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Animal Blood supplemented diet can improve growth performance, body composition and blood profile of Genetically Improved Farm Tilapia (*Oreochromis niloticus*)

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Abstract

Background: Artificial feeding is an effective way to enhance fish production, development and carrying capacity of the culture system to feed the increasing human population. This study was designed to determine and compare the effects of supplementation of basal fish feed with plant (soybean meal) and animal blood as protein sources.

Methods: The experiment was conducted using a completely randomized block design. A total of 135 Genetically Improved Farm Tilapia were randomly divided into three groups comprising three replicates and kept in controlled conditions in nine glass aquaria for a period of ninety days. The animals were provided basal diet, plant and animal protein supplemented diets throughout the experiment. Water quality parameters were recorded on a routine basis while growth performance, blood indices and chemical analysis of the body was recorded after ninety days of trial.

Results: Overall, water quality parameters remained within the normal range, which highlights those diets had no detrimental effect on the quality of the water and in all groups. However, the fish kept on animal-based protein source diet presented higher growth performance, crude protein and lipids contents, red blood cell count and normal serum ALT, AST, and ALP levels In contrast the fish kept on plant protein diet displayed comparatively lower meat quality and signs of toxicity viz., raised level of hepatic enzymes.

Conclusion: From these results, it was concluded that fish fed on diet having blood meal supplementation showed higher performance in comparison to fish groups fed on other diets.

Introduction

Aquaculture sector is expanding its overall fish output in order to meet the globally increasing demand of fish [1]. Genetically Improved Farm Tilapia are being developed in the aquaculture sector because of enhanced immune responses, disease resistance and high growth rate [2]. The success of GIFT has been remarkably noticed in developing countries due to improved food and livelihood status of people [3].

In the aqua feed industry, expensive fishmeal is widely used as the main animal protein source due to presence of balanced essential amino acids such as lysine, which is typically deficient in the grain products. Various feed supplements has health risks to people [4]. However, it is not cost-effective and results in high and unaffordable cost of end product. Because of the high price of fish meal and gradual reduction in supply, researchers are trying to replace it partially or entirely with cheap plant or animal proteins, without influencing the growth performance of cultured species. The comparatively cheaper plant proteins are widely available and are being utilized in aquaculture as an alternative of the fish meal, especially in the developing countries [5]. But plant protein diets have also been recognized to lack essential amino acids and anti-nutritional properties [6]. Similarly, the use of plant protein sources has been reported to induce remarkable changes in the intestinal microbiome which usually result in low growth performance. Consequently, new animal protein sources need to be explored in order to assure the sector's future growth [7]. In fish feed chicken viscera and animal blood can be used. Only few studies are available on animal blood as protein source. In Pakistan, animal blood from slaughterhouse could be utilized as a cheap protein source that would become cost effective for culturing fish if it results in improved growth performance. The growth indices, meat quality, hematological features, fatty acid profile. The level of oxidative stress biomarkers *viz.*, catalase and superoxide dismutase have also been explored as key indicators of fish health [8]. Current study is therefore planned to determine and compare the effects of different diets supplemented by animal and plant proteins on growth performance and fish health as well as meat quality.

Methods

Study site and work design: The current work was accomplished at the Fish Seed Hatchery, Faisalabad, Pakistan, and three-month feeding trial was conducted in glass aquaria for the period of 90 days. GIFT fingerlings were obtained from fish seed hatchery Faisalabad that were hatched from selectively breed.

Experimental design: A total of 135 apparently healthy Genetically Improved Farmed Tilapia (GIFT) fingerlings of mean initial length 8 cm and weight 30 g were acclimatized for 15 days in rectangular glass aquaria measuring (40 × 30 × 40 cm, 96 L capacity containing 90 L of water) with the experimental conditions. Physicochemical parameters (pH, Temperature, DO, Hardness, Alkalinity etc.) of water were monitors in optimum range throughout the experimental duration.

Diets preparation and feeding regimes: The commercially available feed consisting of rice bran, wheat bran, grains, cereal products, vitamins, and trace minerals was used as a basal diet in the present study. Whereas blood was collected from the main slaughterhouse in Faisalabad for diet preparation. The experimental diets were formulated by mixing fish meal with blood meal and soya bean meal as animal and plant protein, respectively. Feed pellets were packed and stored at 4 °C in clean-dry plastic containers until use. The chemical composition of the diet was tested by using standard methods [9] before the experimental diet formulation. All ingredients were ground into a fine powder and then mixed well in a twin shaft paddle mixer until a solid dough was obtained. Subsequently, the dough was passed through a lab extruder (SYSLG30-IV Experimental Extruder) along with the addition of water [10]. The diet was dried for 2 h in a ventilated oven at 60 °C and mixed with oil slowly. Diet ingredients and proximate composition are shown in Table I. *Fish grouping:* Fingerlings were randomly allocated into three groups and kept in 9 aquariums. The first group (control group) was fed on a basal diet with 35% crude protein. The second group (plant protein) was fed on a diet mixed with 35% plant protein. The third group was (animal protein) fed a diet supplemented with 35% animal protein over a period of 90 days.

Fish and tissues sampling: Fish and tissue sampling was performed at the end of the experiment (3-4 from each). Randomly selected fish were euthanized using 0.02% benzocaine [11] and their body length-weight was taken in centimeters and grams respectively. Fish dorsal muscles were frozen in liquid nitrogen and the samples were later on stored at -20°C until evaluated for their proximate composition.

Study of Growth: By using a standard formula, the growth parameters such that, specific growth rate (SGR), weight gain (g) and weight gain percentage of fish were evaluated.

$$\text{Weight gain \%} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{SGR\%} = \frac{\text{ln. final wt. of fish} - \text{ln. initial wt. of fish}}{\text{Test day}} \times 100$$

Hematological analysis: Fish were starved for 24 h before blood sampling at the end of the experimental period. Fish from each group were randomly selected and blood samples were collected, under anesthesia, from the caudal vein using buffered tricaine methane sulphonate (MS-222) (100 µg/mL). A blood sample was divided into two parts to determine hematological and biochemical indices along with antioxidant activities. The first was transferred into a sterile tube containing 0.5% ethylene diamine tetra acetate (EDTA) (EDT001, Bioshop, Canada) and used to estimate white blood cells (WBCs) according to the method of [12] respectively. Furthermore, red blood cells (RBCs) were determined using the standardized cyanomethemoglobin procedure. The other portion of the blood sample was transferred into a sterile centrifuge tube, clotted, and centrifuged at 4000 rpm for 15 min at 4 °C for serum separation. The separated serum was stored at −20 °C until required for estimation of biochemical and antioxidant activities.

Statistical analysis: Differences between treatment groups were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were considered significant at $p < 0.05$. All results are expressed as the mean± standard error. All statistical analyses were performed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA).

Ingredient	Group C (Control)	Group P (Plant Protein)	Group A (Animal Protein)
Fish meal	40	0	0
Blood meal	-	-	35
Wheat brawn	15	12	-
Soybean meal	10	20	5
Corn Gluten-60	a -	25	5
Wheat flour	4	8	13
Rice brawn	8	12	19
Fish oil	10	5	10
Soybean oil	-	5	-
Vitamin premix	5	5	5
Mineral premix	5	5	5
Binder	3	3	3
Proximate Composition			
Moisture	13.5	11.6	8.5
Crude protein	32	33	32
Crude lipid	10	14	11.6
Crude fiber	17.5	18.5	7.5
Ash	12	10	15
NFE4	8.5	7.5	10

Table I: Ingredients and proximate composition (%) of experimental diets

Results

The water pH and temperature, ranged from 6.90 to 9.00 and 25.0 °C to 32.0 °C respectively. Dissolved oxygen, total hardness and alkalinity ranged from 4.0 to 8.0 mg/L, 980 to 1930 mg/L, and 200 to 290 mg/L, respectively. The total dissolved solvents (TDS) and salinity concentrations ranged from 4 to 8.5 mg/L, and 1.00-3.00 ppt, respectively as given by [13]. The conductivity of water varied between 32.00 µS/cm and 89.00 µS/cm in different aquariums.

Growth parameters and somatic indices: The results of growth performance of GIFT fed on different diets are presented in Table III. There was no significant differences in the mean initial body weight (32.89 g) of GIFT among all experimental groups. The final body weight, weight gain, average body weight and specific growth rate (SGR) were significantly varied in fish groups fed on animal protein and plant proteins supplemented diets in comparison with the basal diet throughout the study period. The final body weight, weight gain and specific growth rate (SGR) were 52. 62 ± 0.74 g, 19.84 ± 1.56 g, 62.67 ± 7.06 %, 0.22 ±0.02 g and 0.53±0.05 (% g/day) respectively in group I, 70.36 ± 0.93 g, 37.58±1.95 g, 118.25±10.97 %, 0.42±0.02 g and 0.86 ± 0.06 (% g/day), respectively in group II. In case of animal diet group III, these parameters were found with values of 97.59±1.36 g, 64.48±1.22 g, 197.82±10.69 %, 0.72±0.01 g and 1.21±0.04 (% g/day), respectively. The highest values for growth performance indices were recorded in the animal supplemented group ($p<0.05$). There was no significant increase in total length and but significant in standard length of GIFT among all feeding groups on 90th day of sampling.

The proximate composition of GIFT fed on basal diet and experimental diets are illustrated in Table VI. After three months of feeding trial, crude protein, lipids, moisture, total ash and crude fibers were recorded 11.13±0.39, 1.30±0.09, 74.83±0.19, 0.76±0.05 and 0.66±0.04, respectively in group I. The crude protein (40.90±0.25) and lipid (2.57±0.07) contents of fish fed animal protein containing diet were significantly higher ($p= 0.000$) compared to 32.36±1.40 and 1.91±0.04 values of fish fed on plant protein containing diet. The moisture content showed no significant differences among all groups. Total ash (1.77±0.05) and crude fiber (1.11±0.04) contents were significantly increased in plant protein-based diet group compared to the control group ($p=0.000$), while these contents (ash=1.47±0.01, crude fiber=0.92±0.01) were lower in animal protein diet group when compared to fish fed on plant protein containing diet ($p = 0.000$). The quality of crude protein, lipid, total ash and fiber varied among fish kept on different diets. The amount of crude protein and lipids was highest in fish kept on animal protein diet followed by plant protein diet and basal diet. On the other hand, crude fiber and total ash content were highest in fish kept on plant protein followed by plant protein.

Hematology and serum biochemical profile including blood protein profile, liver function enzymes and

Parameter	Groups			Optimum value for GIFT culture
	G-I	G-II	G-III	
pH	8.56±0.06 (7.90-8.90)	8.29±0.12 (7.00-9.00)	7.84±0.11 (6.90-8.90)	3.70-11.00
Temperature (°C)	29.78±0.28 (27.00-32.00)	30.44±0.31 (27.00-32.00)	28.61±0.33 (25.00-32.00)	25.00-32.00
Dissolved oxygen (mg/L)	5.78±0.17 (4.50-7.00)	6.03±0.18 (4.50-8.00)	5.67±0.19 (4.00-7.00)	2.00-11.00
Hardness (mg/L)	1151.44±0.66 (1005-1240)	1224.41±0.86 (980-1930)	1452.07±2.01 (980-1900)	2800-3000
Alkalinity (mg/L)	236.37±4.57 (210-290)	240.15±4.91 (210-290)	228.7±4.07 (200-290)	20-200
Salinity (ppt)	1.59±0.06 (1.00-1.90)	1.61±0.06 (1.00-1.90)	1.71±0.07 (1.00-3.00)	0-19
Conductivity (µS/cm)	59.44±0.96 (52.00-68.00)	74.82±1.58 (58.00-89.00)	55.28±3.03 (32.00-76.00)	35–87
Total dissolved solvents (TDS, mg/L)	1.66±0.04 (1.37-1.89)	1.75±0.02 (1.47-1.89)	1.32±0.02 (1.20-1.56)	1-3

Data are presented as mean± standard error, minimum and maximum values of different water quality parameters in the fish aquariums. Optimum values as given by Meade (1989) and Alam *et al.* (2014).

Table II: Physio-chemical characteristics in aquariums with normal range

Growth parameter	Groups			*p*<0.05
	G-I	G-II	G-III	
Initial weight (g)	32.78±1.31^a	32.78±1.39^a	33.11±1.30^a	0.979
Final weight (g)	52.62±0.74^a	70.36±0.93^b	97.59±1.36^c	0.000
Weight gain (g)	19.84±1.56^a	37.58±1.95^b	64.48±1.22^c	0.000
WG* (%)	92.67±7.06^a	118.25±10.97^b	197.82±10.69^c	0.000
Average daily weight gain	0.22±0.02^a	0.42±0.02^b	0.72±0.01^c	0.000
SGR# (% g/day)	0.53±0.05^a	0.86±0.06^b	1.21±0.04^c	0.000
Somatic indices				
Total length (cm)	14.00±0.33^a	14.67±0.17^a	15.78±0.32^a	0.135
Standard length (cm)	10.67±0.29^a	11.89±0.20^b	13.94±0.23^c	0.000
Condition factor (g/cm^3)	1.97±0.15^a	2.25±0.09^a	3.08±0.18^b	0.000

*Weight gain, #Specific growth rate. Data are presented as the mean ± standard error. The values within the same row having different superscripts are significantly different at $p < 0$.

Table III: Growth performance of GIFT fed on animal and plant supplemented diets with reference to control for 90 days

Parameter (%)	Groups			*p*<0.05
	GC	GP	GA	
Crude protein	11.13±0.39^a	32.36±1.40^b	40.90±0.25^c	0.000
Crude lipids	1.30±0.09^a	1.91±0.04^b	2.57±0.07^c	0.000
Moisture	74.83±0.19^a	75.74±1.04^a	74.71±0.17^a	0.452
Total ash	0.76±0.05^a	1.77±0.05^c	1.47±0.01^b	0.000
Crude fiber	0.66±0.04^a	1.11±0.04^c	0.92±0.01^b	0.000

Data are presented as mean ± standard error. Mean values (n = 3) in the same row with different superscripts differ significantly ($p < 0.05$).

Table IV: Whole body proximate composition (on % wet weight basis) of GIFT fingerlings reared in aquarium and fed animal and plant protein-based diets for the period of 90 days

Parameter	Groups			*p*<0.05
	GI	GII	GIII	
Liver function enzymes				
ALT (IU)	316.33±2.65^a	899.33±7.44^c	399.67±4.51^b	0.000
AST (IU)	180.89±2.87^a	745.89±4.24^c	242.11±2.53^b	0.000
ALP (IU)	2.00±0.16^a	3.02±0.09^b	1.89±0.07^a	0.000
Blood protein profile				
Albumin (g/dL)	1.09±0.07^a	1.68±0.08^b	1.51±0.14^b	0.001
Globulin(g/dL)	1.93±0.07^a	2.71±0.06^b	2.71±0.10^b	0.000
Hematology				
RBC (10^6 cell/mm^3)	1.24±0.04^a	1.32±0.01^a	1.50±0.02^b	0.000
WBC (10^6 cell/mm^3)	116.67±1.40^a	116.69±0.49^a	117.36±0.84^a	0.855

Data are presented as mean ± standard error. Mean values (n = 3) in the same row with different superscripts differ significantly ($p < 0.005$).

Table V: Hematological and biochemical parameters of GIFT

immunity of GIFT fed on experimental diets in comparison with the control group are presented in Table V. The serum ALT, AST and ALP activities were found increased in diet supplemented with plant protein. These levels were higher than those in fish fed basal and animal protein supplemented diets. There was a significant elevation of serum albumin and globulin levels in experimental diets when compared with the basal diet

group. Meanwhile, no significant differences were observed in WBCs among all experimental groups when compared with the control. The RBC count was significantly higher ($p < 0.05$) in the diet supplemented with animal protein than the other groups.

Discussion

The aqua feed normally contains 25 to 50% crude protein content, which is principal and expensive ingredient in feeds and is essential for growth and maintenance of animal health [14]. Fishmeal is the most common protein source used in aquaculture feeds which account for 68% of global fishmeal consumption. It contains high levels of nucleotides, minerals, vitamins, fatty acids, balanced amino acid profile, high protein palatability and digestibility [15]. The shortage in world production of fishmeal makes it necessary to search for high- protein feed resources.

Micronutrients, which include minerals and vitamins, are required in lower quantity and play essential role in cellular functions. The dietary requirements in terms of composition and quantity of different nutrients can vary considerably among and within species. One of the most important external factors that influence fish feeding behavior and growth is food. These mechanisms are influenced by both food availability and dietary composition. Growth of animal is a polygenic and environmentally (e.g., nutrition) controlled phenotypic expression of muscle hyperplasia [16].

The obtained results clearly revealed that replacing up to 35 % of the fishmeal with animal protein enhance growth parameters including final weight final body weight, weight gain, and average body weight of GIFT when compared to those of control group. Increased growth performance can be attributed to high protein and essential amino acid profiles in blood meal that makes it perfect replacement of fishmeal in fish diet [17]. Blood meal can totally substitute fishmeal with no deleterious effects on growth, survival and feed conversion of Nile tilapia and catfish. The nutrient digestibility values of fishmeal and blood meal are significantly similar for most tilapia species [18] implying that blood meal can be used as a fishmeal substitute in the diet. The fish fed 35% experimental diet of fermented blood meal and unfermented blood meal exhibited significantly higher growth performance than fish fed a control diet of 100% fishmeal [19]. In the current study, although 35% plant protein inclusion level improved the growth performance as compared to control diets. However, growth rate was lower than those fed on animal protein diet. The results suggest that protein and energy digestibility for plant protein is apparently lower than that of animal protein for fish. Possible reasons for the reduced feed utilization and growth parameters recorded for plant protein-based diet may be related to the presence of high-crude fiber, anti-nutritional factors, and poor palatability.

Measures of size and proximate composition of juvenile hatchery-reared fish are necessary to determine if different diets lead to changes in growth or nutrient provisioning, and ultimately survival. The effect of diet on proximate composition in hatchery-reared fish has been determined in a variety of species and it remains one of the most simple and effective means of evaluating dietary quality [20]. In this study, fish fed animal and plant protein-based diets had significantly higher whole-body crude protein and crude lipid content than the control group. However, fish fed the animal protein containing diet had highest whole-body moisture, protein, and lipid contents than those fed the plant supplemented diet. These results are consistent with [21], they found that a high plant protein-based diet reduces protein utilization, carcass protein content, protein retention, and growth.

The hemato-biochemical analysis is an important parameter to determine physiological performance of animal since it can reveal information about hematopoiesis, liver, kidney, and immune system. As a result, hemato-biochemical indices are valuable to detect metabolic disorders, alterations of the functions of various organs and health monitoring [22]. Liver has a complicated structure and plays an important role in the preservation, implementation, and regulation of the body homeostasis [23]. High levels of AST (aspartate aminotransferase) and ALT (alanine aminotransferase) are the crucial markers to detect liver injury. Similarly, serum level of alkaline phosphatase (ALP) is also associated with status and function of hepatic cells since synthesis of this enzyme increases due to rising biliary pressure [24]. In this study, ALT and AST levels were recorded to be higher with inclusion of animal protein in diet. However, ALP levels did not differ significantly amongst the experimental diets. In a similar study, a positive linear relationship was found between incremental amount of animal protein (housefly maggot meal) and serum AST and ALP levels [25]. Fish fed a dietary inclusion of plant protein had higher AST and ALT activity than fish fed a control diet, which is consistent with a study in which significantly higher ALT and AST levels in serum were found in juvenile seabass fed diets supplemented with commercial soya bean (above 30%) as plant protein source The findings indicate that liver of GIFT fish may have been damaged to some extent, which in turn affected the protein metabolism.

Since the liver synthesizes serum proteins, including albumin and a small fraction of globulin, these proteins suffer both quantitative and qualitative alterations in any hepatic disease. The concentration of serum albumin and globulin falls in any disease that causes

hepatocellular injury. Serum albumin and globulin levels change over time, providing useful indicators of hepatic disease severity, progression, and prognosis. GIFT fed on animal protein supplemented diet had considerably higher serum albumin and globulin protein levels compared to fish fed on plant protein supplemented and control diets. These findings suggest that a diet supplemented with animal protein improves fish health. The differential analysis of WBC counts is valuable tool in measuring physiological status and helpful in the evaluation of immunity status of fish [26]. Blood profile is generally linked with physical condition as its values are excellent signals of dietary condition [27]. In the present study, mean RBCs count of GIFT significantly increased with inclusion of 35 % animal protein-based diet compared to the fish fed the control diet. In another study, [28] evaluated the effects of replacing fishmeal with animal protein source on hematological and biochemical parameters in common carp (*C. Carpio)* fingerlings. The authors found that fish fed experimental diets for 14 weeks have improvement in hematological parameters (RBC, WBC, Hb and HCT) and plasma proteins (albumin and globulin) in comparison with control group. In a similar study the effects of replacing fishmeal with different percentages of plant protein (dietary fermented soy pulp) to assess growth performance, hematology, and blood biochemical of African catfish and found the significant increase in RBC and lymphocytosis in fish fed the protein diet. The albumin and globulin were significantly higher ($p < 0.05$) in the experimental diets compared with the control diet [29].

GIFT has the capacity to survive a wide range of adverse climatic conditions. It could be one of the most favorable specie for production in Pakistan. The present study concluded that dietary supplementation with animal protein in the form of cattle blood had positively influence the growth performance, proximate composition, and hematological parameters of GIFT. Expensive fish meal may be replaced with cheap animal blood from slaughterhouse as animal protein source in aqua feed for optimal culturing of tilapia fish.

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Competing Interest

The authors have declared no conflict of interests.

Author Contributions

Hafiza Samra Ambreen conducted trial for research, collected data and prepared manuscript

Najma Arshad assisted in idea conception, Supervised, revision of manuscript critically for important intellectual content.

Muhammad Mudassar Shahzad design experiment, supervised throughout study trial and help in critical review of manuscript preparing

Ghulam Ayesha Javed contributed to analysis, interpretation of data and helped in manuscript preparing

Kiran Shahzadi made contributions in proof reading and review.

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