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Haematological status in *Spirulina* and soyabean supplementation to *Catla catla* fingerlings

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Abstract

The 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. These parameters are also closely related to the response of the animal to the environment, an indication that the environment where fish lives could exert some influence on the blood characteristics. The supplementation of 1% Spirulina, 1% soyabean and 1% combined diet (1% Spirulina and soyabean) on haematological status in *Catla catla*. An experiment was conducted to determine the haematological role of Indian Major Carp *Catla catla*, fingerlings for period of 90 days.There were significant differences between the blood parameters (haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), RBC indices (Mean Corpuscular Haemoglobin concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) and white blood cells (WBC). The present investigation shows that incorporation of a soyabean and spirulina in diets for common carp results in increased haematological performance. Among the various diet, combined diet at 1% concentration produced the best and statistically significant ($p<0.05$) in haematological parameters RBC (1.27±0.07), WBC (6800±421.6), Hb (5.21±0.35), PCV (15.12±1.02), MCV (121.19±0.81), MCH (42.01±2.85) and MCHC (34.45±2.34) compared to other diets and control.

Keywords: *Catla catla*, *Spirulina*, Soyabean, Haematology

1. Introduction

Haematological parameters are therefore ready tools used by fish biologists and researchers in many parts of the world. The 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 [1]. These parameters are also closely related to the response of the animal to the environment, an indication that the environment where fish lives could exert some influence on the blood characteristics [2, 3]. Previous studies on fish haematology have revealed that interpretation of blood parameters is quite difficult since variations in the blood are caused by internal and external factors.

It is well known that blood sampling, laboratory techniques, seasonal variations, size, genetic properties, sex, population density, lack of food supply, environmental stress and transportation could affect haematological data [4, 1].

More recently, the administration of soyabean and spirulina to fish through the diet has appeared as a very promising control measure in fish farms. Soyabean are defined as microbial dietary adjuvents that beneficially affect the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract [5]. *Spirulina* can be considered a nutritional supplement that has various health benefits for humans, and a feed supplement for animals having economic benefits. Nowadays, *Spirulina* can be used to establish immune-potentiating functions in carp [6]. The Indian major carps *Catla catla, Labeo rohita and Cirrhinus mrigala* are the most important commercial fishes in India with a maximum market demand and acceptability as food by the consumers due to their taste and flesh. They contribute about 67% of total freshwater fish production [7]. Among this, *Catla catla* contributes a major portion to the freshwater fish production in South. Therefore, presently an attempt has been made to study the effect of 1% *Spirulina* 1% soyabean and 1% combined diet (1% Spirulina and soyabean) on haematological parameters (RBC, Hb, WBC, PCV, MCV, MCH and MCHC) of fish for the experimental period of 90days.

2. Materials and Methods

2.1 Collection and acclimation of experimental fishes

Fingerlings of *C. catla* (average weight 4.85 ± 1.12 g) were procured from Fish farm, Orathanadu, Thanjavur District, Tamil Nadu, India, using cast net and maintained in the laboratory in a glass aquarium tank and acclimated in aerated tap water with continuous aeration for two weeks prior to experimentation. During this period, fishes were fed with a known amount of fish food.

2.2 Preparation of Diet

Four diets were prepared: an unenriched control diet plus three diets containing either soya bean, *Spirulina. plantensis* and combination of soya bean and *S. plantensis* each at a concentration of 1% (Table 1).

Table 1: Ingredients and proximate composition of formulated diets.

Ingredients (%)	All diets
Fishmeal	35.0
Soybean meal	17.0
Rice bran	11.0
Groundnut oil cake	10.0
Tapioca flour	10.0
Mineral premix	1.5
Vitamin premix	0.5

Ingredients	Control	*Spirulina*	*Soyabean*	*Combined diet*
		1%	1%	1%
Wheat flour	15	14.0	14.0	13.0
Spirulina platensis	-	1.0	-	0.5
Soyabean		-	1.0	0.5
Proximate composition (%)				
Crude protein	36.2	40.6	40.6	40.6
Crude lipid	7.6	8.1	8.1	8.1
Crude carbohydrate	21.2	18.7	18.7	18.7
Ash	8.4	8.8	8.8	8.8

The *spirulina* was obtained from PARRY Nutraceuticals (Division of EID Parry (India) Ltd. at Pannangudi, Pudukkottai Dist. Tamilnadu, and India. The soya bean purchased from General stores, Kelavasal, Thanjavur, and Tamil Nadu, India. Soya bean further formulate a fine powder and used to prepare the experimental diet.

The fingerlings were fed 3% of their body weight twice a day for 90 days. Every third day, tanks were partially cleaned and water was partially changed. The temperature averaged 28±1.5°C, dissolved oxygen 7.4±0.6 mg/l, and total ammonia 0.5±0.2 mg/l.

2.3 Haematological parameters

Lymphocyte and monocyte, RBC and WBC counted by the method of Ochei and Kolhatkar [8]. PCV, MCV, MCH and MCHV determined by the method of Ochei and Kolhatkar [8]. Haemoglobin was estimated by Cyanmethaemoglobin method [8].

2.4 Statistical Analysis

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons. The results were statistically analyzed by Graphpad Instat Software (Graphpad Software, San Diego, CA, USA) version 3 was used $p< 0.05$ were considered to be significant.

3. Results

The results of the haematological parameters of *Catla* fingerlings with different feeding regimes are presented in Table 2. Hemotological parameters of *Catla* fingerlings with different feed clearly showed significant enhancement with 1% combined diet when compared with other concentrations of (1% Spirulina and 1% soyabean) and control. *Catla* fingerlings showed haematological parameters increased maximum in RBC (1.27±0.07), WBC (6800±421.6), Hb (5.21±0.35) while non significant changes in PCV (15.12±1.02), MCV (121.19±0.81), MCH (42.01±2.85) and MCHC (34.45±2.34) were observed in 1% combined diet compared to other soyabean and spirulina diets and control. The control showed that RBC (1.14±0.04), WBC (5700±394.4), Hb (4.42±0.32), PCV (14.2±0.96), MCV (117.35±0.79), MCH (39.00±2.65) and MCHC (33.23±2.25) were observed in 90 days (Table 2).

4. Discussion

There is growing interest in the study of haematological parameters of fish blood cells regarded as important for aquaculture purposes. Blood parameters have been used as indices of fish health status in a number of fish species to detect physiological changes as a result of stress condition such as transportation, handling, hypoxia and acclimation [9, 10]. Haematological studies help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. Haematological parameters are closely related to the response of the animal to the environment, an indication that the environment where fishes live could exert some influence on the haematological characteristics [11]. These indices have been employed in effectively monitoring the responses of fishes to supplementation of diet and thus their health status under such adverse conditions.

The source of fish (wild or cultured) may influence its state of health. This could be revealed by changes in the haematological parameters due to variations in the physico-chemical parameters of habitats, exposure to aetiological agents and environmental pollution among others [12]. Interactions between source (wild or cultured), supplementation of diet and health status indicated that source of fish had a highly significant impact on the health status (Table 2). This is shown in the values of WBC and RBC counts. Besides, fish from the wild had higher Hb values after supplemented fish than control. The significant interactions recorded between source of fish and health status seem to suggest that the source of fish plays an important role in the health status when adjudged by changes in WBC and RBC counts. Changes in WBC and RBC counts have been reported to play important roles in the assessment of the state of health of *Catla catla.*

Table 2: The haematological parameters of freshwater fish *Catla catla* (Fingerlings) with different feeding regimes at 90 days.

Types of Feed	Haematological parameters						
	RBC	WBC	Hb	PCV	MCV	MCH	MCHC
Control	1.14±0.04	5700±394.4	4.42±0.32	14.2±0.96	117.35±0.79	39.00±2.65	33.23±2.25
Soyabean (1%)	1.22±0.06	5890±400.5	4.83±0.32	14.66±0.99	120.16±0.81	39.59±2.69	32.94±2.23
Spirulina (1%)	1.23±0.06	6185±420.5	5.19±0.35	15.11±1.02	122.84±0.83	42.19±2.86	34.34±2.33
Combined (Soyabean and Spirulina) (1%)	1.27±0.07*	6800±421.6*	5.21±0.35*	15.12±1.02	121.19±0.81*	42.01±2.85	34.45±2.34

*Significantly different from control $p<0.05$

Values were expressed as Mean ± standard deviation (number of trials, 3)

The role of blood parameters in the assessment of the health status of fish is underscored by the observation of Omoregie [13] who noted the possibility that changes in the blood will reveal conditions within the body of the fish long before any outward manifestation of diseases. Although there were significant differences between the blood characteristics of apparently healthy fish of supplementation of different diets (1% spirulina, soyabean and combined diet), the values of some of the parameters (Hb, WBC, RBC, MCV, PCV, MCH and MCHC) were higher in the acclimated fish. The above observation reveals that the supplementation of different diets may influence the blood components of apparently healthy *Catla catla* (Table 2). Among the various diet, combined diet at 1% concentration produced the best and statistically significant.

The results of our research provide a contribution to the knowledge of the haematological parameters of the *Catla catla*, under the supplementation conditions employed in this study. This investigation may be helpful as a tool to monitor the health status of fish. In conclusion, the incorporation of *Spirulina* and soyabean in common carp diets improves haematological parameters. Among the various diet supplementations, the 1% combined diet was most effective to improve the haematological parameters in fish as compared with other diets. This might be due to high nutritional content of combined diet (spirulina and soyabean combination).

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