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Haematological indices study of juvenile *Labeo rohita* (Hamilton-1822) fed at varying protein: Energy ratios

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Abstract
Present experiment was pilotedin triplicate to study the haematological indices in *Labeo rohita* (Hamilton-1822) fed at varying Protein: Energy ratios. Ten days old fingerlings of average weight 0.43±0.09(g) and length 3.15±0.28(cm) were collected, acclimatized and shifted in Hapas (8x6x3 ft.), fed @ 10% wet body weight for two weeks and 5% up to the end of experiment. Five fishes from each group were randomly selected at the end of 90 day trial for haematological studies. The blood indices MCV, MCH, MCHC, WBC, LYM, MON, GRA, RBC, HGB, RDW, PLT and MPV were studied. Linear regression analysis was applied to study the statistical relationship. T_1, T_2 and T_0 were indicating isometric growth. Fish fed at low Protein: Energy ratios showed better health status. Fish fed with T_0 and T_4 were more stressed. Thus, low protein diets are not only cost effective but also indicate good health status of fish.

Keywords: Haemotological variants, hapas, protein: energy ratios, fingerlings, isometric growth

Introduction

Current millennium is the era; facing the challenges of low food production, protein energy mal-nutrition, less, expensive and inconsistent supply of quality feed ingredients and health related issues. Aquaculture nutritionists are in unceasing exploration to enhance fish production to cope with these problems for some extent (Hardy, 2008; Lianes *et al.,* 2008) [11, 22]. Efficient feed essences strategy has recently been gained significant attention to enhance fish production and health status (Ibrahem *et al.,* 2010) [15]. Protein energy desire of fish is mostly fulfilled by fishmeal in common aquaculture practices. Aquaculture research, now focusing to lessen the fishmeal percentage in feeds; thus, trying to replace it with substitute diets. New combination of diets will not only provide essential nutrients, but also serve as positive indicator for health (Cazenave *et al*., 2005) [6].

Blood, the most abundant body fluid reflects physiological status of an organism. Haematological parameters are good tools for monitoring and evaluation of fish health status, noticing the illness, following the advancement of disease and fish response to disease treatment (Tilak*et al.,* 2007; Oluyemi *et al*., 2008) [40, 31]. Now a day, it becomes a routine practice to evaluate fish health status through haematological parameters (Tavares-Diasand Moraes, 2006) [39]. Variations in feed ingredients have a drastic effect on fish health. Thus, haematological parameters are influenced by varying protein energy ratios (Ferguson *et al.,* 2010) [10]. A sum of extrinsic and intrinsic factors may have positive or negative effect on haematological data (Naveen *et al.,* 2011) [29]. Blood composition usually altered; during disease, imbalanced diet or in mal-nutrition conditions. Extrinsic factors such as supervision and management, feeding regime, stocking density, different protein energy ratios, diseases and stress conditions may always reflect major alterations in blood composition. Various Protein ratios, glucose level and cholesterol have a direct effect on sex, age and size of the fishes (Dharan Sing *et al.,* 2008) [7].

Haematological indices provide us a valuable tactics to study health status of culture-able species. These indices provide us a reliable statistics about metabolic disorders, chronic stress and nutritional deficiencies before going to clinical settings and therapy. Consequently, serum biochemistry and haematological figures are most valuable in monitoring the health status of fish species, particularly in fisheries management and development projects (Nespolo and Rosenmann, 2002) [30].

Present article reflects the impact of various protein energy ratios of four feeding regime on haematological parameters and health status of *Labeo rohita* (Ham.).

Materials and Methods

Study period and site

Present experiment was piloted from June to August 2015 for 90 days at the Fish Farm Facilities, Al Raheem fish hatchery, Ali Pur Shumali, near Murad Abad, the rural demonstration site (30° 20′ 0″ N, 71° 5′ 0″ E) Muzaffar Garh, Punjab, Pakistan.

Feed formulation

Four diets with varying protein: Energy ratios (T_1 with 25% Crude Protein (CP), T_2 (30%CP), T_3 (35%CP) and T_4 (40%CP) were formulated from cheaper and locally available feed ingredients. Fish meal based diet (T_0); commonly practiced in southern Punjab was served as control. Individual ingredients were dried in convection oven at 60 ^{0}C to a constant weight and then chemically analyzed and calculated on the basis of percentage given by Nakamura (1981) [27] and FAO (2003) [9] (Table 1). The feed ingredients were thoroughly mixed and finally ground to powder form for its easy intake.

Experimental layout

L. rohita (Hamilton) fingerlings were selected for this experimental work because of their prime importance in carp's culture. The study was aimed to observe the effect of different protein: Energy (P: E) diets of cheaper source on growth and haematological indices of this species. All the fingerlings for experiment were obtained from single pair of breeders to prevent from the complexes of genetic variations. Aten days oldfingerlings of average weight 0.43±0.09 (g) and average length 3.15±0.28 (cm) (Table 2) were collected with the help of drag net. All the fingerlings were acclimatized in cemented holding tanks and then shifted in Hapas (8x6x3 ft.); four groups out of five, received the experimental diets while the fifth group was retained on control diet (fish meal). All the treatment groups and control had three replicates each. All the fish groups received the diet @ 10% wet body weight for two weeks and 5% up to the end of experiment. The ration was divided into three parts per day and was given to the fish groups between 08:00-09:00, 13:00-14:00 and 17:00-18:00 h.

Physico-chemical indices

The physico-chemical indices such as Dissolve oxygen, water and air temperature and pH were examined on daily basis while transparency, total hardness and alkalinity were recorded fortnightly.

Haematological parameters

Five fishes from each treatment group (T_1, T_2, T_3, T_4 and T_0) were randomly selected from hapas at the end of 90 days trial. To bleed alive, Fish samples were anaesthetized with MS222 (buffered solution; 30mg/L). The wet body weight (g) and total length (cm) of samples were also recorded at the time of blood collection. Blood samples were drawn by direct puncturing the heart by using 1 ml hypodermic syringe (21 gauge), usually used in insulin administration. A special Attention was taken to inhibit the blood from coming in contact with water. A vial containing the anti-coagulant EDTA (Heparin sodium 1%) was used for blood cell studies (Remya, 2010) [35]. The collected blood samples were rapidly subjected to haematological laboratory for analysis. The blood indices; MCV-*Mean corpuscular volume*, MCH-*Mean corpuscular haemoglobin*, MCHC-*Mean corpuscular haemoglobin concentration,* WBC-*White blood cells,* LYM-*Lymphocytes,* MON-*Monocytes,* GRA-*Granulocytes,* RBC-*Red blood cells,* HGB-*Haemoglobin,* RDW-Red *blood* cell distribution width, PLT-*Platelets* and MPV-*Mean platelets volume*were determined in all the groups by using Mythic 18 automatic hematology analyzer, Orphee, Switzerland.

Statistical analysis

The data of both control and treatment groups based on randomized design was subjected to ANOVA (one-way analysis of variance) at 0.01 significant level. Difference in haemotological and absolute growth indices was analyzed by using SPSS statistical software version 16.0 to distinguish differences in mean values. Linear regression analysis was used to study the statistical relationship between total length (TL) and Wet body weight (W) by using formula;

$\text{Log } W = \log a + b \log TL$

Where "a" is the intercept and "b" is regression coefficient (slope). The relationship of TL v/s blood indices and W v/s blood indices were also calculated by linear regression (Hossain *et al.,* 2006) [13].

Results

Water quality indices

Water quality indices such as Dissolve oxygen, water temperature, pH, transparency, total hardness and alkalinity stayed uniform among all the treatment groups (Table 3). All the parameters were within the acceptable boundaries for ideal growth of *L. rohita* fingerlings as defined by Abid and Ahmed (2009) [1]. This stability and consistency of water indices reveals that various protein energy ratios of diet did not have any side effect on water quality.

Haematological catalogues

Haematological catalogues of experimental fish were studied under the influence of artificial diets of various Proteins: Energy ratios under four treatment groups and a control. The blood of experimental fish showed similarity to the blood of other vertebrates as it comprises of plasma (water, minerals, hormones and electrolytes), cellular constituents (WBC's and RBC's) and platelets. The blood indices values reflect the health status of experimental fish.

RBC's (10^6/µL)

The RBC's count in *L. rohita* (Ham.) under the influence of artificial diet of various proteins: Energy ratios shown in (Table 4). The RBC values in T_0, T_1, T_2, T_3 and T_4 were 2.41±0.29, 2.31±0.31, 2.10±0.21, 2.14±0.23 and 2.32±0.15 (10^6/µL) respectively. Maximum value 2.41±0.29 (10^6/µL) of RBC's was observed in T_0 fed with fish meal. Minimum value 2.10±0.21 (10^6/µL) of RBC was noted in T_2 with 30% crude protein. The order of RBC's count was $T_0 > T_4 > T_1 > T_3 > T_2$.

RBC's Indices (Hgb, MCV, MCH, MCHC, HCT, RDW & RDW-SD)

Hgb-*haemoglobin concentration* (g/dl), MCV-*mean Corpuscular Volume* (µm^3), MCH-*mean Corpuscular Haemoglobin* (pg), MCHC-*mean Corpuscular Haemoglobin Concentration* (g/dl) and HCT-*hematocrit* (%), RDW-*red blood cell distribution width*(%) and RDW-SD -*red blood cell distribution width when measured with standard deviation*(µm^3); the indices of RBC's under the sway of artificial diet of various proteins: Energy ratios in Labeo rohita (Ham.) was intended and mentioned in T_0, T_1, T_2, T_3 and T_4 treatmentgroups (Table 4).

The haemoglobin concentration (Hgb) value was calculated as 8.16±0.4, 8.54±1.17, 7.65±0.7, 7.57±0.88 and 8.58±0.55 (g/dl) inT_0, T_1, T_2, T_3 and T_4 respectively. *L. rohita* made the greatest value of Hgb as 8.58±0.55 (g/dl) in treatment group T_4 (40%CP) and lowest 7.57±0.88 (g/dl) in T_3 (35%CP). The haemoglobin concentration (Hgb) observed in this study was below from normal range (8.5-11.6 g/dl) except T_1 and T_4.

The MCV values in experimental and control groups were 149.7±15.15, 137.86±44.23, 138.35±26.07, 127.7±34.91 and 164.56±14.23 (μm^3) in T_0, T_1, T_2, T_3 and T_4respectively. All the values except T_4 were below the normal range 155.6-226μm^3.

The MCH value in all groups (T_0, T_1, T_2, T_3 and T_4) was 34.13±3.94, 37.02±0.76, 36.53±0.99, 35.32±1.59 and 36.98±1.05pg. All the values mentioned was below the normal range 43.8-75.0pg; however, the treatment group T_1 showed maximum and control group T_0 (Fish meal) showed minimum values in this study.

The MCHC value calculated for all groups viz. T_0, T_1, T_2, T_3 and T_4 was 22.85±2.02, 29.4±10.15, 27.28±6.1, 29.48±8.41 and 22.58±1.84g/dl respectively. Except T_0 and T_4, all the treatment groups had values within normal range (25-39.9g/dl). T_4 treatment group showed minimum value 22.58±1.84g/dl while T_3 representedthe maximum value 29.48±8.41g/dl.

RDW-*red blood cell distribution width* and RDW-SD *red blood cell distribution width when measured with standard deviation*; the blood parameters which indicates the cell volume and size. RDW have shown the variations in normal cell volume. RDW values observed in this study was 10.2±0.63, 12.66±2.45, 11.55±3.25, 12.08±3.25 and 10.26±1.76% viz. T_0, T_1, T_2, T_3 and T_4. RDW-SD values was 73.96±6.09, 99.54±70.51, 62.18±13.17, 58.03±11.79 and 74.32±19.02 (μm^3) viz. T_0, T_1, T_2, T_3 and T_4.

WBC's and its indices (LYM, MON & GRA)

The WBC's count in *L. rohita* (Ham.) under the influence of artificial diet of various proteins: Energy ratios designated as T_0 (Control), T_1, T_2, T_3 and T_4 shown in Table4. The WBC's values in T_0, T_1, T_2, T_3 and T_4 were 115.72±1.24, 111.8±8.65, 111.98±7.96, 114.87±3.15 and 115.66±1.26 ($10^3/\mu L$) respectively. Maximum value 115.72±1.24 ($10^3/\mu L$) of WBC's was also detected in T_0 and Minimum value 111.8±8.65 ($10^3/\mu L$) was observed in T_1 fed with 25% crude protein. The order of observation of WBC's was $T_0 > T_4 > T_3 > T_2 > T_1$.

The lymphocytes count in T_0, T_1, T_2, T_3 and T_4 were 105.94±1.82, 100.38±5.43, 103.90±3.82, 104.98±3.52 and 104.02±5.93 respectively. The percentage of lymphocytes observed in respect to total leukocytes was 91.55±1.17, 90.08±6.51, 93.00±4.29, 91.45±3.89 and 89.94±5.7 respectively. The order of observation of lymphocytes in different treatment groups was $T_0 > T_3 > T_4 > T_2 > T_1$.

The monocytes count in T_0, T_1, T_2, T_3 and T_4 were 6.14±0.32, 5.5±3.3, 4.78±2.83, 5.63±2.2 and 6.76±2.81with percentage 5.31±0.29, 4.76±2.7, 4.18±2.28, 4.87±1.83 and 5.84±2.36 of total leukocytes respectively. The monocyte count observation order was $T_4 > T_0 > T_3 > T_1 > T_2$.

The granulocytes count in T_0, T_1, T_2, T_3 and T_4 were 3.64±1.3, 5.98±4.73, 3.28±2.72, 4.25±2.53 and 4.9±4.05 with percentage 3.15±1.13, 5.16±3.96, 2.83±2.24, 3.68±2.17 and 4.22±3.47 of total leukocytes respectively. The granulocytes observation order was $T_1 > T_4 > T_3 > T_0 > T_2$.

Platelets and its indices (MPV, PCT & PDW)

The numbers of platelets count in *L. rohita* (Ham.) under the influence of artificial diet of various proteins: Energy ratios wasgivenin Table 4. The numbers of platelets count in T_0, T_1, T_2, T_3 and T_4 was 68.5±11.3, 163.6±93.98, 134.25±79.2, 75.17±55.37 and 136.6±49.38 respectively. The minimum value observed was 75.17±55.37 ($10^3/\mu L$) in T_3 cured with 35% CP and maximum value noted was 163.6±93.98 ($10^3/\mu L$) in T_1 fed with 25% CP.

MPV-*mean platelets volume* calculated for all the groups (T_0, T_1, T_2, T_3 and T_4) had values 6.98±0.38, 6.82±0.6, 6.78±0.29, 6.72±0.42 and 6.68±0.65 (μm^3) with minute differences.

PCT-*plateletcrit values* measured as 0.06±0.05, 0.11±0.07, 0.09±0.06, 0.05±0.04 and 0.09±0.04% for T_0, T_1, T_2, T_3 and T_4 respectively. Its maximum value was observed in T_1.

PDW-*platelets distribution width* indicated the variation in size of platelets; its values observed in T_0, T_1, T_2, T_3 and T_4 were 7.94±0.88, 8.46±1.05, 8.13±0.5, 8.25±1.38 and 8.56±0.64% respectively.

Regression exploration and descriptive statistics of various haemotological parameters in relation to total length-TL (cm) and wet body weight-W (g) was also analyzed to study the logarithmic correlations. A logarithmic correlation between total length-TL (cm), wet body weight-W (g) and haemotological parameters in all four diets (T_1, T_2, T_3 and T_4) and control (T_0) shown in Table 5. Independent parameter; the total length, when kept on x-axis and dependent variables on y-axis, the LWR (Length-weight relationship) correlated highly significantly ($P<0.001$). Maximum correlation was observed in T_2 (r = 0.999) and the minimum in T_3 (r = 0.753). The slope value (b) in T_1 (2.894), T_2 (2.828) and T_0 (2.882) was almost equal to 3.0 indicating isometric growth, T_3; b < 2.5 (1.273) reflected an over-proportional growth in length as compared to weight and T_4; b≈3.5 (3.435) stated over-proportional increment in weight relative to length (Table 5).

Condition factor-K, schemed beside total length and wet body weight, it remained constant with increment in length or weight. A highly significant relation was observed in T_2 and T_3, when condition factor piloted against total length (Table 5). When condition factor was piloted against wet body weight, a significant relation was observed in T_2, T_4 and T_0 (Table 6).

A logarithmic correlation between total length-TL (cm), wet body weight-W (g) and Platelets was observed in all treatment groups with control. Platelets correlation with total length-TL (cm) showed highly significant relation in T_1 and T_2; significant relation in T_3, T_0 and non-significant relation in T_4. Platelets correlation with wet body weight-W (g) revealed highly significant relation in T_1 and T_2; significant relation in T_0 and non-significant relation in T_3 and T_4. A non-significant relation was observed in T_3 and T_4, when total length-TL (cm), wet body weight-W (g) piloted against MPV; whereas all other treatment groups denoted a significant relationship (Table 5 and 6).

WBC's, when plotted against total length-TL (cm) and wet body weight-W (g), a highly significant correlation was observed only in T_4(r =0.737 and r =0.845; $P<0.001$). Lymphocytes and monocytes when schemed against total length-TL (cm), a highly significant relationship was observed in T_3 and T_4 (r > 0.781), while granulocytes showed highly significant relation T1, T3and T_4 (r > 0.708) (Table 5).When the correlation of wet body weight-W (g) against Lymphocytes, monocytes and granulocytes was studied a highly significant correlation (r =0.879, r =0.987 and r =0.948) was observed in T_4 (Table 6).

RBCs, when schemed against total length-TL (cm) and wet body weight-W (g), a highly significant correlation was observed only in T_3 viz. 0.730 and 0.981; $P<0.001$. Haemoglobin concentration, when connived against total length-TL (cm), a highly significant relation was observed in T_3 and T_4 and non- significant relation in T_1 and T_0 (Table 5) while wet body weight-W (g) expressed a highly significant relation only in T_3 and non-significant relation in T_1 and T_0 (Table 6). MCV and MCHC relationship with total length-TL (cm) and wet body weight-W (g) denoted highly significant relation in treatment groups T_4 and T_0. RDW-SD against total length-TL (cm) and wet body weight-W (g) showed highly significant relation in T_2, $T_3$and T_4 treatment groups. MCH showed a highly significant relation in T_2 ($r > 0.772$) when piloted against total length-TL (cm) and wet body weight-W (g) (Table 5and 6).

Table 1: Proximate Composition of Various Protein-Energy diets

Group	**% dry weight**					
	Crude protein	Crude fat	Crude fiber	Moisture contents	Ash content	NFE
T1(25% CP)	25.9	9.61	9.73	8.72	10.85	35.19
T2(30% CP)	29.6	8.63	9.23	9.02	10.57	32.95
T3(35% CP)	35.1	7.29	8.21	8.93	11.06	29.41
T4(40% CP)	39.6	6.26	6.5	9.55	13.07	25.02
T0(Controlled)	52.01	6.14	0.9	7.35	14.42	19.18

Table 2: Growth parameters of *Labeo rohita* (Hamilton)reared in earthen ponds at different protein energy ratios(at the time of blood collection)

Parameters	**T1 (25%CP)**		**T2 (30%CP)**		**T3 (35%CP)**		**T4 (40%CP)**		**T0 (Control)**	
	Mean±S.D.	**Range**	**Mean±S.D.**	**Range**	**Mean±S.D.**	**Range**	**Mean±S.D.**	**Range**	**Mean±S.D.**	**Range**
Wet wt. (g)	114.85±10.7	103.07-129.15	103.22±11.97	90.42-118.69	92.64±4.13	88.73-99.57	104.05±11.68	90.86-118.15	99.17±8.39	84.92-106.57
TL (cm)	20.88±0.63	20.3-21.7	20.55±0.83	19.6-21.6	19.28±0.49	18.4-19.8	20±0.62	19.2-20.7	20.06±0.56	19.3-20.7
Condition factor	1.26±0.04	1.21-1.32	1.19±0.01	1.18-1.2	1.29±0.07	1.23-1.43	1.3±0.05	1.24-1.37	1.23±0.05	1.18-1.3

Table 3: Water quality indices of water bodies

TreatmentGroups	**Physico-chemical indices**													
	Air temp.(ºC)		**Water temp.(ºC)**		**pH**		**Dissolved oxygen(mg/l)**		**Total alkalinity(mg/l)**		**Total hardness(mg/l)**		**Transparency(cm)**	
	Mean±S.D.	Range	Mean±S.D.	Range	Mean±S.D.	Range	Mean±S.D.	Range	Mean±S.D.	Range	Mean±S.D.	Range	Mean±S.D.	Range
T1 (25% CP)	35.04±3.19	30-40	31.21±1.62	28-33.5	8.15±0.27	7.6-8.5	6.49±0.22	6.2-6.8	160.08±12.1	142-176	153.17±5.83	145-164	24.0±1.91	21-27
T2 (30% CP)	35.46±3.47	30-41	31.58±1.73	29-35	8.15±0.24	7.8-8.5	6.38±0.23	6.0-6.8	152.92±5.9	142-163	143.75±5.38	133-153	23.33±1.23	22-26
T3 (35% CP)	35.58±3.42	30.5-41	31.5±1.64	28.5-34	8.12±0.32	7.5-8.5	6.43±0.35	6.0-7.1	190.83±7.54	179-201	175.83±7.79	165-189	23.83±2.12	20-27
T4 (40% CP)	35.67±3.32	31-41	31.71±1.54	29-34	8.29±0.32	7.8-8.8	6.08±0.16	5.8-6.3	174.83±6.09	164-183	163.75±6.51	156-177	24.08±1.88	21-27
T0(Control)	35.92±3.37	31-41	32.00±1.76	30-36	8.33±0.21	7.9-8.7	6.24±0.28	5.8-6.9	171.08±3.55	163-175	160.92±4.01	154-167	24.17±2.17	20-27

Table 4: The haematological parameters of *Labeo rohita* reared in earthen ponds at different protein energy ratios

Haematological variables	**Diet variables (Treatments)**									
	T1		**T2**		**T3**		**T4**		**T0**	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
WBC(10^3/µL)	111.8±8.65	97.0-117.9	111.98±7.96	101.0-118.6	114.87±3.15	109.1-118.0	115.66±1.26	114.4-117.7	115.72±1.24	114.6-117.5
LYM(10^3/µL)	100.38±5.43	94.9-108.8	103.90±3.82	98.5-106.6	104.98±3.52	98.3-108.6	104.02±5.93	95.4-108.5	105.94±1.82	103.3-108.3
MON(10^3/µL)	5.5±3.3	1.3-9.0	4.78±2.83	1.6-7.8	5.63±2.2	2.9-9.3	6.76±2.81	4.3-10.2	6.14±0.32	5.8-6.6
GRA(10^3/µL)	5.98±4.73	0.9-12.4	3.28±2.72	0.9-6.7	4.25±2.53	1.3-8.3	4.9±4.05	1.6-10.9	3.64±1.3	2.4-5.2
LYM(%)	90.08±6.51	81.9-97.8	93.00±4.29	88.8-97.5	91.45±3.89	84.8-96.1	89.94±5.7	82.5-94.8	91.55±1.17	90.14-92.77
MON(%)	4.76±2.7	1.3-7.6	4.18±2.28	1.6-6.6	4.87±1.83	2.7-8.0	5.84±2.36	3.8-8.7	5.31±0.29	5.02-5.73
GRA(%)	5.16±3.96	0.9-10.5	2.83±2.24	0.9-5.7	3.68±2.17	1.2-7.2	4.22±3.47	1.4-9.4	3.15±1.13	2.08-4.54
RBC(10^6/µL)	2.31±0.31	1.81-2.57	2.10±0.21	1.82-2.28	2.14±0.23	1.76-2.39	2.32±0.15	2.19-2.5	2.41±0.29	2.11-2.83
HGB(g/dl)	8.54±1.17	6.8-9.6	7.65±0.7	6.7-8.4	7.57±0.88	6.4-8.8	8.58±0.55	7.9-9.2	8.16±0.4	7.6-8.5
HCT(%)	32.82±13.79	15.9-48.4	29.33±7.65	18.7-36.7	27.57±9.1	16.6-39.3	38.3±4.98	32.2-44.1	35.86±2.57	33.1-39.3
MCV(µm^3)	137.86±44.23	87.8-188.3	138.35±26.07	102.7-165.3	127.7±34.91	81.4-164.4	164.56±14.23	145.0-179.3	149.7±15.15	126.5-166.96
MCH(pg)	37.02±0.76	35.7-37.6	36.53±0.99	35.1-37.4	35.32±1.59	32.9-37.4	36.98±1.05	35.6-38.4	34.13±3.94	29.33-40.28
MCHC(g/dl)	29.4±10.15	19.8-42.8	27.28±6.1	21.3-35.8	29.48±8.41	21.4-44.0	22.58±1.84	20.9-24.6	22.85±2.02	20.32-25.68
RDW(%)	12.66±2.45	9.5-16.3	11.55±3.25	9.5-16.4	12.08±3.25	8.2-15.6	10.26±1.76	8.6-12.5	10.2±0.63	9.5-11.2
RDW-SD(µm^3)	99.54±70.51	44.1-196.7	62.18±13.17	53.7-81.7	58.03±11.79	47.7-78.3	74.32±19.02	53.1-99.4	73.96±6.09	66.8-83.1
PLT(10^3/µL)	163.6±93.98	60.0-255.0	134.25±79.2	60.0-222.0	75.17±55.37	38.0-180.0	136.6±49.38	63.0-185.0	68.5±11.3	57.1-82.5
MPV(µm^3)	6.82±0.6	6.2-7.8	6.78±0.29	6.4-7.1	6.72±0.42	6.4-7.5	6.68±0.65	5.8-7.3	6.98±0.38	6.5-7.5
PCT(%)	0.11±0.07	0.04-0.18	0.09±0.06	0.04-0.16	0.05±0.04	0.025-0.121	0.09±0.04	0.04-0.133	0.06±0.05	0.012-0.133
PDW(%)	8.46±1.05	7.1-9.8	8.13±0.5	7.4-8.5	8.25±1.38	6.2-10.2	8.56±0.64	7.8-9.3	7.94±0.88	6.9-8.8

Table 5: Regression analysis and descriptive statistics of various haemotological parameters in relation to total length-TL (cm) of *Labeo rohita* (Hamilton) reared at varying protein: energy ratios in hapas

Equation	Diet variables	Relationship Parameters		95% CI of a	95% CI of b	Standard error	r	r^2
		a	b					
W=a+b TL	T1(25%)	-1.760	2.894	-4.414-0.893	0.883-4.9.5	0.02	0.935***	0.875
	T2(30%)	-1.701	2.828	-2.297- -1.105	2.374-3.282	0.003	0.999***	0.997
	T3(35%)	0.330	1.273	-1.655-2.316	-0.272-2.818	0.01	0.753***	0.567
	T4(40%)	-2.454	3.435	-4.938-0.03	1.526-5.345	0.016	0.957***	0.916
	T0(cntr)	-1.758	2.882	-4.927-1.411	0.449-5.316	0.02	0.909***	0.826
K=a+b TL	T1(25%)	0.239	-0.106	-2.414-2.893	-2.116-1.905	0.02	0.096^{ns}	0.009
	T2(30%)	0.299	-0.172	-0.297-0.895	-0.626-0.282	0.003	0.755***	0.570
	T3(35%)	2.330	-1.727	0.345-4.316	-3.272- -0.182	0.01	0.841***	0.706
	T4(40%)	-0.454	0.435	-2.938-2.03	-1.474-2.345	0.016	0.386*	0.149
	T0(cntr)	0.242	-0.118	-2.927-3.411	-2.551-2.316	0.02	0.088^{ns}	0.008
PLT=a+b TL	T1(25%)	-20.486	17.144	-60.565-19.592	-13.228-47.515	0.25	0.720***	0.518
	T2(30%)	16.795	-11.223	-27.972-61.561	-45.328-22.881	0.24	0.708***	0.501
	T3(35%)	-10.536	9.598	-49.451-28.380	-20.684-39.879	0.27	0.403*	0.162
	T4(40%)	4.375	-1.743	-28.735-37.484	-27.194-23.707	0.22	0.125^{ns}	0.016
	T0(cntr)	4.976	-2.415	-7.681-17.633	-12.134-7.305	0.07	0.415*	0.172
WBCs=a+b TL	T1(25%)	3.603	-1.178	-2.280-9.485	-5.636-3.279	0.04	0.437*	0.191
	T2(30%)	2.020	0.021	-5.154-9.194	-5.444-5.487	0.04	0.012^{ns}	0.0001
	T3(35%)	2.668	-0.473	0.953-4.383	-1.808-0.861	0.01	0.442*	0.195
	T4(40%)	1.729	0.257	1.166-2.291	-0.175-0.689	0.004	0.737***	0.544
	T0(cntr)	2.019	0.034	1.11-2.929	-0.664-0.732	0.005	0.089^{ns}	0.008
RBCs=a+b TL	T1(25%)	2.385	-1.535	-8.589-13.359	-9.851-6.782	0.07	0.321*	0.103
	T2(30%)	0.838	-0.395	-8.983-10.658	-7.876-7.087	0.05	0.158^{ns}	0.025
	T3(35%)	4.336	-3.119	-0.872-9.545	-7.171-0.934	0.04	0.730***	0.533
	T4(40%)	-1.018	1.063	-5.075-3.039	-2.055-4.182	0.03	0.531**	0.282
	T0(cntr)	1.742	-1.045	-7.898-11.382	-8.448-6.357	0.06	0.251^{ns}	0.063
HGB=a+b TL	T1(25%)	2.640	-1.297	-8.535-13.815	-9.766-7.171	0.07	0.271^{ns}	0.073
	T2(30%)	2.085	-0.916	-6.465-10.635	-7.429-5.598	0.05	0.393*	0.155
	T3(35%)	5.422	-3.537	0.550-10.295	-7.329-0.254	0.03	0.792***	0.626
	T4(40%)	-0.876	1.390	-4.565-2.812	-1.445-4.226	0.02	0.669***	0.448
	T0(cntr)	1.549	-0.49	-2.497-5.595	-3.597-2.617	0.02	0.278^{ns}	0.077
LYM=a+b TL	T1(25%)	1.070	0.705	-2.883-5.024	-2.290-3.701	0.02	0.397*	0.158
	T2(30%)	1.863	0.117	-1.779-5.506	-2.658-2.892	0.02	0.127^{ns}	0.016
	T3(35%)	0.701	1.027	-0.767-2.167	-0.114-2.169	0.01	0.781***	0.609
	T4(40%)	4.145	-1.636	1.991-6.298	-3.291-0.019	0.01	0.876***	0.767
	T0(cntr)	2.239	-0.165	0.824-3.655	-1.251-0.922	0.008	0.268^{ns}	0.072
MON=a+b TL	T1(25%)	20.711	-15.201	-31.079-72.502	-54.448-24.045	0.32	0.580***	0.336
	T2(30%)	-1.192	1.369	-71.676-69.293	-52.328-55.066	0.38	0.077^{ns}	0.006
	T3(35%)	18.108	-13.529	5.347-30.869	-23.459- -3.599	0.09	0.884***	0.782
	T4(40%)	-15.071	12.201	-26.288- -3.854	3.578-20.823	0.07	0.933***	0.871
	T0(cntr)	0.223	0.434	-4.084-4.53	-2.874-3.741	0.03	0.234^{ns}	0.055
GRA=a+b TL	T1(25%)	40.882	-30.514	-3.998-85.762	-64.524-3.495	0.28	0.855***	0.731
	T2(30%)	5.800	-4.130	-86.332-97.933	-74.319-66.059	0.5	0.176^{ns}	0.031
	T3(35%)	23.442	-17.808	-8.216-55.099	-42.443-6.826	0.22	0.708***	0.502
	T4(40%)	-31.757	24.849	-55.073- -8.441	6.927-42.772	0.15	0.931***	0.866
	T0(cntr)	-6.136	5.126	-34.23-21.957	-16.447-26.699	0.16	0.4*	0.160
MCV=a+b TL	T1(25%)	4.445	-1.762	-22.952-31.843	-22.524-18.999	0.17	0.154^{ns}	0.024
	T2(30%)	0.750	1.055	-18.654-20.155	-13.728-15.838	0.1	0.212^{ns}	0.045
	T3(35%)	8.046	-4.633	-9.905-25.997	-18.602-9.335	0.13	0.418*	0.175
	T4(40%)	-1.440	2.810	-2.145- -0.735	2.268-3.352	0.005	0.994***	0.989
	T0(cntr)	-1.124	2.532	-7.689-5.441	-2.509-7.574	0.04	0.678***	0.460
MCH=a+b TL	T1(25%)	1.232	0.255	-0.328-2.791	-0.926-1.437	0.001	0.369*	0.136
	T2(30%)	2.248	-0.523	0.531-3.966	-1.831-0.786	0.01	0.772***	0.596
	T3(35%)	2.051	-0.392	-0.985-5.088	-2.755-1.971	0.02	0.224^{ns}	0.050
	T4(40%)	1.144	0.326	-0.905-3.140	-1.250-1.901	0.01	0.355*	0.126
	T0(cntr)	0.807	0.556	-8.784-10.399	-6.81-7.921	0.06	0.137^{ns}	0.019
MCHC=a+b TL	T1(25%)	-1.220	2.022	-27.971-25.530	-18.249-22.293	0.17	0.180^{ns}	0.033
	T2(30%)	3.472	-1.557	-16.769-23.714	-16.978-13.863	0.11	0.294^{ns}	0.086
	T3(35%)	-3.968	4.221	-20.882-12.946	-8.941-17.382	0.12	0.407*	0.165
	T4(40%)	4.555	-2.461	2.553-6.556	-4.000- -0.923	0.01	0.947***	0.896
	T0(cntr)	3.931	-1.976	-1.954-9.817	-6.496-2.543	0.03	0.626***	0.392
RDW-SD =a+b TL	T1(25%)	-7.774	7.339	-61.36-45.812	-33.268-47.946	0.33	0.315^{ns}	0.099
	T2(30%)	-3.757	4.224	-13.056-5.542	-2.861-11.308	0.05	0.876***	0.767
	T3(35%)	6.869	-3.978	-4.307-18.046	-12.675-4.719	0.08	0.536**	0.287
	T4(40%)	-6.897	6.732	-18.027-4.232	-1.823-15.287	0.07	0.822***	0.676
	T0(cntr)	1.732	0.105	-5.218-8.681	-5.232-5.441	0.04	0.036^{ns}	0.001
MPV=a+bTL	T1(25%)	-0.735	1.188	-7.031-5.560	-3.582-5.959	0.04	0.416*	0.173
	T2(30%)	1.779	-0.723	-1.279-4.438	-3.053-1.607	0.02	0.686***	0.471
	T3(35%)	1.612	-0.611	-2.368-5.591	-3.708-2.486	0.03	0.264^{ns}	0.070
	T4(40%)	0.087	0.566	-7.479-7.653	-5.25-6.382	0.05	0.176^{ns}	0.031
	T0(cntr)	3.027	-1.676	0.612-5.441	-3.531-0.177	0.01	0.857***	0.734

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

Table 6: Regression analysis and descriptive statistics of various haemotological parameters in relation to wet body weight-W (g) of *Labeo rohita* (Hamilton) reared at varying protein: energy ratios in hapas

Equation	Diet variables	Relationship Parameters		95% CI of a	95% CI of b	Standard error	r	r^2
		a	b					
CF=a+b W	T1(25%)	-0.092	0.093	-1.389-1.205	-0.537-0.723	0.02	0.262^{ns}	0.069
	T2(30%)	0.19	-0.06	-0.151-0.532	-0.228-0.112	0.003	0.719***	0.517
	T3(35%)	0.771	-0.335	-2.416-3.958	-1.956-1.285	0.02	0.276^{ns}	0.076
	T4(40%)	-0.29	0.2	-1.187-0.606	-0.245-0.645	0.01	0.637***	0.406
	T0(cntr)	-0.192	0.141	-1.64-1.255	-0.585-0.866	0.017	0.335*	0.113
PLT=a+b W	T1(25%)	-11.944	6.439	-25.299-1.41	0.353-13.326	0.16	0.889***	0.789
	T2(30%)	10.333	-4.111	-12.953-33.619	-15.684-7.462	0.23	0.734***	0.539
	T3(35%)	-2.508	2.189	-40.511-35.496	-17.136-21.515	0.3	0.155^{ns}	0.024
	T4(40%)	1.069	0.515	-13.209-15.347	-6.569-7.599	0.2	0.132^{ns}	0.017
	T0(cntr)	3.927	-1.05	-1.579-9.434	-3.811-1.709	0.07	0.573**	0.328
WBCs=a+b W	T1(25%)	2.443	-0.192	-0.774-5.66	-0.755-1.37	0.04	0.22^{ns}	0.049
	T2(30%)	2.026	0.011	-1.856-5.909	-1.919-1.94	0.04	0.017^{ns}	0.0003
	T3(35%)	2.677	-0.314	1.174-4.179	-1.078-0.451	0.01	0.495*	0.245
	T4(40%)	1.898	0.082	1.706-2.09	-0.013-0.177	0.003	0.845***	0.715
	T0(cntr)	2.01	0.027	1.58-2.44	-0.189-0.242	0.005	0.222^{ns}	0.049
RBCs=a+b W	T1(25%)	0.704	-0.167	-5.105-6.513	-2.988-2.654	0.07	0.108^{ns}	0.012
	T2(30%)	0.583	-0.131	-4.741-5.906	-2.776-2.515	0.05	0.159^{ns}	0.022
	T3(35%)	5.199	-2.477	3.448-6.551	-3.164- -1.79	0.01	0.981***	0.962
	T4(40%)	0.053	0.155	-1.932-2.039	-0.83-1.14	0.03	0.277^{ns}	0.077
	T0(cntr)	1.897	-0.76	-2.024-5.818	-2.725-1.205	0.05	0.579**	0.336
HGB=a+b W	T1(25%)	1.039	-0.054	-4.812-6.889	-2.896-2.788	0.07	0.035^{ns}	0.001
	T2(30%)	1.526	-0.32	-3.11-6.162	-2.624-1.984	0.05	0.389*	0.152
	T3(35%)	5.33	-2.265	1.613-9.047	-4.155-0.375	0.03	0.857***	0.735
	T4(40%)	0.43	0.249	-1.504-2.364	-0.71-1.209	0.03	0.431*	0.186
	T0(cntr)	1.111	-0.1	-0.889-3.113	-1.013-0.903	0.02	0.181^{ns}	0.033
LYM=a+b W	T1(25%)	1.513	0.237	-0.465-3.491	-0.724-1.198	0.02	0.413*	0.171
	T2(30%)	1.95	0.034	-0.028-3.926	-0.949-1.016	0.02	0.103^{ns}	0.011
	T3(35%)	1.625	0.201	-0.427-3.677	-0.842-1.245	0.02	0.259^{ns}	0.067
	T4(40%)	2.938	-0.457	2.02-3.856	-0.913-0.002	0.01	0.879***	0.773
	T0(cntr)	2.142	-0.059	1.466-2.818	-0.397-0.28	0.008	0.303*	0.092
MON=a+b W	T1(25%)	6.271	-2.729	-24.08-36.62	-17.47-12.01	0.37	0.322*	0.104
	T2(30%)	-0.523	0.561	-38.631-37.586	-18.379-19.501	0.38	0.09^{ns}	0.008
	T3(35%)	13.342	-6.417	-4.075-30.759	-15.274-2.439	0.14	0.709***	0.503
	T4(40%)	-6.444	3.595	-8.6- -4.288	2.526-4.665	0.03	0.987***	0.974
	T0(cntr)	0.169	0.31	-1.644-1.983	-0.599-1.219	0.02	0.531**	0.282
GRA=a+b W	T1(25%)	16.44	-7.687	-16.09-48.97	-23.49-8.12	0.4	0.666***	0.444
	T2(30%)	2.847	-1.227	-47.25-52.94	-26.13-23.67	0.5	0.148^{ns}	0.022
	T3(35%)	9.924	-4.763	-28.52-48.367	-24.31-14.786	0.3	0.320*	0.103
	T4(40%)	-13.642	7.052	-22.395- -4.889	2.71-11.395	0.13	0.948***	0.899
	T0(cntr)	-3.536	0.043	-16.31-9.237	-4.359-8.444	0.16	0.506**	0.256
MCV=a+b W	T1(25%)	1.354	0.372	-12.56-15.27	-6.38-7.13	0.17	0.101^{ns}	0.01
	T2(30%)	1.418	0.356	-9.105-11.94	-4.874-5.586	0.1	0.203^{ns}	0.041
	T3(35%)	5.385	-1.675	-11.903-22.673	-10.466-7.116	0.14	0.256^{ns}	0.065
	T4(40%)	0.714	0.745	-0.229-1.657	1.213-0.277	0.01	0.946***	0.895
	T0(cntr)	0.286	0.946	-2.282-2.854	-0.341-2.233	0.03	0.804***	0.646
MCH=a+b W	T1(25%)	1.321	0.12	0.608-2.034	-0.226-0.467	0.01	0.537**	0.289
	T2(30%)	1.945	-0.19	1.059-2.83	-0.63-0.25	0.01	0.796***	0.634
	T3(35%)	1.096	0.229	-1.652-3.845	-1.168-1.627	0.02	0.222^{ns}	0.049
	T4(40%)	1.375	0.095	0.497-2.253	-0.34-0.531	0.01	0.374*	0.139
	T0(cntr)	0.214	0.66	-3.789-4.218	-1.346-2.666	0.05	0.517**	0.267
MCHC=a+b W	T1(25%)	1.963	-0.25	-11.72-15.64	-6.89-6.39	0.17	0.069^{ns}	0.005
	T2(30%)	2.512	-0.539	-8.464-13.488	-5.994-4.916	0.11	0.288^{ns}	0.083
	T3(35%)	-2.235	1.877	-18.185-13.714	-6.233-9.987	0.12	0.306*	0.094
	T4(40%)	2.653	-0.645	0.006-1.433	-1.25- -0.04	0.02	0.891***	0.793
	T0(cntr)	1.928	-0.286	-1.564-5.421	-2.037-1.464	0.04	0.288^{ns}	0.083
RDW-SD =a+b W	T1(25%)	-3.725	2.738	-30.25-22.8	-10.14-15.62	0.31	0.364*	0.132
	T2(30%)	-1.265	1.517	-6.0-3.471	-0.836-3.871	0.05	0.891***	0.794
	T3(35%)	9.02	-3.694	2.553-15.488	-6.983- -0.405	0.05	0.842***	0.709
	T4(40%)	-2.21	2.02	-6.131-1.71	0.075-3.965	0.06	0.886***	0.785
	T0(cntr)	1.626	0.121	-1.703-4.956	-1.548-1.789	0.04	0.132^{ns}	0.017
MPV=a+b W	T1(25%)	-0.082	0.444	-3.142-2.978	-1.042-1.93	0.04	0.481*	0.232
	T2(30%)	1.369	-0.268	-0.212-2.949	-1.053-0.518	0.02	0.719***	0.518
	T3(35%)	1.453	-0.318	-2.178-5.084	-2.165-1.528	0.03	0.233^{ns}	0.054
	T4(40%)	0.286	0.266	-2.882-3.454	-1.305-1.838	0.05	0.297^{ns}	0.088
	T0(cntr)	1.554	-0.356	-0.293-3.4	-1.282-0.569	0.02	0.577**	0.333

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

Discussion

Haematological profile is an important, effective and sensitive monitoring tool to determine the physiological, pathological and functional status (health) of an organism (Kohanestani*et al.,* 2013) [20]. Haematological indices have an uttermost value in representing the deviation and disturbance in physiological conditions of fish, clinical diagnosis, and degree of ecosystem contaminations and effects of toxic substances (Southamani*et al.,* 2015) [38]. Variations in haematological characteristics, mostly depends on wide range of nutritious and anti-nutritious dynamics of the feed ingredient (Osuigwe*et al.,* 2007) [32]. Fish fed with various plant origin diet shows significant variation in blood characteristics (Anyanwu*et al.,* 2011) [4]. Nile tilapia (*Oreochromisniloticus*) fed with different increment levels of maltose diet also show variations in blood characteristics (Ighwela*et al.,* 2012) [16]. A non-significant correlation among various haematological indices was observed in Nile tilapia (*O. niloticus*) fed with synthetic feed with fish oil (El-Kasheif*et al.,* 2011) [8]. In state of above findings, present study was arranged to establish normal value range of haematological characteristics of fish fed with varying protein energy ratio diets.

Hrubec*et al.* (2000) [14] stated the reference values of blood indices in *Oreochromis* hybrid treated with varying protein diets viz. RBCs (1.91 - 2.88x106/µl), Hgb (7.0 - 9.8g/dl), MCV (115 - 183µm^3), MCH (28.3 - 42.3pg), MCHC (22 - 29g/dl), WBCs (21.56 - 154.69×103/µl), lymphocytes (31.43 - 88.17%), neutrophils (2.58 - 6.38%) andmonocytes (1.9 - 2.8%). In our findings, *L. rohita* shows best related results with the findings of Hrubec*et al.* (2000) [14] in all blood indices of all the treatment groups.

Stress conditions of an organism usually measured through erythrocyte and leukocytes count and its indices values as these are reliable indicator. Mature erythrocytes (RBCs) have a red pigment containing iron as a central element to carry oxygen to various tissues (Rehulka, 2002) [34]. In present study, RBCs and WBCs count deviate significantly on varying protein diets. A highly significant increment in RBCs and WBCs was observed in diet containing high proportion of proteins viz. T_0 (Fish meal) and T_4 (40%CP) which is contrary to the conclusion of Yue andZhou(2008) [43] and Iqbal*et al.* (2016) [17] as they observed low proportion ofRBCs and WBCs in juvenile hybrid tilapia and juvenile *L. rohita*respectively, fed at high protein sources, but agreement with the findings of Nasir and Al-Sraji (2013) [28] as they observed high proportion of RBCs and WBCs fed at higher protein content diet instead of low protein content diet. Thus, higher the proportion of RBCs and WBCs in blood, the fish is in stressful condition. Number of RBCs and WBCs decreases in fish blood with the increase in estradiol-17 hormones and Moringaoleifera leaf meal (Khara*et al.,* 2013; Ozovehe, 2013) [19, 33]. Reduction in RBCs represents the anemic condition due to stress situations (Li *et al.,* 2011) [21]. Amplitude of toxicants in diet causes hemolysis which results in dwindling in RBCs count (Kavitha*et al.,* 2010; Saravanan*et al.,* 2011) [18, 37]. Decrease in leukocytes may be harmful to fish as it indicates stressful state of the fish which trigger the immune system in contaminated environment (Martins *et al.,* 2004a) [23].

Hgb concentration in T_1, T_4 and T_0 treatment groups of fish was significantly higher than T_2 and T_3 groups, which represents good health status of fish while lower concentration represents that oxygen carrying capacity may be influenced by treatment in water and pollution (Ahmed, 2011) [2]. Wepener*et al.* (1992) [42] reported RBCs swelling may indicated by MCHC, which is ratio of blood Hgb as apposite to PCV, not influenced by blood volume or cells. Alwan*et al.* (2009) [3] founds MCV as indicator representing the status or size of RBCs. The decreased Hgb content is directly proportional to decreased MCHC indicating decreased hemoglobin synthesis.

In our results, MCH and MCHC concentrations are fluctuating continuously and best values were in T_1 group indicating low hemoglobin content in the shrunken RBCs of other treatment groups. The raised values of RBCs, Hgb, MCV and hematocrit in the fish indicate stressful manufacturing of RBC and Hgb. The percentage of red blood cells (Hematocrit) also indicates the stressful condition in its higher concentration (Barton, 2000) [5].

Lymphocyte (immune competent cells) number expresses the pathological changes which activate the immune system. A significant correlation was observed in T_3 and T_4. Low percentage of lymphocytes was found in T_4 which is harmful and is similar to the findings of Martins *et al.* (2004) [24] and designated stressed fish. Platelets indicate the morbidity process and disease resistance, present study observed the higher concentration in T_1 while decreased trend in all other treatment groups. The decreased no. may harm the blood capillary or bone marrow or spleen, so higher protein: energy ratios are more susceptible to diseases.

When weight length relationship was studied in all the treatment groups, estimated value of b was almost equal to "3.0" indicating isometric growth (T_1, T_2 and T_0) while it was much lower in T_3 indicating negative allometric growth and much greater in T_4 representing positive allometric growth. Variation in "b" value as compared to constant "a" are due to various reasons like temperature, food (ration, size, quality), maturity, age, sex, salinity, pH, season, habitat, daily changes and time of years; except all these factors fatness of the species and shape also have reasonable influence. Condition factor-K, schemed beside total length and wet body weight, it remained constant with increment in length or weight. These results were matching with the findings of Naeem*et al.* (2010 and 2011) [25, 26] in hybrid (*Catlacatla XLabeo rohita*) and *Oreochromismossambicus*.

A rare and unusual research work regarding fish nutrition in relation to haemotology is in progress but still this field has a vast vacuum to be filled.Artificial diets with varying protein: energy ratios show noticeable variations in haematological indices. Environmental and individual factors may also have a noticeable impact on hematological parameters. Our findings are contrary to Welker *et al.* (2007) [41]; Sado*et al.* (2008) [36] and (Hisano*et al.,* 2007) [12] as they do not find noticeable differences in haematological indices of channel catfish and Nile tilapia fed with increasing mannan oligosaccharides diet and dietary yeast.

Conclusion

In overall observations, the fish fed at low protein: energy ratios show better health status as compared to high protein: energy ratios.Low protein: energy ratio diets increases the physiological responses and health status of the experimental fish. The fish in control (T_0) and T_4 were more stressed as compared to other treatment groups. An increase in haemotological parameters RBCs, WBCs, HCT, MCV and lymphocytes at high protein: energy ratio diet indicates stressful health status while higher concentration of platelets, MCH, MCHC and PCT in Low protein: energy ratio diet represents good health status of the fish.

References

1. Abid M, Ahmed MS. Efficacy of feeding frequency on growth and survival of *Labeo rohita* (ham.) fingerlings under intensive rearing. J. Anim. Plant Sci. 2009; 19:111-113.
2. Ahmed Z. Acute toxicity and haematological changes in common carp (*Cyprinuscarpio*) caused by diazinon exposure. African Journal of Biotechnology. 2011; 10(63):13852-13859.
3. Alwan SF, Hadi AA, Shokr AE. Alterations in haematological parameter of fresh water fish. *Tilapia Zilli* exposed to Aluminium. Journal of Science and Its Applications. 2009; 3(1):12-19.
4. Anyanwu DC, Udedibie ABI, Osuigwe DI, Ogwo VO. Haematological responses of hybrid of *Heterobranchusbidorsalis* and *Clariasgariepinus* fed dietary levels of Carica papaya leaf meal. World Rural Observ. 2011; 3:9-12.
5. Barton BA. Stress, in: Stickney RR.(Ed.), Encyclopedia of aquaculture: Wiley, New York. 2000; 892-898.
6. Cazenave J, Wunderlin DA, Hued AC, de los Angeles-Bistoni M. Haematological parameters in a neotropical fish, *Corydoraspaleatus* (Jenyns, 1842) (Pisces, Callichthyidae), captured from pristine and polluted water. Hydrobiologia. 2005; 537:25-33.
7. Dharan Sing, KamleshNath SP, Thrivedi, Sharma YK. Impact of copper on haematological profile of freshwater fish *Channapunctatus*. Journal of Environmental Biology. 2008; 29(2):253-257.
8. El–Kasheif MA, Amal SS, Seham A. Effects of varying levels of fish oil on growth performance, body composition and haematological characteristics of Nile tilapia (*Oreochromisniloticus* L). Egypt. J. aquat. Biol. Fish. 2011;15:125-141.
9. FAO. The role of aquaculture in improving food security and nutrition. Committee on World Food Security, Twenty-ninth Session. Rome. 2003; 14:12-16.
10. Ferguson RMW, Merrifield DL, Harper GM, Rawling MD, Mustafa S, Picchietti S.*et al*. The effect of *Pediococcusacidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromisniloticus*. Journal of Applied Microbiology doi: 10.1111/j, 2010. 1365-2672.
11. Hardy RW. Farmed fish diet requirements for the next decade and implication for global availability of nutrients, in: Lim C, Webster CW, Lee CS. (Eds.), Alternative Protein Sources in Aquaculture Diets: The Haworth Press, New York, 2008, 1-15.
12. Hisano H, Barros MM, Pezzato LE. Levedura e zincocomopró-nutrientesemraçõesparatilápia do Nilo (*Oreochromisniloticus*: aspectoshematológicos. Bol. Inst. Pesca. 2007; 33(1):35-42.
13. Hossain MY, Ahmed ZF, Leunda PM, Jasmine S, Oscoz J, Miranda R. *et al*. Condition, length-weight and length-length relationship of the Asian striped catfish *Mystusvittatus* (Bloch, 1794) (Siluriformes: Bagridae) in the Mathbhanga River, southwestern Bangladesh. J. Appl. Ichthyol. 2006; 22:304-307.
14. Hrubec TC, Cardinale JL, Smith SA. Haematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis hybrid*). Veterinary Clinical Pathology. 2000; 29(1):7-12.
15. Ibrahem MD, Fathi M, Mesalhy S, Abd El-Aty AM. Effect of dietary supplementation of inulin and vitamin C on the growth, hematology, innate immunity, and resistance of Nile tilapia (Oreochromisniloticus. Fish Shellfish Immunol. 2010; 29:241-246.
16. Ighwela KA, Ahmad AZ, Abol-Munafi AB. Haematological changes in nile tilapia (*Oreochromisniloticus* fed with varying dietary maltose levels. World J. Fish. mar. Sci. 2012; 4:376-381.
17. Iqbal KJ, Ashraf M, Javid A, Khan N, Abbas F, Hafeez-ur-Rehman M. *et al.*Effect of Different Plant and Animal Origin (Fishmeal) Feeds on Digestive Enzyme ActivityandHaematology of Juvenile Labeo rohita. Pakistan J. Zool. 2016; 48(1):201-207.
18. Kavitha C, Malarvizhi A, Kumaran SS, Ramesh M. Toxicological Effects of Arsenate Exposure on Hematological, Biochemical and Liver Transaminases Activity in an Indian Major Carp, *CatlaCatla*, Food and Chemical Toxicology. 2010; 48:2848-2854.
19. Khara H, Falahatkar B, Meknatkhah B, Ahmadnezhad M, Efatpanah I, Poursaeid S. *et al.* Effect of dietary estradiol 17β on growth, hematology and biochemistry of stellate sturgeon, *Acipenserstellatus*. World J. Fish. mar. Sci.2013; 5:113-120.
20. Kohanestani ZM, Hajimoradloo A, Ghorbani R, Yulghi S, Hoseini A, Molaee M. Seasonal Variations in Hematological Parameters of *Alburnoideseichwaldii* in Zaringol Stream-Golestan Province, Iran. World J. Fish & Marine Sci. 2013; 5(2):121-126.
21. Li Z, Velisek H, Zlabek J, Grabic V, Machova RJ, Kolarova J. *et al.* Chronic Toxicity of Verapamil on Juvenile Rainbow Trout (*Oncorhynchusmykiss*): Effects on Morphological Indices, Hematological Parameters and Antioxidant Responses, Journal of Hazardous Materials. 2011; 185:870-880.
22. Lianes J, Toledo J, Vega JD.Comportamiento del bagreafricano(*Clariasgariepinus*) alimentado con dieta semi-húmeda, basada en ensiladobiológico de pescado. RevistaCubana de CienciaAgrícola. 2008; 42(3):269-263.
23. Martins ML, Nomura DT,Miyazaki DMY,Pilarsky F,Ribeiro K, Castro MP.*et al.* Physiological and hematological response of *Oreochromisniloticus* (Osteichthyes: Cichlidae) exposed to single and consecutive stress of capture. Acta Sci. Anim. Sci. 2004a; 26:449-456.
24. Martins ML, Tavares-Dias M, Fujimoto RY, Onaka EM, Nomura DT.Haematological alterations of *Leporinusmacrocephalus* (Osteichtyes: Anostomidae) naturally infected by *Goezialeporini* (Nematoda: Anisakidae) in fish pond.ArquivosBrasileiro de MedicinaVeterinária e Zootecnia, Belo Horizonte. 2004b;56:640-646.
25. Naeem M, Salam A, Baby R, Ali M, Ishtiaq A. Study of Body Composition of Female Population Of Farmed *Oreochromismossambicus* In Relation To Body Size And Condition Factor From Pakistan, World Academy of Science, Engineering and Technology. 2011; 78:1154-1157.
26. Naeem M, Salam A, Ishtiaq A, Shafique S. Length-Weight And Condition Factor Relationship Of Farmed hybrid (*Catlacatla* ♂ X *Labeo rohita* ♀) From Multan, Pakistan. Sindh Univ. Res. Jour. (Sci. Ser.). 2010; 42(2):35-38.
27. Nakamura H. Why is Spirulina such an astonishing miracle multipurpose food? After twelve years of intensive research, the essence of spirulina is finally

Revealed, in: Hills C. (Ed.), Thesecretes of spirulina: University of the Trees Press, Boulder Creek, CA, 1981, 21-25.

28. Nasir NA, Al-Sraji AYJ. Effect of Different dietary protein and fats on some biochemical blood parameters in common carp fingerlings (*Cyprinuscarpio* L.) reared in float cages. Asian J. exp. Biol. Sci. 2013; 4:293-296.
29. Naveen AP, Venkaeshwaslu, Janaiah C. Biochemical alteration induced by triazophos in the blood plasma of fish *Channapunctatus* (Bloch.). Annals Biol. Res. 2011;2(4):31-37.
30. Nespolo RF, Rosenmann M. Intraspecific allometry of haematological parameter in *Basilichthysaustralis* J. Fish Biol. 2002. 60:1358-1362.
31. Oluyemi KG, Adeparusi EA, Olanrewaju J. Basic haematological parameters in African catfish, *Clariasgariepinus*(Burchell, 1822) fed on ascorbic acid supplemented diets. Research Journal of Animal Sciences. 2008; 2(1):17-21.
32. Osuigwe DI, Nwosu C, Ogunji JO. Preliminary observations on some haematological parameters of juvenile *Heterobranchuslongifilis* fed different dietary levels of raw and boiled jackbean (*Canavaliaensiformis*) seed meal. Proceedings of Conference of International Agricultural Research Development, Tropentag, University of Kassel-Witzenhausen and University of Göttingen, 2007, 6.
33. Ozovehe BN. Growth performance, haematological indices and some biochemical enzymes of juveniles *Clariasgariepinus* (Burchell, 1822) fed varying levels of Moringaoleifera leaf meal diet. J. aquacul. Res. Develop.2013; 4:166.
34. Rehulka J. Aeromonas causes severe skin lesions in rainbow trout (*Oncorhynchusmykiss*): Clinical pathology, haematology and biochemistry. Acta Vet Brno, 2002; 71:351-360.
35. Remya V. Biochemical Effects of different Phenolic compounds on O. mossambicus. Ph.D. Thesis. Cochin University of Science and Technology, Cochin, 2010.
36. Sado RY, Bicudo AJA, Cyrino JEP. Feeding Dietary Mannan Oligosaccharides to Juvenile Nile Tilapia *Oreochromisniloticus*, Has No Effect on Hematological Parameters and Showed Decreased Feed Consumption. J World Aquacult Soc. 2008; 39(6):821-826.
37. Saravanan M, Karthika S, Malarvizhi A, Ramesh M. Ecotoxicological Impacts of Clofibric Acid and Diclofenac in Common Carp (*Cyprinuscarpio*) Fingerlings: Hematological, biochemical, Ionoregulatory and Enzymological Responses, Journal of Hazardous Materials. 2011; 195:188-194.
38. Southamani C, Shanthi G, Deivasigamani M. Hematological response in three Indian major carps in relation to supplementary feeding. International Journal of Fisheries and Aquatic Studies. 2015; 3(2):287-294.
39. Tavares-Dias M, Moraes FR. Haematological parameters for the *Bryconorbignyanus* Valenciennes, 1850 (Osteichthyes: Characidoe) intensivelybred. *Hidrobiologica.* 2006;16(3):271-274.
40. Tilak KS, Veeraiah K, Butchiram MS. Effect of phenol on haematological components of Indian major carps *Catlacatla*, *Labeo rohita* and *Cirrhinusmrigala*. Journal of Environmental Biology. 2007; 28(2):177-179.
41. Welker TL, Lim C, Yildirim-Aksoy M, Shelby R, Klesius PH. Immune response and resistance to stress and *Edwardsiellaictaluri*, challenge in channel catfish, *Ictaluruspunctatus* fed diets containing commercial whole-cell yeast or yeast subcomponents. J. World Aquacult. Soc. 2007; 38(1):2435.
42. Wepener V, Van Vuren JHJ, Du Preez HH. Effect of manganese and iron at neutral and acidic pH on the hematology of the banded tilapia (Tilupiuspurrmunii). Bull. Environ. Cotam. Toxicol. 1992; 49:613-619.
43. Yue YR, Zhou QC. Effect of replacing soybean meal with cottonseed meal on growth, feed utilization, and hematological indices for juvenile hybrid tilapia (*Oreochromisniloticus*. Aquaculture. 2008; 284:185-189.