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Surjya Narayan Datta
Department of Fisheries Resource Management, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Ajeet Singh
Department of Harvest and Post- Harvest technology, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Amit Mandal
Department of Aquaculture, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Geeta Jassal
Department of Fisheries Resource Management, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Correspondence
Surjya Narayan Datta
Department of Fisheries Resource Management, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Effect of different dietary protein sources on hematological parameters of striped catfish *Pangasianodon hypophthalmus*

Surjya Narayan Datta, Ajeet Singh, Amit Mandal and Geeta Jassal

Abstract
The present study was conducted with the aim to evaluate performance of different protein source on growth, survival and hematological parameters in striped catfish *P. hypophthalmus*. The experiment was conducted at College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, during July- October, 2017 for a duration of 120 days in indoor glass aquaria (60× 30 ×30cm) maintaining stocking density of 8 no of *P. hypophthalmus* fingerling (9.80±0.08 cm and 11.0±0.06 g) in each aquaria. Four iso-proteinous (≈ 32% crude protein) experimental diets (D1 – D4) were prepared containing poultry waste (D1), fish meal (D2), fish silage (D3) and soybean (D4) as major protein source in diets in four treatments T1- T4, respectively. No significant differences were observed in water quality parameters among different treatments fed with different sources of protein. Maximum net weight gain was recorded in T4 (19.329 g) followed by T2 (16.154 g), T3 (14.583 g) and T1 (11.937 g), respectively indicating diet containing soybean meal as major protein source gained highest weight in *P. hypophthalmus.* Hemoglobin value ranged 5.8-7.1g dL^{-1} while erythrocyte count 1.44-2.75 mill $cu.mm^{-1}$, PCV 16.4-33.4%, MCV 113.9-123.0 fL, MCH 24.7-32.6 pg, MCHC 20.6-28.7 g dL^{-1} recorded in fish blood. Among leukocytes, range of count for neutrophils 60.2-84.7%, lymphocytes 8.2-25.5%, monocytes 0.8-4.0% and eosinophils 0.0 – 22.3% recorded in fish blood, fed with different diets. *P. hypophthalmus* appeared to exhibit a better response towards survival, growth and acquired immunity of fish when fed with soybean rich diet as compared to fish silage, poultry slaughter waste and fish meal.

Keywords: *Pangasianodon hypophthalmus*, iso-proteinous diets, carcass composition, haematological parameters

1. Introduction

In intensive aquaculture fish are exposed to numerous stressors; during the production cycle such factors directly or indirectly affect their immune response and resistance to infectious microorganisms present in the culture environment [1]. Numerous studies have been conducted trying to identify management practices that may minimize disease loss due to stress factors and strengthen their immune response [1]. Among the different approaches used to develop immunity, nutritional modulation is one of the ways for fish health management. Protein is the major macronutrient in fish which provides essential and non-essential amino acids for required protein synthesis and energy for maintenance and growth [2]. However, protein is the most expensive component in fish feed [3, 4]. For successful aquaculture practices, it is needed to determine the minimum level of protein at which fish can attain maximum growth as well as the operational costs can be saved [5]. It is well documented that dietary protein requirements for most of the fish species are found to be between 30 and 55% of the diet; however, it depends on the fish species, fish size, dietary protein sources and environmental conditions [3, 6]. Blood parameters are clear indicator to reveal the health status of living organism of animal origin, while leukocytes count reflects the level of infection and status of immune response [7]. Development of hematocrit components largely depend on the nutrient taking through feed. Therefor balance of different nutrient in optimum quantity is prime requirement in developing quality feed. Protein is such vital nutrient required for blood cell formation and its supply largely depends on optimum combination of amino acids. Traditionally, fish meal has provided a major part of protein sources in formulated feeds because of its suitable protein quality [8]. Since the recent scarcity and uncertain consistency of supply encourage its replacement by alternative protein sources that are of high quality and biological values, but less expensive has been investigated in many fish species.

Asian striped catfish *Pangasianodon hypophthalmus* is native of Mekong basin of Indochina [9]. Over the last ten years, the striped catfish *Pangasianodon hypophthalmus* has emerged as a new aquaculture whitefish product on the world market [10]. It has been introduced for cultivation in different countries of the world (Singapore, Philippines, Taiwan, Malaysia, China, Myanmar, Bangladesh, Nepal and India) because of its high growth, easy culture practice, high disease resistance and high resistance to crowding and low oxygen [11] while the market for *Pangasianodon* has expanded globally, the production of fish has remained exclusively in Eastern Asia and centered principally in Vietnam. *P. hypophthalmus* has proven particularly adaptable for intensive production in several countries [12]. In India the fish was introduced in 1997 from Bangladesh and is being cultured both in monoculture as well as in polyculture system [13]. Cost on feeding is major investment and predominantly affects the margin of profit in all culture systems to raise animal protein, and aquaculture is not an exceptional. It has been estimated that feed costs accounts for 70–90% of total investment cost [14]. Therefore, in order to reduce feed costs and the use of fish meal in aquafeeds, more extensive use of alternative feed ingredients is needed [15]. The present study was conducted with the aim to evaluate the efficacy of conventional and non-conventional protein source like poultry slaughter waste, fish meal, fish silage and soybean meal on survival and hematological parameters in striped catfish *P. hypophthalmus* to find out the better protein source in diet and effect on survival, growth and haematology of *P. hypophthalmus*.

2. Materials and Methods

The present experiment was carried out at College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. Experiment was conducted during July- October, 2017 in triplicate with four treatments i.e.T1, T2 T3 and T4 providing the diet D1, D2, D3 and D4, respectively in indoor glass aquaria (60× 30 ×30cm) maintaining stocking density of 8 no of *P. hypophthalmus* fingerling (9.80±0.08 cm and 11.0±0.06 g) in each aquaria for the duration of 120 days. Four iso-proteinous experimental diets (≈ 32% crude protein) (D1 – D4) were prepared containing poultry waste (D1), fish meal (D2), fish silage (D3) and soybean (D4) as major protein source in diets. Details of feed ingredients for the preparation of experimental diets are given in Figure 1. Fishes were fed with formulated diets @ 3% of body weight in total (two meals of 1.5% body weights) twice a day (9.00 am and 5.00 pm). Proximate composition of feed for moisture, crude protein, crude fat and ash was evaluated as per standard method given in AOAC [16], Nitrogen free extracts (NFE) was calculated by the formula i.e. 100 -% (moisture + crude protein + crude lipid + ash + crude fibre). Details of proximate composition of experimental diets are given in Figure 2. The physico-chemical parameters of the water were estimated as per standard method described in APHA [17]. The values of basic hematological parameters were evaluated according to standard methods described by Svobodova *et al* [18]. Statistical software SPSS 16 was used for analyzing the data.

3. Results and Discussion:

There were no significant differences were observed in water quality parameters viz. temperature, pH, total dissolved solids, dissolved oxygen, total alkalinity, total hardness, NH_3-N, NO_3-N, NO_2- N and PO_4-P observed among different treatments and all these parameters were within the range as suggested by Boyd and Pillai [19]; Rowland [20] and Boyd and Tucker [21]. Details of the water quality parameters range in different treatments recorded during experimental period are given in Table 1.

For present study iso-proteinous (≈ 32.0%) diets were prepared using different conventional and non-conventional feed ingredients to evaluate the performance of other dietary protein sources replacing fish meal in diet of *P. hypophthalmus*. Protein is the most important component of fish feeds providing essential amino acids for tissue repair and growth [22]. Hung *et al.* [23] reported that the protein requirement in *P. hypophthalmus* was approximately 32.5% on dry matter (DM) basis for optimum growth. Fish survival in all treatments was 100% indicating water quality and culture environment was suitable and feed provided in different treatments containing different dietary protein source were acceptable by the species. At the end of the experiment maximum net weight gain was recorded in T4 (19.329 g) followed by T2 (16.154 g), T3 (14.583 g) and T1 (11.937 g), respectively indicating diet containing soybean meal as major source of protein D4 gained highest weight in *P. hypophthalmus* (which is 11.69%, 18.55% and 32.27% more weight gain recorded than that of diet prepared with fish meal, fish silage and chicken waste, respectively). However soybean meal is deficient in methionine and lysine but rich in arginine, serine and phenylalanine amino acids while fish meat and poultry waste is rich in methionine and lysine [24]. Methionine plays major role in liver function and wound healing rather than growth while lysine is essential requirement for growth and arginine plays role in growth and immune boosting.

The hematological parameters are an important tool to express the conditions of fish health. Hemoglobin (Hb) is the protein contained in red blood cells and responsible for delivery of oxygen to the tissues. To ensure continuous supply of oxygen, a sufficient hemoglobin level must be maintained [25]. Average haematological values in *P. hypophthalmus* young ones fed with diets containing different protein sources are depicted in Table 2. In present study, highest value of hemoglobin (7.1g dL^{-1}) was recorded in fishes fed with soybean (T4) as major protein source, while it was recorded lowest in fish fed with fish silage (D3) as source of dietary protein. Hedayati and Tarkhani [26] reported the average value of hematological parameters as Hb (g L^{-1}) 7.17 in *P. hypophthalmus* and the value are quite near to values recorded in this study. Fish erythrocyte is a permanently nucleated, hemoglobin-ladened, oval shaped, flattened, biconvex disc (II). Primary function of erythrocytes is to transport oxygen as it contains the pigment hemoglobin. Similar to hemoglobin content, highest value of erythrocytes (mill $cu.mm^{-1}$) was recorded in fishes fed with soybean (T4) i.e. 2.75, followed by fish meal (D2) 2.67, poultry slaughter waste (D1) 2.09, fish silage (D3) 1.44. Change in erythrocyte values were in accordance to changes in hemoglobin parameters in different treatments.

Average leukocyte count in different treatments is presented in Table 3. The packed cell volume (PCV) of fish blood is an important indicator of fish health. The hematocrit or packed cell volume (PCV) is a part of the complete blood count (CBC) and denotes the percent of whole blood that is composed of red blood cells. In present study PCV (%) value was recorded highest in fish fed with diet containing soybean meal (33.4%) followed by fish meal (32.0%), poultry slaughter waste (25.7%) and fish silage (16.4%). Mean corpuscular volume (MCV) (fL) is the average volume of a red blood cell. This is a calculated value derived from the

hematocrit and red cell count. The values for MCV in soymeal (D4), fish meal (D2), poultry slaughter waste (D1) and fish silage (D3) were 121.5, 123.0, 119.9 and 113.9, respectively. Mean Corpuscular Hemoglobin (MCH) is the average amount of hemoglobin in the average red cells, while Mean Corpuscular Hemoglobin Concentration (MCHC) is the average concentration of hemoglobin in a given volume of red cells. This is a calculated volume derived from the hemoglobin measurement and the hematocrit. The values for MCH (pg) and MCHC (g dL^{-1}) in soymeal (D4), fish silage (D3), poultry slaughter waste (D1) and fish meal (D2) were 25.8 and 21.3, 32.6 and 28.7, 27.8 and 22.6, 24.7 and 20.6, respectively. Hedayati and Tarkhani [26] reported the average value of MCV (fl) 139, MCH (pg) 52.3, MCHC (g/dL) 36.4 in healthy *P. hypophthalmus*. The values recorded for PCV and MCV denoted that fish was not under the condition of hypoxia and anoxia.

Leukocytes are indicator of infection and give some idea regarding infection. Teleost neutrophils are large round cells with abundant clear cytoplasm and an eccentric, condensed nucleus that is either round or multi-lobed. The cytoplasm is often light gray and in some cells, contains very fine azurophilic granules, while lymphocytes are small round cells with a high N: C ratio and a rim of smooth light blue cytoplasm around the large oval-round condensed nucleus [27]. In present investigation highest average value of different leukocytes were recorded as neutrophils (84.7%) in D1, lymphocytes, monocytes and basophils (25.5%, 4.0%, 10.3%, respectively) in D3, eosinophils (22.3%) in D2. Variation in number of different cells may be due to incorporation of protein from different sources. Hedayati and Tarkhani [26] reported the average value of neutrophil (5.00%), lymphocyte (93.3%), eosinophil (0.33%) in healthy *P. hypophthalmus*. It can be stated that the diet containing the soybean meal as major protein source improved the hemoglobin (g dL^{-1}) and erythrocyte count (mill $cu.mm^{-1}$) in striped catfish *P. hypophthalmus*. The leukocytes count also indicated that fish fed with soybean meal increased the acquired immunity in fish. Moreover no abnormal haematological parameters recorded in diets prepared with fish silage and poultry slaughter waste as major protein source.

4. Conclusion

The results of this study conclude *P. hypophthalmus* has very good capacity to utilize the different conventional and non-conventional protein sources in diet preparation. The disposal problem of poultry slaughter waste and fish waste generated in markets could be solved by utilizing these waste as valuable protein source in diet preparation of this catfish. No adverse effect on survival, growth and blood parameters were observed in fish, fed with fish silage and poultry slaughter waste. Considering the parameters measured in this study *P.hypophthalmus* appeared to exhibit a better response towards survival, growth and immunity of fish when fed with soybean rich diet as compared to fish silage, poultry slaughter waste and fish meal.

Table 1: Range of water quality parameters recorded during experimental period

Parameters	Range
pH	**8.37 – 8.58**
Total dissolved solids (mg l^{-1})	635.27 - 653.94
Dissolved Oxygen (mg l^{-1})	8.14 – 8.24
Total alkalinity (mg l^{-1})	295.78 – 307.89
Total hardness (mg l^{-1})	303.44 309.56
Ammonia (mg l^{-1})	0.112- 0.123
Ortho-phosphate (mg l^{-1})	0.136 - 0.148

Table 2: Hematological values (average) in *P. hypophthalmus* young ones fed with diets containing different protein sources.

Parameters	Control	D1	D2	D3	D4
Hemoglobin (g dL^{-1})	4.6	5.8	6.6	4.7	7.1
Erythrocyte count (mill $cu.mm^{-1}$)	1.82	2.09	2.67	1.44	2.75
PCV (%)	23.8	25.7	32.0	16.4	33.4
MCV (fL)	130.8	123.0	119.9	113.9	121.5
MCH (pg)	25.3	27.8	24.7	32.6	25.8
MCHC (g dL^{-1})	19.3	22.6	20.6	28.7	21.3

Table 3: Leukocyte count (average) in *P. hypophthalmus* youngones fed with diets containing different protein sources.

Treatments / Parameters	Control	D1	D2	D3	D4
Neutrophils (%)	60	84.7	61.2	60.2	81.2
Lymphocytes (%)	31.5	8.2	10.9	25.5	17.5
Monocytes (%)	4.1	3.6	1.6	4.0	0.8
Eosinophils (%)	0.0	0.0	22.3	0.0	0.0
Basophils (%)	4.4	3.5	4.0	10.3	

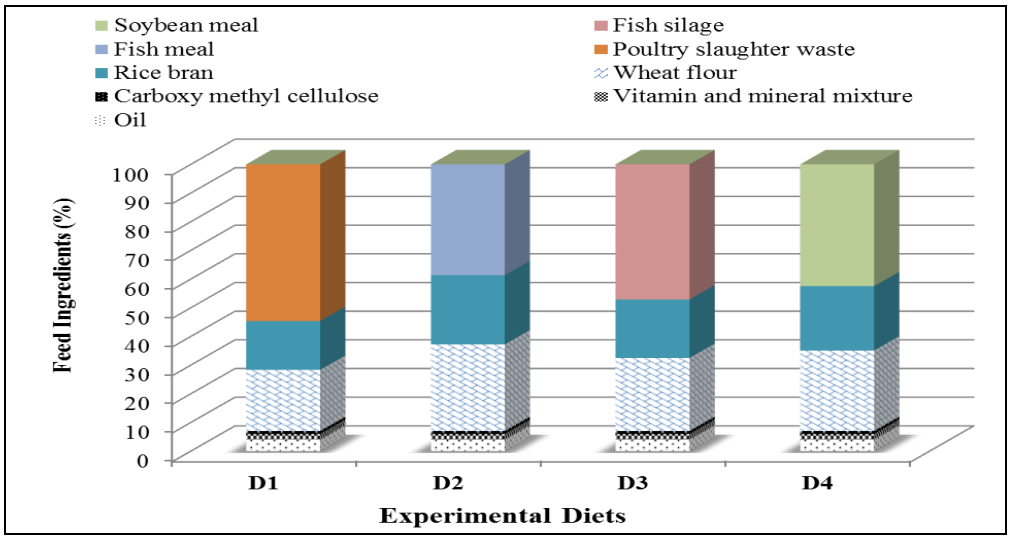

Fig 1: Levels of feed ingredients (%) in experimented diets (D1 – D4)

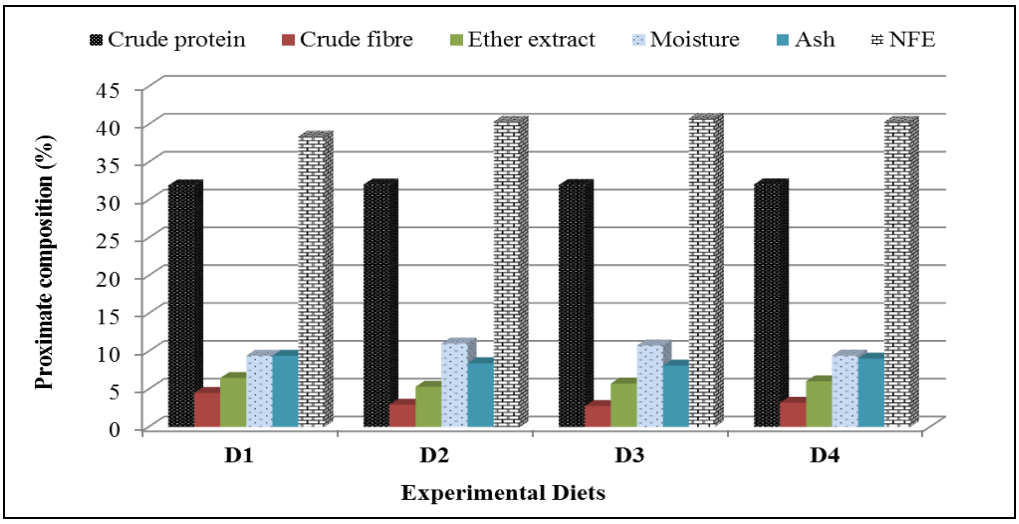

Fig 2: Proximate composition (%) of experimental diets (D1 – D4) (% dry matter basis)

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