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Changes in haematological and serum biochemical indices of Nile tilapia (*Oreochromis niloticus*) fry fed dietary shrimp head meal

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Abstract
A total of 200 acclimatized fry (1.56 ± 0.10 g) were randomly distributed in quadruple into five distinct experimental groups with five isonitrogenous (40% crude protein) diets : T1 (control) containing fish meal; and T2, T3, T4 and T5 replacing 25%, 50%, 75% and 100% of fish meal with shrimp head meal for 60 days. The haematological parameters (haemoglobin, erythrocytes, leucocytes, differential leucocyte count and haematocrit, mean cell volume, Mean corpuscular haemoglobin, mean cell haemoglobin concentration) showed significant (p<0.05) difference among various experimental groups. Serum total protein, total immunoglobulin, albumin and globulin and albumin/globulin ratio were significantly (p<0.05) higher in T3 treatment. In conclusion, replacement of fish meal by shrimp head meal in practical diet of tilapia showed a slight difference in the haematological and biochemical parameters without impact on the health status of the fish. Therefore, use of shrimp head meal as alternative feed should be encouraged.

Keywords: Fish meal, shrimp head meal, haematological, biochemical, Nile tilapia

Introduction
The overall growth in aquaculture production stays relatively strong owing to the growing demand for food fish among most producing countries [1]. Protein is the basic requisite for cultured species. Thus, feeding of protein rich feed leads in rise of feed cost making a great proportion of the total expenses of aquaculture feed [2]. Nutrient requirements for growth, reproduction and normal physiological functions in fish are alike to other animals. However, fish have much higher requirements in proteins, so feed mixtures with 25 to 45% of raw proteins are mainly used [3]. Nile tilapia (*Oreochromis niloticus*) is one of the most economically significant species in aquaculture to culture because of its rapid growth, good survival in high density culture and disease tolerance [4]. Tilapia culture has been growing globally in tropical and subtropical areas and is mitigated to new disputes involving well-balanced diets to uplift the growth performance and feed efficiency of fish.
Biochemical and haematological parameters are a expression of health and physiological responses that may be determined by various stressors. In general, increase susceptibility of fish to disease is associated to stress caused by unfavorable environmental conditions, inadequate management practices or in response to dietary limitation of essential nutrients [5]. Hematological indices are closely associated with the response of the animal to the environment, an indication that the environment where fishes live could utilize some effect on the hematological characteristics [6]. These indices have been employed in effectively monitoring the responses of fishes to the stressors and thus their health status under such adverse environments. Fish are known to be in close relationship with the aqueous environment, hence, the blood will expose conditions within the body of the fish long before there is any visible reflection of disease [7, 8], haematological indices are therefore broadly apply by fish biologists and researchers the world over. However, at present, only few reports are accessible regarding the effect of shrimp head meal as a feed additive on haematological characteristics. Therefore, this study directed at revealing the influence of substitution of fish meal with different levels of shrimp head meal on hematological and biochemical characteristics of the Nile tilapia (*O. niloticus*) fry.

Materials and methods

Experimental fish and Experimental design

Fry of tilapia (*O. niloticus*) were selected for the experiment. Fry were procured from the fish hatchery of Kachchh Vikas Trust, Bhuj, Gujarat, India. The fishes were brought to Aquaculture Wet Laboratory at College of Fisheries Science, JAU, Veraval, and were allowed to remain in plastic pools (500 L) with continuous aeration and feeding for 15 days. After acclimatization, tilapia fry (average weight 1.56 ± 0.10 g) were assigned into groups of 10 fry in each experimental tank. The experimental set-up consisted of 20 plastic tanks (40 L capacity). Two hundred (200) fishes were distributed in five distinct experimental groups after length-weight measurement of individual fish. Each plastic tank containing 30 L chlorine free water was stocked with 10 fishes. Fry of tilapia (*O. niloticus*) were selected by measuring their length and weight individually and distributed in five distinct experimental groups in quadruple, following a completely randomized design.

Feed ingredients

Ingredients such as fish meal, shrimp head meal, wheat bran, wheat flour, rice flour, tapioca powder, sunflower oil, vitamin and mineral mixture (Valaenza Pharmaceuticals Private Limited) and cod liver oil (Sanofi India Limited, Sanofi Consumer Healthcare Division) were used for feed formulation (Table 1).

Chemical analysis

Prior to formulation of diets all ingredients were analyzed in triplicate for proximate composition (Table 1) following the methods of AOAC [9]. Crude protein was obtained by the semi- automatic Micro-Kjeldahl digestion method, crude lipid by soxhlet solvent extraction unit, carbohydrate by difference method, ash by muffle furnace and moisture by oven after drying at 105 ºC till constant weight [10]. Feed formulation and proximate composition of treatment diets are given in Table 2. The following calculations were made to evaluate the proximate composition:

Crude protein (%) = N2 (%) × 6.25

Ether extract (%) = (Weight of the ether extract/Weight of the sample) x 100

Moisture (%) = [(Wet weight of sample - Dried weight of sample)/Wet weight of sample] x 100

Ash (%) = (Weight of ash/ Weight of sample) x 100

Total carbohydrate = 100 - [weight in grams (protein + lipid + moisture + ash) in 100 g of food]

Table 1: Proximate composition of ingredients used for preparation of treatment diets

Ingredients	Moisture	Dry matter	Ash	Protein	Ether extract	Nitrogen free extract
Fish meal[1]	8.42	91.58	23.93	44.01	8.41	15.23
Shrimp head meal	6.52	93.48	27.84	43.12	6.45	16.07
Wheat flour[2]	5.74	94.26	1.92	11.46	2.68	78.20
Wheat bran[2]	6.62	93.38	3.88	14.83	2.95	71.72
Rice flour[2]	5.45	94.55	1.76	11.75	0.97	80.07

1 Star fish meal plant, Veraval, Gujarat, India

2 Local market, Veraval, Gujarat, India

Table 2: The formulation of control and treatment diets

Ingredients	Diets				
	T1 (Control)	T2	T3	T4	T5
Shrimp head meal (43.12 CP)	0	23	46	69	92
Fish meal (44.01 CP)	92	69	46	23	0
Wheat bran (14.83 CP)	1	1	1	1	1
Rice Flour (11.75 CP)	1	1	1	1	1
Wheat Flour (11.46 CP)	1	1	1	1	1
Tapioca	1	1	1	1	1
Sun flower oil[1]	1	1	1	1	1
Fish Oil[2]	2	2	2	2	2
Vitamin Mixture[3]	1	1	1	1	1
Total	100	100	100	100	100
Proximate analysis (determined on dry matter basis)					
Crude protein (CP) (%)	40.48	40.25	40.02	39.79	39.56
Ether extract (%)	10.07	9.84	9.78	9.53	9.28
Ash (%)	21.94	22.62	22.94	23.28	23.37
Moisture (%)	7.57	7.75	7.97	8.44	9.52
Nitrogen free extract (%)	19.94	19.54	19.29	18.96	18.27

1Gemini sunflower oil

2 seven sea cods

3 Vitamin and mineral mixture/Kg premix: Vitamin A-7,00,000IU, VitaminD3-70,000IU, Vitamin E-250 mg, Nicotinamide-1000 mg, Cobalt-150mg, Copper-1200mg, Iodine-25g, Iron-1500 mg, Magnesium-6000 mg, Manganese-1500 mg, Potassium-100mg, Sodium- 5.9mg, Sulphur-0.72%, Zinc-9600 mg, Calcium-25.5%, Phosphorus-12.75%.

Feed preparation

Five treatment diets with 40% protein were formulated viz. T1 = control (100% fish meal as the primary protein source; zero% shrimp head meal), T2 = (75% of fish meal and 25% shrimp head meal), T3 = (50% of fish meal and 50% shrimp head meal), T4 = (25% of fish meal and 75% shrimp head meal) and T5 = (0% of fish meal and 100% shrimp head meal). Shrimp head was collected from Star Fish Meal Plant, Veraval, Gujarat, India, it was sun-dried for 3 days. All the ingredients were grinded first then mixed together and made a dove and cooked in an autoclave for 10 minutes separately for each experiment. Then pelleted those into 1 mm sizes and

dried in mechanical drier and then dry feeds were stored in air tight plastic bottles.

Feeding

Feeding was done at the rate of 10% of body weight initially, and after 10 days fishes were fed *ad libitum* till the end of the experiment. The daily ration was divided into three equal parts and was given at 09.00, 13.00 and 18.00 hrs. The experimental tanks were cleaned manually and siphoning was done every day in order to remove excess feed pellets and the remaining faecal matter. An equal volume of clean water replaced the siphoned water.

Collection of blood samples

At the end of the feeding experiment, blood samples of about 1 ml was collected from the caudal peduncle with the aid of a 24 no. niddle, 0.5 ml of blood was dispensed into ethylene diamine tetra-acetic acid (EDTA) anticoagulant for haematological studies, while 0.5 ml transferred into a tube containing lithium heparin anticoagulant to obtain plasma for biochemical analysis and kept at 20˚C until analysis.

Hematological analysis

Haematocrit (HCT) and haemoglobin (Hb) concentration (cyanmethaemoglobin method) were analysed within two hours after collection. Mean corpuscular haemoglobin (MCH), mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) were calculated respectively using following formula [11]. Red blood cells (RBC) and white blood cells (WBC) were counted by Neubauer's improved haemocytometer using Hyem's and Turk's solution.

MCH (pg) = [Haemoglobin (g dl^{-1}/)/RBC x (10^{-6} C mm^{-1})] x 10

MCV (fl) = [Haematocrit (%)/RBC x (10^{-6} C mm^{-1})] x 10

MCHC (g/dl) = [Haemoglobin (g dl^{-1})/Haematocrit (%)] x 100

Total protein (g/dl) = (Absorbance of sample/Absorbance of standard) × 6

Total immunoglobulin = Protein in blood serum – Protein content in the supernatant

Albumin (g/dl) = (Absorbance of sample/Absorbance of standard) × 5

Statistical analysis

One way Analysis of Variance (ANOVA) and least significantly difference (LSD) [12] was applied to test the level of significance amongst the treatments.

Results

Significantly (p<0.05) higher MCV value (144.24±1.21), MCH (59.36±0.89) and MCHC (45.43±1.01) were found in T1 treatment (Table 3). The values of, HCT (31.44±0.80), RBC (2.14±0.10), Hb (9.31±0.10), WBC (68.40±0.10), lymphocytes (92.7±0.61) and basophils (0.25±0.04) were found significantly (p<0.05) higher in diet T3 (Table 3). In case of, monocytes (10.07±0.64), neutrophils (2.98±.05) and eosinophils (0.55±0.03), significantly higher values were found in T1 treatment (Table 3). Biochemical parameters like total plasma protein (4.87±0.06), total immunoglobulin (0.92±0.05), albumin (1.48±0.05), globulin (2.31±0.16) and A/G ratio (0.65±0.04) were found significantly (p<0.05) higher in T3 treatment (Table 4).

The whole body proximate composition of fish is presented in Table 5. Whole body moisture of tilapia increased from 20.57% to 20.93% in T5 diet. No difference in crude protein, lipid, ash and NFE were found among treatments.

Table 3. Haematological parameters of *O. niloticus* fed the test diets for 60 days (Mean ± SE)

Parameters	Treatment				
	T_1 (Control)	**T_2**	**T_3**	**T_4**	**T_5**
RBC (x 106 mm^{-3})	1.76±0.03^{b}	2.09±0.05^{a}	2.14±0.10^{a}	2.05±0.04^{a}	1.79±0.04^{b}
Hb (g dl^{-1})	7.59±0.06^{b}	9.23±0.06^{a}	9.31±0.10^{a}	9.18±0.05^{a}	7.64±0.08^{b}
HCT (%)	20.98±0.81^{c}	29.89±0.97^{a}	31.44±0.80^{a}	28.54±0.86^{b}	22.43±0.90^{c}
MCV (fl)	144.24±1.21^{a}	123.22±0.86^{c}	118.76±1.03^{d}	134.68±0.90^{b}	137.68±1.74^{b}
MCH (pg)	59.36±0.89^{a}	48.42±0.76^{bc}	46.78±0.90^{c}	49.64±1.05^{b}	57.43±0.84^{a}
MCHC (g dl^{-1})	45.43±1.01^{a}	36.65±0.86^{b}	34.84±0.95^{b}	42.86±0.87^{a}	44.38±0.85^{a}
WBC (x10^{3} mm^{-3})	56.80±1.26^{c}	66.90±0.10^{ab}	68.40±0.10^{a}	64.70±1.20^{b}	58.30±1.30^{c}
Eosinophils (% mm^{-3})	0.55±0.03^{a}	0.23±0.03^{c}	0.13±0.03^{c}	0.40±0.04^{b}	0.51±0.03^{a}
Basophils (% mm^{-3})	0.1±0.03^{c}	0.22±0.04^{ab}	0.25±0.04^{a}	0.22±0.03^{a}	0.12±0.03^{bc}
Neutrophils (% mm^{-3})	2.98±.05^{a}	2.12±0.02^{c}	1.98±0.02^{c}	2.37±0.04^{b}	2.82±0.13^{a}
Lymphocytes (% mm^{-3})	86.3±0.75^{c}	91.8±0.47^{ab}	92.7±0.61^{a}	90.60±0.88^{b}	87.2±0.79^{c}
Monocytes (% mm^{-3})	10.07±0.64^{a}	5.63±0.06^{bc}	4.94±0.05^{c}	6.43±0.44^{b}	9.35±0.92^{a}

*Mean±SE within a row followed by with different superscripts are significantly (p<0.05) different from each other

RBC- Red Blood Cells; Hb- Haemoglobin; WBC- White Blood Cells; HCT- Haematocrit; MCV- Mean corpuscular volume; MCH- Mean corpuscular haemoglobin; MCHC- Mean corpuscular haemoglobin concentration

Table 4. Blood serum biochemical parameters of *O. niloticus* fed the test diets for 60 days (Mean ± SE)

Parameters	Treatment				
	T_1 (Control)	**T_2**	**T_3**	**T_4**	**T_5**
Total protein (g dl^{-1})	3.77±0.10^{b}	4.76±0.09^{a}	4.87±0.06^{a}	4.72±0.06^{a}	3.85±0.10^{b}
Total immunoglobulin (g dl^{-1})	0.76±0.02^{b}	0.89±0.04^{a}	0.92±0.05^{a}	0.87±0.04^{a}	0.77±0.01^{b}
Albubin (g dl^{-1})	1.09±0.07^{b}	1.45±0.06^{a}	1.48±0.05^{a}	1.41±0.04^{a}	1.12±0.03^{b}
Globulin (g dl^{-1})	2.11±0.07^{b}	2.30±0.16^{a}	2.31±0.16^{a}	2.28±0.04^{a}	2.14±0.06^{b}
A/G	0.52±0.02^{c}	0.64±0.05^{a}	0.65±0.04^{a}	0.62±0.01^{ab}	0.52±0.02^{bc}

*Mean±SE within a row followed by with different superscripts are significantly (p<0.05) different from each other

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Discussion

No studies have been performed to assess the effect of dietary shrimp head meal on fish haematological values [13]. stated that the most common blood characteristics constantly affected by diet are the RBCs, Hct, TPP, and glucose levels. Previous hematological studies of nutritional effects [14, 15], infectious diseases, and pollutants [16, 17] suggeted that erythrocytes are a major and reliable indicator of several sources of stress. Red blood cells transport Hb that, in turn, transports oxygen. The amount of oxygen received by tissue relies on the maturity of RBCs and the amount of Hb. The higher hemoconcentration observed in the present study for Nile tilapia fed diet T3 containing 50% of shrimp head meal recommends greater hematological function of fish.

Table 5: Proximate analysis of fish carcass (Dry weight basis)

Parameters	T1 (Control)	T2	T3	T4	T5
Crude protein	40.82±0.71	40.73±1.02	40.60±0.53	40.47±1.57	40.35±0.61
Ether extract	14.93±0.72	14.87±0.54	14.73±0.26	14.67±0.76	14.58±0.74
Ash	21.95±1.02	22.17±1.54	22.26±0.63	22.45±0.62	22.56±1.24
Moisture	20.57±0.94	20.61±0.73	20.74±0.97	20.85±0.34	20.93±0.04
Nitrogen free extract	1.73±0.03	1.62±0.04	1.67±0.03	1.56±0.03	1.58±0.04

*Mean±SE within a row followed by with different superscripts are significantly (p<0.05) different from each other

White blood cell, haemoglobin and haematocrit raised as dietary shrimp head meal levels increased from 0% to 50%, and then reduced when the dietary shrimp head meal increased from 50% to 100%. The values of RBC, HCT and Hb are within the range reported for normal, healthy juvenile channel catfish [18, 19]. Moreover, studies will be led to attain some data between the relationship of haematological indices and fish health.

All fish fed shrimp head meal supplemented diets had significantly (p<0.05) higher levels of total plasma protein (TPP), total plasma albumin (TPA), total plasma globulin (TPG) and total immunoglobulin in the diet T3 compared to fish in the control diet [20]. stated that the increase in serum protein would result when anabolic processes surpassed catabolic ones, and reserved protein is being produced in greater magnitude to meet increased metabolic requirements of the fish. They contributed that an increased catabolic rate would describe the decreases in serum protein level. Furthermore, the cyclic nature of the total serum protein is a sign of the changes happening in the serum globulin fraction. However [21], reported that most techniques employed to examine the immune state of an animal are those that depend on finding and measurement of antibody in blood serum and other body fluids. Globin is the source of antibody in blood serum. The total serum globulin level possibly reveals the level of specific immunoglobulin (antibody) [22].

Generally, nutrients are deposited in fish body at a rate proportional to their levels in diets [23]. Carcass protein contents were higher than the initial; revealing of the fact that experimental treatments favored body protein deposition as much as the control, and confirmed an acceptable protein digestibility in the dietary treatments [24].

Conclusion

The present study showed that significant (p<0.05) difference was observed in the haematological and biochemical parameters for tilapia fed shrimp head meal but it has no harmful impact on the health profile of fish. Therefore, direct use of shrimp head meal as sole supplementary feed should be encouraged. The findings of this research advise that shrimp head meal has no adversarial effects on haematological and biochemical profile on tilapia and consistently cut down a large proportion of the feed production cost.

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