabet in superscript represent no significance difference

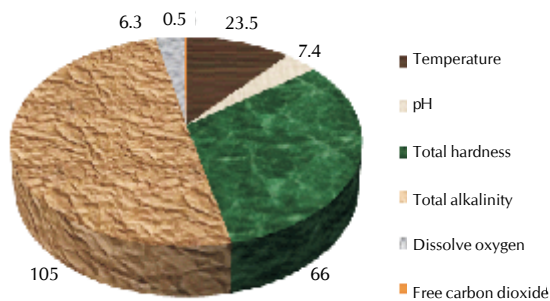


Figure 1: Physico-chemical parameters of water

0.2 ± 0.1 by 0.5% and 0.2% from earthworm fed group 0.5 ± 0.4 observed.

In the present study, total RBC (10⁶/uL), haemoglobin (g/dL) and haematocrit (%) of earthworm fed group was more significant (p < 0.01) increase from molluscan fed group and molluscan fed group showed significant (p < 0.05) increase from goat liver fed group. It was 3.85 ± 0.1, 10.1 ± 2.45 and 8.5 ± 0.6706 in earthworm fed group increased by 102.63%, 18.82% and 0.85% respectively from molluscan fed group 1.9 ± 0.6, 8.2 ± 2 and 7.65 ± 0.9 while MCV (fl) and ESR was 100.1 ± 7.245 and 10 ± 2 significantly increase from goat liver fed group 91.35 ± 11.4 by 9.57% and 8 ± 3 by 25% respectively but, on other hand total RBC (10⁶/uL) and haematocrit (%) of molluscan fed group increased from goat liver fed group 1 ± 0.25 and 6.88 ± 1 by 90% and 0.77% was observed.

There was no significant difference recorded among monocytes (%), MCHC (%), RDW (fl), PDW (%), Platelet (10³/uL) and MPV (%) of all three fed groups. But, among these groups earthworm

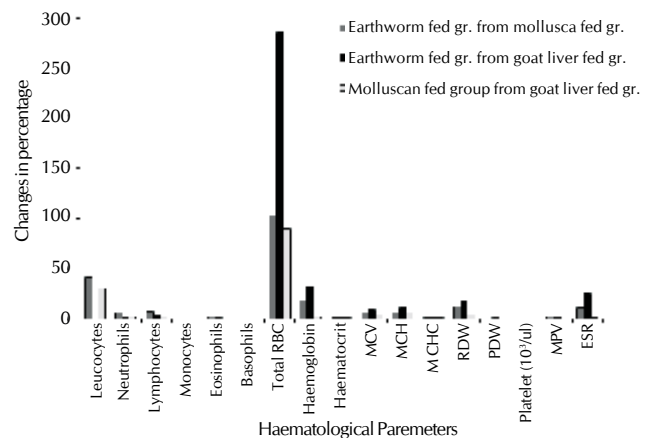


Figure 2: Changes in percentage of haematological parameters of three different feeding groups

fed group showed higher values of these haematological parameters than rest of two fed groups *i.e* goat liver fed and molluscan fed group. It was 1.1 ± 0.5, 34 ± 6, 16.9 ± 7.63, 14.4 ± 1.4, 251 ± 0.6 and 8.7 ± 2.5 respectively in earthworm fed group which increased by 0.2%, 0.7%, 12.66%, 0.5%, 0.42% and 1.8% from molluscan fed group 0.9 ± 0.4, 33.3 ± 4.5, 15 ± 2, 13.9 ± 0.4, 249.93 ± 3.5 and 6.9 ± 0.5 as well as from goat liver group *i.e* 0.6 ± 0.5, 32 ± 5, 14.4 ± 7.55, 13.2 ± 4, 249 ± 10 and 6.2 ± 2.2 by 0.5%, 2%, 17.36%, 1.2%, 0.6%, 2.5% were obtained. In the present study no mortalities occurred in all three fed groups throughout the period and, therefore, the meal *i.e.* earthworm, pila and liver of goat did not have any deleterious effects on the fish.

It is well known that certain blood parameters serve as reliable indicators of fish health Therefore, the changes associated with haematological parameters due to different types of meal establish a database, which could be used in diseases diagnosis and in guiding the implementation of treatment or preventive measures. These measures are essential in fish

farming and fish industry also. In the present study Table 1 shows the variation in haematological values of fish due to proteineous diets. The values obtained in fish fed diets viz. earthworm, pila and goat liver were within the normal ranges for catfish. This suggests that provided diets can be beneficial to fish. Earthworm fed group provide best results compare to molluscan fed group and goat liver fed group because earthworm is rich source of protein as well as pila and liver of goat are also good source of protein but, less than earthworm. Blood composition is usually altered during diseases or malnutrition conditions (Feist *et al.*, 2000). Aletor and Egberongbe (1998) reported that red blood cell counts and packed cell volume (PCV) are mostly affected by dietary treatment. Under normal conditions the composition of blood is reasonably constant for any particular species with changes falling with fairly narrow limits as observed by Banerjee *et al.*, (2002).

Dada and Ikuerowo, 2009 reported that catfish (*Clarias gariepinus*) fed with dietary ethanolic extracts of *Garcinia kola* seeds at 1.0g/kg level had the highest mean Hb concentration and PCV compare to other while the least mean Hb concentration was obtained in fish fed with diet at 0.25 g/kg. The same variation was recorded by them for WBC, RBC as the values increased with increasing dietary ethanolic extracts *G. kola* levels up to 1.0 g/kg and decreased as the inclusion levels increased up to 2.0 g/kg feed. The reduction in RBCs, Haemoglobin, PCV, MCH and MCHC in the infested catfish (*Clarias gariepienus*) with *Henneguya branchialis* while WBC and MCV was increased (Sabri, *et al.*, 2009). Coad, *et al.*, 2012 conducted an experiment on the effect of feeding with different protein levels on haematological profile and leucocytes population of juvenile paddlefish, *Polyodon spathula* and they recorded that RBC, Hb, Ht and MCV were increased in fish feed with 41% crude protein while MCH and MCHC decreased compared to fish feed with 54% crude protein. We can say that a higher percentage of protein fish diets can lead to a decrease of red blood cells number, of haemoglobin quantity and also of hematocrit value. This finding was agreed with present study in case of goat liver fed group of *Clarias batrachus* compare to earthworm fed group and pila fed group. Ayoola, *et al.*, 2013 reported that fish, *Clarias gariepinus* fed with *Moringa oleifera*, the haematological value of Packed cell Volume, Haemoglobin, Red Blood Cell and White Blood Cell was increase which agreed with the finding of present study in case of earthworm fed group of experimental fish. There was reduction in MCHC and MCH of fish fed with *Moringa oleifera* diet was also noted by Ayoola, *et al.*, 2013 and they concluded that using *M.oleifera* leaves as feed for *Clarias gariepinus* enhances the growth of the fish and has no negative impact on the health status of the fish. Therefore partial replacement of feed with *M.oleifera* should be encouraged. Similarly, in the present study, the provided protein diets viz. earthworm, pila and goat liver used as feed for *Clarias batrachus* has no negative impact on the health status of fish.

The increase that was observed in the haematological parameters of fish in the present investigation is agreement with the findings of Joshi *et al.*, 2002 who stated that survival of fish can be correlated with increase in antibody production which helps in the survival and recovery.

The increase in WBC may be due to increase in leucopolesis as a means of combating stressor in the body system of the fish, similar findings were recorded by Gabriel *et al.* (2004) in *Clarias gariepinus*. These changes in white blood cell have been reported to play important roles in the assessment of the state of health of *C. gariepinus* (Ezeri, 2001; Gabriel *et al.*, 2004).

Maheswaran, *et al.*, 2008 reported that in fish *Clarias batrachus* exposed to mercuric chloride, the leucocytes (WBC) was increased which could be attributed to a stimulation of the immune system in response to tissue damage caused by mercuric chloride. Gill and pant, (1985) have reported that the stimulation of immune system causes an increase in lymphocytes by an injury or tissue damage. Dhanekar, *et al.*, 1985 reported increase in large lymphocytes, reduction in small lymphocytes and thrombocytes populations as also elevation in monocytes, neutrophil and eosinophils cells in *Heteropneustes fossilios*, *Channa punctatus* and *Mastacebalus puncalus* on long-term exposed to least effective concentrations of mercuric chloride. Exposure to mercuric chloride induced variations in differential leucocytes counts and caused lymphocytosis, neutrophillia monocytosis, eosinophilia and thrombocytopenia in *Anabas testudineus* (Kumar, and Patri, 2004). An increase in lymphocyte number may be the compensatory response of lymphoid tissues to the destruction of circulating lymphocytes (Shah and Altindag, 2005). In the present study the leucocytes (WBC) in molluscan fed group was decreased compare to rest fed groups goat liver fed group and earthworm fed group of *Clarias batrachus* which attributed to a condition of leucopenia. Due to this the immunity of fish is severely weakened and the individual is at a greater risk of infections. It may be caused by diseases, food quality, and genetic deficiencies. But these types of situation is not applied for rest of the fed groups.

The increase in the red blood cell and heamoglobin concentration may be attributed to the increase in the size of the fish as a result of growth in the fish. This is in agreement with Das (1965) who reported that both the haemoglobin contents and Erythrocyte counts (red blood cell) tend to increase with length and age of the fish. The increase in haemoglobin concentration as a results of increase in the activity of the fish, *Clarias batrachus* are naturally active. This agrees with Eisler (1965) who suggested that there was a correlation between haemoglobin concentration and activity of fish. The more active fishes tend to have high haemoglobin values than the more sedentary ones. In the present investigation, increase value of haematological parameters was not exceed or lower than the normal value which present in *Clarias batrachus* indicate rich blood production in body and experimental fish was not in anemic condition. Anemia made the fish weaker and sluggish causing retarded feeding activities (Kumar and Banerjee, 2012). No mortality was recorded in our investigation and also noticed that the supplied feeds was completely finished within few minutes of administration. This illustrates voracious predatory nature of experimental fish *Clarias batrachus*, which is a nutritionally very important fish in the Indian sub continent. Similar investigation was also made by Hossain *et al.*, 2006 on *Clarias batrachus* larva fed with *Moina* (D-1), chopped *Tubifex* (D-2) and both *Moina* and chopped *Tubifex* (D-3) at 4 h interval for first 6 days and at 6

hour interval for another 6 days. The finding of present investigation indicate the suitability of provided meals to fish.

CONCLUSION

The demand for animal protein has gone far beyond supply as a result of the rapid human population in the developing countries. This therefore necessitates an urgent and immediate need to increase the production of protein sources and fish is one of the main animal protein sources which has been cultured using different sources of feed ingredients. It is true that, fish meal has been the main source of animal protein in fish feed but, its high cost and dwindling availability has called for the search for alternative sources of protein. So, in the present study earthworm, pila and goat liver were used to determine the health status of fish and after investigation it was concluded that the provided these three different protein meals to fish was completely finished within few minutes of administration which indicate that it was digest properly by fish and changes in level of haematological parameters was noted within the normal range of *Clarias batrachus* i.e. value of haematological parameters was not exceed or lowered than normal value present in fish. This findings indicate that these meals does not affect on fish health, rich production of blood in body, no anaemic condition of fish and good quality of food So, these meals are beneficial for fish production in aquaculture industry.

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