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IMPACT OF GRADED DIETARY PROTEIN ON HAEMATOOGICAL PARAMETERS OF GENETICALLY IMPROVED FARMED TILAPIA (GIFT)

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ABSTRACT

The objective of the present study was to evaluate the impact of graded levels of plant protein diets (15%, 20% and 25%) on haematological profile of mono sex (male) Genetically Improved Farmed Tilapia (GIFT), a developed strain of Nile tilapia (*Oreochromis niloticus*). Three treatment groups (T1=15%, T2=20% and T3=25%) were formed and experiment was carried out in duplicate. Five days old GIFT tilapia fingerlings of mean weight 0.24±0.03 (g) and length 2.01±0.27 (cm) were collected, acclimatized for two weeks, and transferred to hapas (8x6x3 ft) at random. Fingerlings were fed @ 10 % during acclimatization period and 5% up to the end of experiment. At the end of 90 days feeding trial, ten fishes were randomly selected from each treatment hapa for the estimation of haematology. Haematological parameters viz. WBCs, LYM, MON, GRA, RBCs, HGB, HCT, MCV, MCH, MCHC, PLT, MPV were studied. The RBCs values in T1, T2 and T3 were 0.93±0.30, 1.40±0.48 and 1.47±0.16 (10^6/μL) respectively. The WBCs values in T1, T2 and T3 were calculated as 55.51±10.25, 59.29±12.86 and 104.32±18.82 (10^3/μl) respectively. In T1, T2 and T3 the platelets count was estimated as 196.04±80.31, 225.87±59.32 and 290.00±186.00 respectively. Statistical relationship was studied applying linear regression analysis.RBCs, WBCs exhibited non-significant correlation against total length and weight in all groups.While, platelet count yielded non-significant correlation in T1, T2 and T3 with weight and length except for T2 which was found least significant against length. T3 fish fed at 25% CP exhibited more WBCs, RBCs and their indices with in the range as compared to T1 and T2 fish. Thus, protein diet (25%) is cost effective as well as keep the fish in good health condition.

Keywords: Haematological profile, GIFT, Fingerling, Hapas, Graded levels of protein, Fish size

INTRODUCTION

Fish nutrition and feeding is one of the major requirements for sustainable aquaculture production. In fish culture, the most expensive component is feed. It represents about 30% to 60% of the total variable cost [1]. Traditionally, in aquaculture, the major source of protein used in formulated feeds is fish meal because of its good quality protein, high protein contents, palatability and balanced amino acid profile [2,3,4]. However, fish meal supplies and prices have witnessed significant fluctuations [2]. The health status of living organisms of animal origin can be revealed by blood parameters. While, the level of infection and immune response status is a reflection of leukocytes count. [5]. The nutrients intake through feed represents the development of hematocrit component. So, it is of prime importance to maintain a balance, in the optimum quantities, of different nutrients in formulating quality feed. For blood cell formation, requirement of protein is vital and its supply is dependant on the optimum combination of amino acids [4]. Utilization of new feed combinations to provide essential nutrients may serve as positive health indicator and reduction in utilization of fish meal percentage in feeds [6].

The haematological characteristics play an integral part in the evaluation of fish health status [7]. The main factors responsible for alteration in haematological profile of fish include sex, species, stress, diet composition, metabolic adaptation and variation in fish physiology [2, 8, 9]. Haematological analysis is considered a valuable tool for monitoring physiological responses, assessment of diet composition, nutritional status and fish health [10].

Blood, constituting the most abundant body fluid, represents physiological condition of an organism including fish. Valuable information can be obtained by the analysis of blood cells regarding fish diseases [11]. The blood of fish is similar with other vertebrates as it consists of plasma comprising of 97% water, electrolytes, minerals, hormones and cellular constituents (WBCs, RBCs) and platelets [3].

According to [12, 13] analysis of haematological and biochemical indices act as useful tools in the assessment of performance, viability and health status of farmed fish and animals. Analysisof haematological profile provides reflection of physiological responsiveness of animals to their internal and external environments. So, external environmental fluctuations may lead to blood dysfunction and severely influence the physiological activities like metabolism, resistance to disease and breeding performance [14].

Some studies have investigated the influence of variations in quality and composition of feedon the blood parameters indices of fish [6, 7, 15, 16, 17]. A wide variety of nutritional and antinutritional factors in the feed ingredients could be a cause of haematological profile variations [18]. These parameters are also closely related to the environmental and biological factors [19]. The good health status of fish is the main element for theirwelfare thus it is of great importance [20]. Therefore, the objective of the present study was to evaluate the impact of plant protein diets of three feeding regimes (15%, 20% and 25% crude protein) as well as body size of treated fish on the haematological profile of Genetically Improved Farmed Tilapia.

MATERIALS AND METHODS

Feed formulation

Feed formulation of three experimental diets incorporated with graded levels of crude protein (15%, 20% and 25%) using locally available cheaper feed contents i.e. sunflower meal, canola meal, sarson meal, soya bean meal, wheat bran, rice polish was carried out in Fish Feed Laboratory, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan.

Experiment site

Five days old fingerlings of monosex (male) Genetically Improved Farmed Tilapia (GIFT), a strain of Nile tilapia (*Oreochromis niloticus*), to evaluate the influence of graded levels of protein diets haematological profile, were collected from the Fish Farm Facilities, Tawakkal Tilapia Hatchery, Tawakkal Nagar (30º 11' 27" N, 71º 15' 4" E), 18 Km Jhang Road, Muzaffar Garh, Punjab, Pakistan. Fingerlings were acclimatized for two weeks andfish were fed @10% body weight. While, through out the 90 days feeding trial, fishwere fed @ 5% body weight. Experiment was carried out in hapas (8x6x3ft) in duplicate from June to August 2017. Fish were fed twice daily between 8:00 – 9:00 am and 7:00 – 8:00 pm.

Sampling

At the end of 90 days feeding trial, ten fish from each treatment hapa (T1, T2, T3) were selected randomly. All fish samples were anesthetized using MS-222 (buffered solution; 30mg/L) in order to bleed the fish samples alive. Prior to blood sampling, wet body weight (W) and total length (TL) were also recorded. Blood samples were collected directly by puncturing fish heart using 1 ml hypodermic syringe (21 Gauge). EDTA (ethylene diamine tetra acetic acid, an anticoagulant) vials were used for collection of blood.

Determination of haematological profile

Blood samples were rapidly subjected to haematological laboratory for the determination of haematological analysis. Blood cell and their various indices were estimated in all the treatment groups. Indices of RBCs includes haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. The types of WBCs (White blood cells) comprise of LYM (Lymphocytes), GRA (Granulocytes) and MON (Monocytes), platelets (PLT) and mean platelets volume (MPV) were also estimated in all the treated groups using Mythic 18 automatic heamatology analyzer, Orphee, Switzerland.

Data analysis

Descriptive statistics for each haematological parameter was expressed as Mean ±Standard Deviation. All the results, based on randomized design, were subjected to linear regression, to calculate the relationship of total length (TL) versus blood parameters and wet body weight (W) versus blood parameters using MS Excel. [6].

RESULTS

Means, Standard deviations and ranges of the RBCs, WBCs and platelet count and their indices in studied GIFT under the influence of graded levels of protein in T1, T2 and T3 fed at 15%, 20% and 25% CP respectively are summarized in Table 1. The RBCs values in T1, T2 and T3 were 0.93±0.30, 1.40±0.48 and 1.47±0.16 (106/µL) respectively. The order of RBCs count was T3 > T2 > T1. The WBCs values in T1, T2 and T3 were calculated as 55.51±10.25, 59.29±12.86 and 104.32±18.82 (10^3/µl) respectively. In T1, T2 and T3, the platelets count was 196.04±80.31, 225.87±59.32 & 290.00±186.00 respectively. The observation order for platelets count was found as T3 > T 2 > T1.

Relationship between haematological parameters and body size

Linear regression was applied to sudy the correlation ofhaematological parameters with total length (TL, cm) and wet weight (W, g) with respect to three different feeds of graded protein levels (15%, 20% and 25%) and results are presented in Table 2 and 3 respectively.

Red blood cells (RBCs, 10^3/µL) on plotting against TL (cm) and W (g) exhibited non-significant correlation in all the three groups (T1, T2 and T3). A highly significant correlation (r=0.881) between HGB (g/dl) and TL (cm) was observed in T3 ($p<0.001$). While, T1 and T2 showed non- significant values for HGB. Similarly, HGB exhibited least significant relationship in T3 (r=0.687, $P<0.05$) against wet body weight (Table 3), while non-significant relations were observed in T1 and T2 (Table 2 and 3). MCV relationship denoted non-significant values in T1, T2 and T3 with TL (cm) and W (g) (Table2 and 3). MCH expressed least significant correlation in T1($r=0.696$, $P<0.05$) when analyzed against both total length (Table 2) and Wet body weight ($r=0.668$, $P<0.05$) (Table 6.3). However, non-significant results were observed in both T2 and T3. MCHC values expressed non-significant relationships in T1, T2 and T3 with total length and wet body weight (Table 2 and 3).

A non-significant relation was found between white blood cells (WBCs, $10^3/\mu L$) and total length (TL, cm) as well as with wet body weight in all the treated groups T1, T2 and T3 (Table 2 and 3). Study of WBCs indices i.e. lymphocytes, monocytes and granulocytes indicated non-significant relationship in T1, T2 and T3 when analyzed against both total length (cm) and wet body weight (g).

Regression between platelets and TL provided least significant results in T2 ($r=0.703$, $P<0.05$) and non-significant in T1 and T3. Platelets correlation with wet weight exhibited non-significant relationship in T1, T2 and T3. A non-significant correlation ($P>0.05$) was exhibited by MPV with both TL (cm) and W (g) in T1 and T3 while least significant correlation was examined in T2 with total length (TL) but non-significant with wet weight (Table 2 and 3).

Table 1: The haematological parameters of Giftreared in hapas at graded levels of protein

Hematological Variables	Diet Variables					
	T1		T2		T3	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
WBC ($10^3/\mu L$)	55.51±10.25	42.80-72.00	59.29±12.86	42.50-99.40	104.32±18.82	84.50-136.30
LYM ($10^3/\mu L$)	61.99±18.84	41.70-98.40	69.54±18.06	46.30-95.20	85.23±11.14	70.40-97.30
MON ($10^3/\mu L$)	1.36±0.48	41.70-98.40	1.92±0.51	1.50-2.90	1.57±0.74	0.80-2.80
GRA ($10^3/\mu L$)	7.70±6.76	1.50-23.00	4.41±1.29	1.70-34.20	12.70±10.22	1.70-34.20
LYM (%)	89.38±6.15	78.00-97.40	84.28±6.92	70.60-91.90	89.68±4.35	82.30-96.70
MON (%)	1.99±1.02	0.70-3.80	2.76±1.04	1.00-4.30	1.82±0.84	0.80-3.40
GRA (%)	8.63±5.28	1.90-18.20	13.08±6.18	6.20-25.10	8.22±3.96	2.50-14.30
RBC ($10^6/\mu L$)	0.93±0.30	0.53-1.53	1.40±0.48	0.77-1.99	1.47±0.16	1.22-1.68
HGB (g/dl)	4.42±0.99	2.70-6.50	5.10±8.33	2.60-7.90	6.16±0.66	5.70-7.90
HCT (%)	11.65±8.71	3.20-27.80	15.94±8.33	7.50-29.80	24.07±3.93	16.80-30.40
MCV(μm^3)	150.01±30.36	97.40-181.70	140.75±46.73	72.30-87.60	119.20±61.14	48.50-206.50
MCH (pg)	40.87±3.41	34.90-47.90	59.29±12.86	42.50-83.00	39.99±3.02	35.70-45.00
MCHC (g/dl)	63.41±39.44	21.10-128.10	28.86±6.83	22.40-40.10	27.01±9.48	13.70-42.30
PLT ($10^3/\mu L$)	196.04±80.31	100.00-357.00	225.87±59.32	162.70-325.00	290.00±186.00	107.00-653.00
MPV (μm^3)	5.71±0.43	5.00-6.40	6.17±0.79	5.20-7.40	5.85±0.53	5.10-6.80

SD=Standard deviation

Table: 2 Statistical analysis of various haematological parameters in relation to total length (cm) of GIFT reared at graded levels of protein in hapas

Equation	Diet Variables	Relationship Parameters		95% CI of a	95% CI of b	Standard error	r	r^2
		A	B					
PLT=a+b TL	T1(15%)	307.5271	-9.69	21.298- 593.756	-34.048-14.659	10.561	0.309ns	0.095
	T2(20%)	34.646	15.25	-126.557-195.849	2.660-27.837	5.459	0.703*	0.494
	T3(25%)	10.7563	23.51	-869.599-891.111	-49.666-96.677	31.731	0.253ns	0.064
WBCs=a+b TL	T1(15%)	0.5212	3.16	-18.195-59.237	-0.135-6.453	1.428	0.616ns	0.379
	T2(20%)	136.3074	-2.55	69.528-203.086	-7.766-2.664	2.261	0.370ns	0.137
	T3(25%)	117.4293	-4.18	51.434-183.425	-9.669-1.302	2.379	0.528ns	0.279
RBCs=a+b TL	T1(15%)	0.7255	0.03	-1.011-1.011	-0.119-0.176	0.0641	0.155ns	0.024
	T2(20%)	1.4903	-0.002	0.864-2.116	-0.051-0.047	0.021	0.032ns	0.001
	T3(25%)	1.5997	-0.06	0.242-2.958	-0.169-0.056	0.049	0.379ns	0.143
HGB=a+b TL	T1(15%)	1.3976	0.32	-5.209-8.004	-0.240-0.884	0.244	0.423ns	0.143
	T2(20%)	5.4263	-0.08	0.634-10.218	-0.483-0.314	0.173	0.171ns	0.029
	T3(25%)	3.4921	0.21	2.298-4.687	0.119-0.306	0.040	0.881***	0.776
LYM=a+b TL	T1(15%)	44.1734	1.55	-23.449-111.796	-4.204-7.303	2.495	0.214ns	0.046
	T2(20%)	102.7339	-1.39	-1.396-142.724	-4.519-1.727	1.354	0.342ns	0.117
	T3(25%)	130.48	-5.13	57.854-203.106	-11.166-0.907	2.618	0.569ns	0.324
MON=a+b TL	T1(15%)	0.5707	0.07	-1.099-2.240	-0.073-0.211	0.061	0.367ns	0.134
	T2(20%)	4.2398	-0.11	-1.044-9.524	-0.509-0.286	0.172	0.223ns	0.049
	T3(25%)	3.1736	-0.13	-0.176-6.524	-0.413-0.143	0.121	0.368ns	0.135
GRA=a+b TL	T1(15%)	8.9344	-0.12	-16.770-34.639	-2.303-2.071	0.948	0.043ns	0.002
	T2(20%)	20.3043	-0.59	-9.332-49.941	-2.821-1.636	0.966	0.211ns	0.045
	T3(25%)	1.93879	0.21	-4.017-7.893	-0.287-0.703	0.215	0.324ns	0.105
MCV=a+b TL	T1(15%)	184.2326	-2.98	74.119-294.345	-12.344-6.393	4.063	0.251ns	0.063
	T2(20%)	216.5441	-5.90	93.507-339.582	-15.154-3.348	4.012	0.462ns	0.213
	T3(25%)	56.9642	5.24	-237.65-351.579	-19.249--29.729	10.619	0.172ns	0.029
MCH=a+b TL	T1(15%)	30.1984	0.93	21.026-39.371	0.146-1.708	0.338	0.696*	0.484
	T2(20%)	44.6831	-0.37	33.834-55.532	-1.221-0.473	0.367	0.339ns	0.115
	T3(25%)	21.5133	3.18	-33.101-76.128	-1.359-7.719	1.968	0.496ns	0.246
MCHC=a+b TL	T1(15%)	10.4399	1.44	-22.296-43.176	-1.344-4.226	1.193	0.389ns	0.151
	T2(20%)	21.5592	0.58	-3.819-46.937	-1.399-2.564	0.859	0.233ns	0.054
	T3(25%)	103.2052	-3.35	-86.900-293.311	-19.151-12.451	6.852	0.170ns	0.029
MPV=a+b TL	T1(15%)	5.3759	0.03	3.776748-6.975057	-0.10701-0.165114	0.059	0.171ns	0.029
	T2(20%)	34.6460	15.25	-126.557-195.8491	2.660351-27.83789	5.459	0.703*	0.494
	T3(25%)	4.1564	0.14	1.999941-6.3129	-0.03668-0.321795	0.078	0.544ns	0.296

Correlation coefficient-r, coefficient of determination-r^2, intercept-a, regression coefficient-b, Confidence intervals-Cl, standard error-S.E, *** $P < 0.001$, **$P<0.01$, * $P < 0.05$, ^{ns} $P > 0.05$.

Table 3: Regression analysis and descriptive statistics of various haematologicalparameters in relation to weight (g) of GIFT Tilapia reared at graded levels of protein in hapas

Equation	Diet Variables	Relationship Parameters		95% CI of a	95% CI of b	Standard error	r	r^2
		A	B					
PLT=a+b W	T1(15%)	280.5838	-3.99	108.571- 452.597	-11.649- 3.668	3.321	0.391ns	0.153
	T2(20%)	112.7479	4.17	-72.080- 297.576	-2.472- 10.802	2.878	0.455ns	0.207
	T3(25%)	173.3192	3.83	-106.509- 453.148	-4.229- 11.888	3.495	0.361ns	0.130
WBCs=a+b W	T1(15%)	40.9895	0.75	13.719- 68.259	-0.465- 1.963	0.527	0.449ns	0.202
	T2(20%)	144.1648	-1.50	87.325- 201.004	-3.508- 0.574	0.885	0.506ns	0.256
	T3(25%)	79.9544	-0.33	39.850- 120.059	-1.489- 0.820	0.501	0.230ns	0.053
RBCs=a+b W	T1(15%)	0.8781	0.01	-0.203- 1.959	-0.039- 0.056	0.021	0.138ns	0.019
	T2(20%)	1.0623	0.02	0.598- 1.527	0.032- -0.002	2.055	0.588ns	0.346
	T3(25%)	1.1639	-0.01	0.733- 1.595	-0.020- 0.005	0.005	0.455ns	0.207
HGB=a+b W	T1(15%)	4.7768	-0.01	3.208- 6.346	-0.057- 0.033	0.019	0.207ns	0.043
	T2(20%)	2.9032	0.10	-1.208 -7.014	-0.079- 0.288	0.079	0.419ns	0.176
	T3(25%)	4.2608	0.07	2.581- 5.941	0.009- 0.130	0.026	0.687*	0.472
LYM=a+bW	T1(15%)	50.2545	0.55	8.471- 92.039	-1.306- 2.414	0.807	0.236ns	0.056
	T2(20%)	99.7355	-0.53	62.666- 136.805	-1.865- 0.797	0.577	0.311ns	0.097
	T3(25%)	88.9033	-0.64	65.986 111.821	-1.296-0.025	0.286	0.618ns	0.381
MON=a+b W	T1(15%)	0.8621	0.02	-0.166- 1.890	-0.022-0.069	0.019	0.386ns	0.149
	T2(20%)	2.2984	-0.01	0.261- 4.336	-0.083-0.064	0.032	0.105ns	0.011
	T3(25%)	1.0416	3.19	1.042- 3.189	-0.049-0.013	0.013	0.427ns	0.182
GRA=a+b W	T1(15%)	8.4217	-0.04	-7.542- 24.385	-0.749-0.672	0.308	0.044ns	0.002

	T2(20%)	4.6641	0.07	-5.509 14.837	-0.290-0.440	0.158	0.165ns	0.027
	T3(25%)	3.6728	0.02	1.713- 5.633	-0.032-0.081	0.024	0.329ns	0.109
MCV=a+bW	T1(15%)	177.5437	-1.29	111.031- 244.057	-4.261-1.662	1.284	0.337ns	0.114
	T2(20%)	101.6689	0.67	56.095- 147.243	-0.970-2.303	0.709	0.315ns	0.099
	T3(25%)	110.1940	0.29	11.932- 208.456	-2.534-3.125	1.227	0.085ns	0.007
MCH=a+bW	T1(15%)	34.7391	0.29	28.835- 40.644	0.552-0.027	0.114	0.668*	0.446
	T2(20%)	21.2995	0.28	-1.763- 44.362	-0.549-1.107	0.359	0.264ns	0.069
	T3(25%)	46.1249	0.43	29.376- 62.874	-0.050-0.914	0.209	0.589ns	0.348
MCHC=a+bW	T1(15%)	15.1271	0.56	-4.40168- 34.656	-0.30853-1.430	0.377	0.466ns	0.217
	T2(20%)	21.2996	0.28	-1.763- 44.362	-0.549-1.107	0.359	0.264ns	0.069
	T3(25%)	67.9504	-0.15	4.472- 131.428	-1.978-1.679	0.793	0.066ns	0.004
MPV=a+b W	T1(15%)	5.4365	0.01	4.457- 6.417	-0.031-0.057	0.019	0.235ns	0.055
	T2(20%)	4.8843	0.05	2.319- 7.449	-0.045-0.139	0.039	0.386ns	0.149
	T3(25%)	5.3219	0.02	4.631- 6.013	-0.003-0.037	0.009	0.579ns	0.335

Correlation coefficient-r, coefficient of determination-r^2, intercept-a, regression coefficient-b, Confidence intervals-Cl, standard error-SE, ***$P<0.001$, **$P<0.01$, * $P < 0.05$, ^{ns} $P > 0.05$

DISCUSSION

Haematological profile analysis of fishes is an integral part of evaluating their health status [6, 7, 15]. The values of haematological parameters observed in the present study are supported by findings of [9] in *Cyprinus carpio*, [21] reference interval values in hybrid tilapia and [22] in African catfish. [23] reported a non-significant correlation in various blood indices in Nile tilapia (*Oreochromis niloticus*) fed with synthetic feed with limited inclusion of fish oil. Non-significant increasing trend was observed for WBCs and lymphocytes with increasing level of %CP. The increased number of WBCs shows good health status of fish [6]. Higher number of WBCs in the studied fish is related to findings of [9, 15, 24]. Although, significantly higher WBCs count points at a possible immunomodulatory effect or due to stressor effect [15, 24]. Reduced WBCs count may be attributed to stress imposed by handling the fish [9].[25] affirmed that lymphocytes are the most abundant leukocytes in fish followed by monocytes, neutrophils and eosinophils. Lymphocytes count (T3=89.38±6.15) is supported by [6] worked out the effect of different protein energy ratios in juvenile *Labeorohita* (90.08±6.51 in 25% CP). Contrarily, [15] found low lymphocyte count in Nile tilapia fed with high levels of maltose (35%). Monocytes showed the similar non-significant differences in all groups. Monocyte count are in agreement with findings of [15].[26] reported monocytes comprises less than 10% of the total WBCs in animals of all species.

In the present study, RBCs count revealed non- significant variations in T1, T2 and T3 and corroborates [9, 22]. [9] stated high number of WBCs and RBCs shows good health status. Contrarily, [3, 27] noted low RBCs at high CP. Mostly the reduced RBCs count in fish may indicate the anemic status of fish under stressful conditions [28]. Hemoglobin, concentration gives reflection of oxygen supply of an organism and organism itself attempts to keep it as stable as possible [3]. An increasing trend of RBCs, HGB and HCT with increasing CP is in line with [16]. The levels of HGB and HCT are best related to [22]in African catfish. Red blood cell indices viz. MCV, MCH and MCHC seem to be altered being more sensitive causing changing in the homeostatic system of fish [29]. Fish fed 15% CP diet showed least significant result of MCH (P<0.05) and non-significant MCV (20%, 25%) and MCH values (15%, 20% and 25%) are related with [3,21,22]. MCHC is an indication of shrinkage or swelling of red blood cells [30]. In present study, T1 and T2 and T3 showed non-significant results (P>0.05) of MCHC. The greater values of MCHC in the treated fish (T1) is probably an indication of RBCs shrinkage and/or reduced hemoglobin synthesis [3]. The most recognized role of platelets is to initiate blood clotting in the process of hemostasis. Platelet count is an indication of disease resistance and morbidity process of an organism [6]. An increasing trend of platelet level was exhibited. Least Significant (P<0.05) platelets against total length were noted in T2 while non-significant in T1 and T3. Platelets count against wet body weight in T1, T2 and T3 yielded non-significant correlation (Table 2 and 3).

CONCLUSION

In state of present investigation, it can be concluded that response of organisms varies with varying feed stuffs, and feed ingredients have pronounced influence on haematological profile of GIFT fingerlings. Protein level of 25% diet enhances the health status and physiological responses of treated fish.

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